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IMPACT OF SOLE CROPPING AND MULTIPLE CROPPING ON SOIL HUMIFIED CARBON FRACTIONS

IRFAN AZIZ[1], TARIQ MAHMOOD[2*] AND KHANDAKAR RAFIQ ISLAM[3]

[1]*Department of Agronomy, PAMS Arid Agriculture University, Rawalpindi, Pakistan,*
[2]*Department of Environmental Sciences, PMS Arid Agriculture University, Rawalpindi, Pakistan,*
[3]*Soil and Water Resources, Ohio State University South Centers, Piketon, Ohio, USA, and Soil Drainage Research, USDA-ARS, Columbus, Ohio, USA*
[*]*Corresponding author e-mail: tamahmood@hec.gov.pk*

Abstract

The present study was planned to improve our understanding how crop rotation can enhance humified C fractions. A long term experiment was conducted on Vanmeter farm of the Ohio State University South Centers at Piketon Ohio, USA from 2002 to 2007. Crop rotation treatments included were continuous corn (CC), corn-soybean (CS) and corn–soybean-wheat-cowpea (CSW) rotations. Randomized complete block design with 6 replications was used under natural field conditions. The findings of this long-term study revealed that multiple cropping had significantly improved humified carbon fractions compared to mono-cropping system. Although total humified carbon (THOC), sugar free humified carbon (HOC) concentration were non-significant however, humin (NH) contents, humic (HA), fulvic acids (FA), humic and fulvic acid associated glucose (HA-NH and FA-NH) were significantly affected by various crop rotations within five years. The soil under CC had 22-52% significantly greater NH concentration than CSW and CS rotations respectively. Similarly all crop rotations had shown 5–16 increase in HA and 5-17% decreased in FA over time. Likewise soil under CC had 16 and 54% greater HA-NH concentration as compared to CSW and CS rotations. The FA-NH concentration increased significantly by 27– 51% in soil under all treatments over time. The soil under CSW had greater HA/FA (1.6) fallowed by CC (1.4) and CS (1.1). Soils under CSW had significantly greater HA/HOC (12–18%) as compare to CC and CS respectively. Conversely, the value of FA/HOC decreased (1–23%) in soil under all crop rotation treatments within five years. Degree of humification (DH) had shown a significant increase (7–12%) in soil under all treatments as compared to 2002. Irrespective of crop rotation THOC, HOC, NH, humin, HA, HR and FA/HOC concentration decreased significantly with increase in soil depth. While fulvic acid concentration HA/HOC in all crop rotation increased with increase in soil depth. The effect of crop rotation on humified C fractions could be because of variations in type, amount and quality of C returned by different plants into the soil. So replacing mono-cropping with multiple cropping can enhance humified C fractions and can improve soil functional properties.

Key words: Sole Cropping and Multiple Cropping, Total Humified Carbon, Humin, Humic Acid, Fulvic acid

Introduction

Humus constitutes the bulk of agricultural soil organic matter (Stevenson, 1982) and plays a significant role in nutrients recycling of soil ecosystems, regulate carbon cycling and interact with inorganic components and pesticides (Tan 1994; Lal 2004). As soil organic matter is thermodynamically unstable with respect to CO_2, it is part of balance among the natural processes of primary production, decomposition and transformation which largely contribute to the formation of humified C fractions (e.g. humic, fulvic, and humin) in soil (Wander & Traina 1996). Moreover, the quantity and quality of humus is as well affected by cultural operations (Slepetiene, 2001; Gonzalez *et al.,* 2003). Several researchers investigated that crop rotation has probably influence on quality of soil organic matter (Wood & Edwards, 1992). The use of traditional plowing and sole-cropping has resulted in reduction OM contents, which ultimately resulted in decline soil quality (Wander *et al.,* 1994). Wood & Edwards, (1992) also reported that changes in soil C quality were more in continuous corn as compared to corn-soybean rotation due to greater C sequestration than continuous soybean. Therefore, replacing mono-cropping with multiple cropping and less-disruptive systems like no-till could enhance humic C fractions and improve soil functional properties. Higher fulvic acid contents were reported in soil having low HA/FA ratio probably due to management strategies. Moreover, wheat-wheat had favorable environment for humification process than wheat- grass as result of variations in bulk densities (Andriulo *et al.,* 1990) might be the result of decreased HA: FA in former. Conversely, the greater stable fraction of HS (humic substances), humin, was reduced under wheat-grass, most likely due to variations in physical conditions of by conservation tillage. Doane *et al*. (2003) reported that HS pools have significant turnover of C, verify the idea that each humic fraction possibly have unique role in C cycling based on substrate and environments. The variation might be recognized to soil physical properties where humification occurs. Greater bulk density and variations in porosity of grazed lands may decrease microbial activity due to less space for microbes (Andriulo *et al.,* 1990; Iglesias *et al.,* 1996).

It is believed that functionality of humic substances is because of their physical structure and chemical composition (Hayes, 1989). There are continues processes undergoing in SOM by soil biota and chemical reactions and during humification process labile pools are converted into recalcitrant fractions (Zech *et al.,* 1997). The remainder is a non-humic discrete material composed of mainly of polysaccharide and protein-like structures (Flaig *et al.,* 1975).

The impact of management practices on C dynamics in humified organic matter is limited because these fractions have not been regularly utilized to elucidate dynamics of soil organic matter related to C sequestration (Spaccini *et al.,* 2000; Doane *et al.,* 2003; Olk, 2006). Soil humus consists of mostly organic materials and semi-stable fractions. Therefore the study of fate of C in soil humus can reveal important information for development of sustainable management system that can lead to long-term soil C sequestration (Murage *et al.,* 2008).

There is a serious dearth of knowledge which could address the impact of crop rotation practices on concentration, mass and storage of humified organic matter into various C fractions. Hence present study was planned to investigate impact of various crop rotation on concentration of humified organic substances at different soil depths under rainfed condition (2002-2007).

Materials and Methods

Experimental treatments: The study was conducted on Vanmeter farm known as the Ohio Management Systems Evaluation Area (MESA) of the Ohio State University South Centers at Piketon Ohio, USA with different tillage and crop rotations with randomized block design in factorial arrangement from 2002 to 2007. The treatments were (CC) continuous corn (*Zea mays* L.), (CS) corn-soybean (*Glycine max* L.) and (CSW) corn–soybean-wheat (*Tritium aestivum* L.) - cowpeas rotation with conventional and no–till. Soil was silt loam is covering 60 percent of the study site and contains 21 g/kg sand, 55 g/kg silt and 24 g/kg clay.

Soil collection and processing: Ten soil core samples were taken up to 30 cm (segmented at 0-7.5, 7.5-15, 15-22.5 and 22.5-30 cm) using a soil probe (1.9 cm internal diameter) by following systematic random sampling from each replicated plot in 1st week of November, 2007. The cores were pooled in a plastic bucket; each replication plot was made composite in field immediately, placed in plastic bags, and sealed. Soils were brought from the field to the laboratory in plastic bags kept on ice in a white cooler. In the laboratory, soil cores were lightly sieved to pass through a 4 mm mesh to separate roots, stones, and large organic residues. After sieving, the composite field moist soil was halved, and each sub sample was placed in a separate plastic bag. The soil from one sub sample bag was sieved by a 2 mm sieve and immediately homogenized. soil samples were spread on a polyethylene sheet and air dried for 72 h with a fan at room temperature, and analyzed for humified C fractions.

Detemination of humified organic carbon fractions

Extraction of humified organic carbon: Soil humified organic matter was extracted with 0.5M NaOH (pH 13.5). A 10 g sample of oven dried equivalent (ODE) of air dried soil was suspended in 20 mL of 0.5M NaOH and from flask air was removed by N_2 and it was shaken flask for @ 250 rpm kept overnight at room temperature. The soil suspensions were transferred to 50 mL plastic tubes, centrifuged at 3000 rpm for half an hours, and filtered to get dark colored aliquots free of soil. The aliquot was fractionated into humic acid (HA) and fulvic acid (FA) fractions (Ghosh & Schnitzer, 1979). A 20 mL portion of humified organic matter was acidified with concentrated H_2SO_4 to around pH 2 and allowed over night to facilitate the precipitation of HA fraction. The tubes were then centrifuged at 2000 rpm for 10 min to separate HA and FA fractions. The soluble FA fraction was separated from precipitated HA fraction by filtration using 0.4 µm Millipore membrane. The precipitated HA was dissolved in 20 mL of 0.1 M NaOH and purified from silica and ash contents by repeated dissolution and precipitation. Glucose equivalent total reducing sugars as non-humic C (NH) in both HA and FA were determined.

Total humified organic carbon and Humic and fulvic acids: Humified organic matter fractions were analyzed for total extractable humified organic C (THOC), HA, and FA by following rapid microwave digestion and colorimetric method (Islam & Weil 1998a). Exactly 5 mL of the THOC, HA and FA aliquots were taken in Folin-Wu glass tubes, mixed with 1 mL of 0.17$\underline{M}$ $K_2Cr_2O_7$ and 5 mL concentrated H_2SO_4, and digested in a microwave oven at 480 J/mL of solution (Islam & Weil (1998b). The absorbance of the digestate was determined colorimetrically at 590 nm using sucrose C standards for calibration.

Glucose equivalent total reducing sugars (NH) associated NH-HA, NH-FA: Glucose equivalent total reducing sugars NH were determined in both HA and FA using anthrone sulfuric acid (Brink *et al.,* 1960). Exactly, 1 mL of THOC, HA or FA sample was taken into a glass tube with 5 mL of cold Anthrone and sulfuric reagent. The mixture was vortex for uniform mixing followed by heating in a boiling water bath for 10 min. After cooling, the absorbance of the aliquot as against the standard glucose equivalent C solutions was measured on a spectrophotometer at 607 nm.

Humin: The humin was calculated after subtracting the THOC from TC content.

Sugar free humified organic carbon: The sugar free humified organic C (HOC) was calculated after subtracting NH from THOC.

Calculation of humification index (HI), humification ratio, and degree of humification: Using the data, several quotients of humification such as humification index (HI), humification ratio, and degree of humification (Tan, 1994; Islam *et al.*, 1999) were calculated as follows:

$$\text{HI (\%)} = \frac{\text{NH carbon}}{\text{THOC}} \times 100$$

$$\text{HR (\%)} = \frac{\text{HA + FA}}{\text{TC}} \times 100$$

$$\text{DH (\%)} = \frac{\text{HA + FA}}{\text{THOC}} \times 100$$

Statistical analysis: Simple and interactive effects of crop rotation, soil depth and their interaction were analyzed by using a PROC ANOVA procedure of the SAS (Anon., 2008). Treatment means were separated by using an F-protected least significant difference (LSD) test with $p<0.05$.

Result and Discussion

Crop rotation impact on concentration of soil humified carbon fractions: Although crop rotations had non-significant effect on total humified organic carbon (THOC), sugar free (glucose) humified organic carbon (HOC) and humin (Table 1) however the inclusion of time as a factor with crop rotation significantly influenced THOC, HOC and humin concentrations. The Non-humified carbon (Total glucose), (NH) increased significantly by 3–58% in all crop rotation as compared to their initial values determined in 2002. The continuous corn (CC) had significantly 22–52% more NH than corn-soybean-wheat (CSW) and corn-soybean rotation (CS) respectively. Humification index (HI) was significantly affected by crop rotation and continuous corn had 11 and 37% higher HI than corn-soybean-wheat and corn-soybean respectively. Moreover, CC had significantly higher (HR) humification ratio (10 and 14%) as compared with CS and CSW (Table 1). Crop rotations had non- significant effect on HA concentration however all crop rotations had shown 5–16% increase in HA concentration over time. The FA concentration significantly decreased by 5–17% in all crop rotations in five years. Soils under continuous corn had a net increase in fulvic acid by 10 and 16% compared to CS and CSW rotations respectively. Fulvic acid concentration in soil under different crop rotation decreased with increase in soil depth. The HA/FA was non-significantly influenced by crop rotation. The HA/FA value in soil under all crop rotation significantly increased by 11–24% compare to initial values measured in 2002. The CSW had the highest (1.6) fallowed by CC (1.4) and CS had the lowest (1.1) HA/FA value (Table 2). The soil under continuous corn had 16 and 18% more concentration of (HA-NH) humic acid associated non-humic carbon (glucose) as compared to corn-soybean-wheat (CSW) and corn-soybean (CS) rotation respectively. The fulvic acid associated with non-humic carbon (glucose) concentration significantly decreased by 1–23 percent in treatments over time. The soil under continuous corn had greater fulvic acid associated non-humic carbon (glucose) concentration (349 mg/kg) fallowed by corn-soybean-wheat (274 mg/kg) and corn-soybean (231 mg/kg) rotations. The soil under corn-soybean-wheat rotation had non-significant high value of HA/FA fallowed continuous corn by and corn-soybean rotations. Soils under CSW had significantly higher (12–18%) HA/HOC compared to CC and CS respectively. Soils under CSW had significantly higher HA/HOC by 11–15% compared with CC and CS respectively. On the other hand, the value of FA/HOC decreased (1 –23%) in all crop rotations as compare to initial value of 2002. Soil under all crop rotation treatments had shown as significant decrease (23–34%) in HA-NH/NH over time. However degree of humification (DH) in soil under all crop rotation increased by 7–11% over five years (Table 3). The humification ratio (HR) was not-significantly affected by crop rotations however HR decreased significantly with increase in soil depth. Irrespective of crop rotations, the HA decreased significantly with increased in soil depth. The FA/HOC and HA/HOC increased and decreased respectively with increasing soil depth in all crop rotation treatments.

Total humified organic carbon (THOC), sugar free (glucose) humified organic carbon HOC and humin concentration all crop rotation treatments decreased significantly with increasing soil depth. Irrespective of crop rotation non-humified carbon (Total glucose) (NH) decreased with increased in soil depth. The humification ratio HR was not affected by crop rotation however HR decreased significantly with increase in soil depth. Irrespective of crop rotations, the humic acid (HA) decreased significantly with increased in soil depth. The FA/HOC and HA/HOC increased and decreased respectively with increasing soil depth in all crop rotation treatments (Tables 1-3).

A change in concentration of humified organic C fractions by crop rotation is related to continuous cover and variations in organic matter inputs and placement. Crop rotations effect chemical composition of humus altering the amount and quality organic material inputs in soil. Crop rotation can affect SOM because of variations in quality and quality of carbon added into soil (McGill *et al.,* 1986). However, differences in humus contents attributed by type biomass added to soil are very hard to estimate as compared to amount of residue. In general, organic matter enters in the soil through crop residues is mainly composed of polysacchriarides, lipids, proteins and other aliphatic and aromatic compounds that differ in their biodegradability (Dinel & Gregorich, 1995). Type of crop bimass and variations in vegetation can also influence nature of SOM (Drijber & Lowe, 1990). The HI, HR, and HA/FA are important indices of humification of C. A significantly high HI under CSW suggests slightly less humification of OM. Similarly, the ratio of humic acid and fulvic acids to TC is commonly denoted as humification ratio (HR). A low HR under CSW usually indicates a less humidification of SOM (Jimenez and Gracia, 1992; Saviozzi *et al.,* 1994).

Several studies suggested that HA/FA is affected by vegetation (Hobson 1983). In a study including continuous corn and native grassland for 40 years, a higher HA/FA ratio showed a greater degree of humification (DH) of SOM in grassland (Saviozzi *et al.,* 1994). While, Oades *et al*. (1988) reported similar OM composition in soils with three diverse crop management practices. They discribed this as a result of lack of variation in climatic and microbial biomass activity which determine humic end products as result their action on plant biomass. Our results have shown a non-significant increase in HA/FA ratios in response to crop rotations. A non-significant increase in THOC and HOC with significantly higher NH content and significant decrease in HR, HA/FA, and DH suggests that soils in CSW under NT were less humified as compared with other crop combinations. A longer duration of plant biomass on top layer of soil is most probably related to greater exposure of organic matter to be more bio-chemically changed by abiotic processes.

Table 1. Crop rotation impacts on on humified organic carbon, humin, non-humic carbon (glucose) concentration and humification index, humification ratio at different soil depths (Averaged across tillage).

Crop rotation	Depth of soil (cm)	THOC (g/kg)	HOC (mg/kg)	Humin (%)	NH (%)	HR	HI
Initial ($_{2002}$)		4.2X*	3.8Y	9.0X	391.9Y	31.4Y	9.6Y
CC_{2007}		4.5Xa	3.8Xa	9.0Xa	617.7Xa	34.1Xa	14.3Xa
CS_{2007}		4.1Xa	3.7Xa	9.5Xa	405.3Yc	30.8Yab	10.4Yc
CSW_{2007}		4.3Xa	3.8Xa	8.9Xa	505.0Xb	29.2Yb	12.9Xb
Crop rotation and soil depth interaction							
Initial ($_{2002}$)	0-7.5	6.0#	3.3#	11.0#	485.6#	35.9#	8.4#
	7.5-15	4.4	3.3	8.4	467.1	34.4	9.6
	15-22.5	3.2	2.9	8.3	410.2	28.0	11.2
	22.5-30	3.0	3.2	8.1	346.3	27.2	9.3
CC_{2007}	0-7.5	6.2	5.5	11.7	723.1	35.4	11.6
	7.5-15	4.3	3.8	9.4	558.3	32.8	12.3
	15-22.5	3.8	3.0	7.9	818.5	33.1	20.0
	22.5-30	3.5	3.0	7.0	552.3	35.2	13.2
CS_{2007}	0-7.5	5.3	4.8	11.2	488.8	33.0	9.2
	7.5-15	4.3	3.9	9.5	423.6	32.3	9.7
	15-22.5	3.4	3.0	7.9	427.8	32.2	11.7
	22.5-30	3.3	3.0	9.4	403.5	28.5	11.0
CSW_{2007}	0-7.5	6.5	6.0	11.0	508.7	27.7	7.8
	7.5-15	4.1	4.0	8.4	582.1	31.5	13.0
	15-22.5	3.7	3.2	8.3	519.5	31.5	12.7
	22.5-30	3.0	2.4	8.1	676.3	37.5	18.1

Initial=Data collected from conventionally tilled (CT) continuous corn (CC) plots in 2002, CC_{2007}=Data collected from continuous corn plots in 2007, CS_{2007}=Data collected from corn-soybean rotation plots in 2007, CSW_{2007}=Data collected from corn-soybean-wheat rotation plots in 2007, THOC=Total humified organic carbon, HOC=Sugar free (glucose) humified organic carbon, NH =Non-humified carbon (Total glucose), HI=Humification index, HR=Humification ratio, and ns=Non-significant.

*Means followed by same upper case letter (X to Z) in the column were not significantly different at $p\leq0.05$ over time (2002 vs. 2007). +Means followed by same lower case letter (a to c) in the column were not significantly different at $p\leq0.05$ among crop rotation treatments. # indicates significant crop rotation and soil depth interaction.

Table 2. Crop rotation impacts on humic and fulvic acids, and their associated non-humic carbon (glucose) concentration at different soil depths (Averaged across tillage).

Crop rotation	Depth of soil (cm)	HA/HOC (%)	FA/HOC	NH (%)	NH (%)	FA-NH/DH (%)
Initial ($_{2002}$)		1.8Y*	2.0Y	236.7Y	155.2Y	1.0Y
CC_{2007}		2.0Xa+	1.9Xa	268.8Xa	348.9Xa	1.4Xa
CS_{2007}		1.9Xa	1.8Xab	174.9Zc	230.5Xc	1.1Xa
CSW_{2007}		2.1Xa	1.7Yb	231.2Yb	273.8Xb	1.6Ya
Crop rotation and soil depth interaction						
Initial ($_{2002}$)	0-7.5	2.1#	3.5#	266.3ns	238.3ns	0.6#
	7.5-15	2.0	2.1	260.4	161.2	1.0
	15-22.5	1.5	1.4	227.9	134.7	1.1
	22.5-30	1.6	1.2	192.2	86.7	1.4
CC_{2007}	0-7.5	3.1	2.4	317.5	417.2	1.6
	7.5-15	1.8	2.0	240.7	288.2	1.1
	15-22.5	1.7	1.3	332.1	407.2	2.1
	22.5-30	1.2	1.8	184.6	283.2	0.7
CS_{2007}	0-7.5	2.6	2.2	164.7	322.6	1.2
	7.5-15	2.2	1.7	169.2	229.6	1.2
	15-22.5	1.5	1.5	191.0	196.3	1.1
	22.5-30	1.3	1.6	174.5	173.5	0.8
CSW_{2007}	0-7.5	3.4	2.6	225.7	281.0	1.3
	7.5-15	2.1	1.5	257.1	257.4	1.9
	15-22.5	2.1	1.1	200.0	254.8	2.2
	22.5-30	0.9	1.4	242.0	302.1	0.8

Initial=Data collected from conventionally tilled (CT) continuous corn (CC) plots in 2002, CC_{2007}=Data collected from continuous corn plots in 2007, CS_{2007}=Data collected from corn-soybean rotation plots in 2007, CSW_{2007}=Data collected from corn-soybean-wheat rotation plots in 2007, HA=Humic acid, FA=Fulvic acid, HA-NH=Humic acid associated non-humic carbon (glucose), FA-NH=Fulvic acid associated with non-humic carbon (glucose), and ns=Non-significant.

*Means followed by same upper case letter (X to Z) in the column were not significantly different at $p\leq0.05$ over time (2002 vs. 2007). + Means followed by same lower case letter (a to c) in the column were not significantly different at $p\leq0.05$ among crop rotation treatments in 2007. # indicates significant crop rotation and soil depth interaction.

Table 3. Crop rotation impact on distribution of humic and fulvic acids in humified organic carbon, their associated with non-humic carbon (glucose), and the degree of humification of organic carbon at different soil depths (Averaged across tillage).

Crop rotation	Depth of soil (cm)	HA (g/kg)	FA (g/kg)	HA-NH (mg /kg)	FA-NH rotation	HA/soil (cm)
Initial ($_{2002}$)		48.3Y*	50.4X	61.4X	38.6Y	80.0Y
CC$_{2007}$		52.9Yb+	47.1Xa	40.9Ya	59.1Xa	85.7Xa
CS$_{2007}$		50.3Yb	49.7Xa	43.8Ya	56.2Xa	89.6Xa
CSW$_{2007}$		59.3Xa	40.7Yb	47.3Ya	52.7Xb	87.1Xa
Crop rotation and soil depth interaction						
Initial ($_{2002}$)	0-7.5	47.6#	52.4#	52.3ns	47.7ns	75.0ns
	7.5-15	46.3	51.0	61.7	38.3	81.3
	15-22.5	51.7	48.6	62.8	37.2	79.5
	22.5-30	47.7	49.6	69.0	31.0	84.2
CC$_{2007}$	0-7.5	65.9	34.1	38.5	61.5	88.4
	7.5-15	48.1	51.9	43.0	57	87.7
	15-22.5	56.8	43.2	43.9	56.1	80.0
	22.5-30	40.9	59.1	38.2	61.8	86.8
CS$_{2007}$	0-7.5	57.0	43.0	33.9	66.1	90.8
	7.5-15	54.5	45.5	43.9	56.1	90.3
	15-22.5	47.0	53.0	48.9	51.1	88.3
	22.5-30	42.7	57.2	48.4	51.6	89.0
CSW$_{2007}$	0-7.5	75.2	24.9	45.3	54.7	92.2
	7.5-15	58.7	41.3	48.9	51.1	87.0
	15-22.5	65.4	34.6	48.9	51.1	87.3
	22.5-30	37.8	62.2	46.1	53.9	81.9

*Means followed by same upper case letter (X to Z) in the column were not significantly different at $p \leq 0.05$ over time (2002 vs. 2007). +Means followed by same lower case letter (a to c) in the column were not significantly different at $p \leq 0.05$ among crop rotation treatments in 2007. # indicates significant crop rotation and soil depth interaction.

Conclusions

Crop rotation had variable effects on humified carbon fractions at different soil depths over time. Among the crop rotations, corn–soybean-wheat-cowpea had a significantly lower non-humic carbon (NH) concentration then continuous corn and corn-soybean. A low humification ratio (HR) under corn–soybean-wheat-cowpea suggests a less humidified organic matter than other crop rotations. Irrespective of crop rotation treatments, the humified carbon fractions decreased with increase in soil depth. Similarly, the crop rotations had a significant increase in degree of humification (DH) over time. Total humified organic carbon (THOC), Sugar free (glucose) humified organic carbon (HOC) and concentration all crop rotation treatments decreased significantly with increasing soil depth. Irrespective of crop rotations, the humin, humic acid and FA/HOC decreased significantly with increased in soil depth. The and HA/HOC decreased with increase in soil depth in all crop rotation treatments. So replacing monocropping with multiple cropping can enhance humified C and improve soil functional properties. The effect of crop rotation on humified C sequestration is because of variations in type, amount and quality of C returned.

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