



{Effect of organic matter application on CP-MAS- ^{13}C -NMR spectra of humic acids from a brown soil

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The humified SOM or humic substances (HS) composed of humic acid (HA), fulvic acid (FA) and humin (HM) represent the most microbially recalcitrant and stable reservoir of organic carbon in soil (Piccolo *et al.*, 2004). OM applications can influence the amount and structural characteristics of HS (Dou *et al.*, 2008). During the past few decades, there has been much research on HS, but their chemical structure is still not fully understood (Dong, 2006). CP-MAS- ^{13}C -NMR spectroscopy was considered as an effective method to study structures of HS without dissolving problem compared with liquid ^{13}C -NMR (Conte *et al.*, 1997; Dou *et al.*, 2008). It can directly measure the carbon framework and reflect the nature of HS transformation after OM application (Spaccini *et al.*, 2000). For that reason, this method was applied in this study. The objective of this paper was to clarify the effect of long term OM application on the changes of structural characteristics in HAs, which provided new information for improving soil fertility by OM application.

The experiment was carried out on a brown soil (Paleudalf in USDA Soil Taxonomy) at Shenyang Agricultural University, Liaoning province, China (N41°48'E123°25'). The experiment included 3 treatments: zero-treatment (CK_{br}), and two pig manure (PM) treatments (O_1 and O_2) at the rates of 0.9 t ha^{-1} and 1.8 t ha^{-1} of organic carbon, respectively. The samples of the HA fraction were extracted, separated and purified according to the method described by Dou *et al.* (1991). Elemental composition, Differential thermal analysis (DTA), $\lg K$ value, FT-IR and CP-MAS- ^{13}C -NMR of HAs were performed.

Effects on the contents of organic carbon and its composition. The contents of TOC were from 8.77 g kg^{-1} to 12.25 g kg^{-1} . The relative contents in TOC for WSS, HA, and FA were 6.87%, 14.2% and 19.8%. Comparing the CK_{br} , the contents of WSS, HA and FA for O_1 and O_2 increased, but relative contents of WSS and FA decreased. The content of the HA increased after OM application, which was consistent with other studies (Wang *et al.*, 2001). The content of the WSS increased after the OM application indicating that the increase of labile organic carbon. The C/H mole ratio of the HS could reflect the degree of condensation (Dou *et al.*, 1995).

Effects on HA chemical and optical properties. The chemical and optical properties of HA were listed. The C/H ratios decreased after OM application, from 0.830 (CK_{br}) to 0.754 (O_2). While $\lg K$ increased, from 0.623 (CK_{br}) to 0.658 (O_2). The HA structure tended to become simpler. The C/H ratio of the HA decreased after OM application. This indicates that OM application decreased the degree of condensation. The $\lg K$ values can be used as the index of HA molecule complexity in the soil. If $\lg K$ increased, the molecular structure becomes simpler. After OM application, $\lg K$ increased indicating that the molecular structure became simpler.

Effects on HA thermal properties. It could be seen that HA had exothermic peaks in moderate and high temperature regions. After OM application, heat (H_2) of exothermic peak increased in moderate temperature region, while heat (H_3) of exothermic peak decreased in high temperature region. The the heat ratio of exothermic peaks in high temperature region to moderate (H_3/H_2) decreased. From CK_{br} to O_2 , H_3/H_2 decreased from 4.31 to 0.86. The HA had moderate and high temperature exothermic peaks. The heat of exothermic peaks in the moderate temperature region might show that aliphatic compounds decomposed and peripheral functional groups decarboxylated. The heat of the exothermic peaks in the high temperature region might show that the HA was oxidized completely and inter-aromatic structures in the molecule decomposed. The heat ratio of the high to moderate temperature exothermic regions (H_3/H_2) decreased significantly after PM application, indicating that the proportion of aromatic structure decreased and the HA molecular structure simplified.

Effects on CP-MAS- ^{13}C -NMR spectrum of HA. The CP-MAS- ^{13}C -NMR spectra of the HA were quite similar to each other. These spectra exhibited signals for alkyl (0~50 ppm), O-alkyl (50~110 ppm), aromatic (110~160 ppm) and carbonyl (160~200 ppm) regions. The signals in carbonyl C region concentrated between 172 ppm and 173 ppm, and with a small signal occurred in the region of 190~200 ppm, indicating that there was carbonyl C of carboxylic acid, ester and amide, but a little amount carbonyl C of ketonic compounds. In the region of aromatic C, the most obvious peaks were the absorption at 131~133 ppm and 114~117 ppm. The former was mainly the aromatic C substituted by -COOH or -COOMe and the unsubstituted aromatic *meta* to carbons bearing an oxygen or nitrogen atom; the latter was mainly the unsubstituted aromatic C *ortho* and *para* to carbons bearing an oxygen and nitrogen atom. There was a small peak at 152~154 ppm, which was the signal of phenolic OH. The signal at 55~56 ppm was methoxyl C. The signals at 71~73 ppm were due to the -CH(OH)- in carbohydrate. The peak at 102~103 ppm was generally assigned to double oxygen-C in polysaccharide (possibly acetal). The maximum absorption at 30 ppm was the contribution of the polymethylene chain $-(\text{CH}_2)_n-$ in saturated hydrocarbons (Wilson, 1981).

After OM application, the contents of alkyl C and O-alkyl C increased and the contents of aromatic C and carbonyl C except to 1986 decreased. Compared with 1986, the contents of O-alkyl C increased and the contents of alkyl C decreased for the same treatment CK_{br} and O_2 . Aromaticity decreased significantly in OM treatments, indicating that the OM decreased the content of aromatic C and was simplified the molecular structure. The relative content of O-alkyl C increased indicating that OM application increased the content of methoxyl C and -CH(OH)- in carbohydrate. Alkyl C was probably derived from compounds of plants with high resistance to degradation, such as cutin and suberin (Baldock *et al.*, 1992; Preston, 1996), or from newly synthesized products from soil micro-organisms, which are likely to represent the most persistent fraction of stable OM (Baldock *et al.*, 1990; Lichtfouse *et al.*, 1998; Piccolo, 2002). The alkyl C increased after the OM applications, indicated by the increase of hydrophobic components content and aliphatic character. Compared with 1986, the contents of O-alkyl C increased and the contents of alkyl C decreased for the same treatment CK_{br} and O_2 (Table 4), indicating that a simplification trend took place in the aliphatic fraction of HA molecular with cultivation time in the tested soil.

Conclusions. We have found that: 1) The contents of HAs increased after OM application; 2) OM application increased the contents of alkyl C and O-alkyl C, and decreased the C/H ratio, aromaticity, and the H_3/H_2 ratio of the HA, which indicated that the HA structure tended to become simpler and more aliphatic. 3) The results obtained by CP-MAS- ^{13}C -NMR spectroscopy were mainly corresponding with those obtained by chemical analysis, thermal analysis, optical properties and IR spectroscopy, which indicated that ^{13}C -NMR spectroscopy had a potential in characterizing the structural changes of HA after long-term OM application into soils.

(Table 4; Fig. 2; References 16).