Humic substances comprise 50%-70% of soil organic matter and play an important role in global C cycling. The stabilization of soil organic matter in terrestrial ecosystems is intimately associated with organic C structural chemistry. Composition of soil humic substances varies considerably depending on the climate and origin and age of organic material. Although it is well recognized that land conversion from natural forest or grassland to agriculture leads to a reduction in the soil C pool, studies on how land-use affects the composition of soil C are limited. Moreover, most investigations focus only on the variations of absolute C concentrations at different landscape topographic positions without attention to the alterations that may occur in C composition at the molecular level. In this study, we targeted a mixed-land use watershed in southeast Pennsylvania. At a natural forest and an adjacent agricultural site, soil samples were collected from the top of the hillslope. At an adjacent grassland site, soil samples were taken at the top, back and base of the hillslope. We collected soil samples at two soil depths: 0-5 cm and 20-25 cm and the humic and fulvic acids were extracted from the soils. The C functional group composition of humic acids and fulvic acids were characterized by Carbon near-edge x-ray absorption fine structure (NEXAFS), Nuclear Magnetic Resonance (NMR) and Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopic techniques. This study will help to improve our understanding of organic matter dynamics in soils.