

## Potential origin and formation for molecular components of humic acids in soils

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Humification is defined as the process by which plant and microbial debris are transformed into humic substances. Proposed pathways for the formation of humic substances, include the lignin and lignin decomposition theories, the lignin-polyphenol theory as well as the melanoidin pathway. It is generally accepted that a combination of several of these pathways with some modifications may be responsible for producing humic substances. The current study examines humic acids from numerous soil samples to demonstrate their molecular composition. In addition we provide an explanation for the formation of these molecules that introduces a new perspective of the humification process. Our work utilizes advanced analytical techniques such as ESI-FTICR-MS and solid state NMR to more completely characterize humic acids at the molecular level.

### Methods

Humic acids were extracted from soils using 0.5 M NaOH followed by treatment with a Dowex™ ion-exchange resin to remove sodium ions. Solid State <sup>13</sup>C NMR spectra were obtained on a Bruker 400 MHz Avance II spectrometer equipped with a 4 mm solid state MAS probe. ESI-FTICR-MS analysis was conducted in the negative ion mode on a Bruker Daltonics 12 Tesla Apex Qe FTICR-MS instrument equipped with an Apollo II ESI source.

### Results:

Soil humic acids from numerous soils were investigated in this study. The molecular formulas calculated from ultrahigh resolution mass spectra of well humified soils fall clearly into two predominant regions consisting of condensed aromatic molecules as well as high H/C, low O/C carboxyl-containing aliphatic molecules (CCAM). In contrast, the spectral data for humic acids from a poorly humified spodosol soil show a less dramatic separation of these regions, with relatively more molecular formula plotting in the lignin-like region and relatively fewer condensed aromatic molecules. From the mass spectral observations made for the humic acids, we can readily discern a relationship based on degree of humification. The less humified samples contain relatively more components having a relationship to lignin. The more humified samples are composed of relatively more molecular formulas in the CCAM and condensed aromatic regions and also contain relatively more carboxylated molecular formulas than the less humified samples.

To explain the molecular formulas observed we propose a humification process that involves photo- or microbially-generated reactive oxygen species in soils which are responsible for transforming the materials supplied to soil as fresh organic matter, mainly lignin, to the molecules observed in ESI-FTICR-MS data. When plotted on the van Krevelen diagram, the H/C and O/C ratios of molecular formulas from humic acids predictably plot in the same regions as the newly produced formulas discovered by Chen, et al. (2014) when natural organic matter was photoirradiated or when lignin-derived humics were subjected to Fenton chemistry (Waggoner et al., 2015).

### References:

- Chen H., Abdulla H.A.N., Sanders R.L., Myneni S.C.B., Mopper K. and Hatcher P.G. (2014) Production of Black Carbon-like and Aliphatic Molecules from Terrestrial Dissolved Organic Matter in the Presence of Sunlight and Iron. *Environmental Science & Technology Letters* 1, 399-404.
- Waggoner D.C., Chen H., Willoughby A.S. and Hatcher P.G. (2015) Formation of black carbon-like and alicyclic aliphatic compounds by hydroxyl radical initiated degradation of lignin. *Organic Geochemistry* 82, 69-76.