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Changes in the chemical composition of soil organic matter including water-soluble component during incubation: A case study of coniferous and broadleaf forest soils

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ABSTRACT

The objective of this study was to clarify what changes occurred in the amount and chemical properties of the soil organic matter (SOM) and dissolved organic matter (DOM) in the soil following isothermal incubation for 170 days. In this study, the soil samples were collected from coniferous and broadleaf forests in Toyama, Japan. The changes in the amount of carbon dioxide generation, the composition of the organic components of the SOM and its chemical properties during the incubation period were investigated. Likewise, DOM was extracted and examined for changes in chemical properties. For that purpose, pyrolysis-gas chromatography–mass spectrometry using tetramethylammonium hydroxide (TMAH-Py-GC/MS), absorption spectrophotometry, and fluorometry were used. From the amount of CO_2 generated during incubation, it was found that 10%–15% of the SOM was mineralized. More CO_2 was found to have been generated by the soil of the coniferous forests than by the soil of the broadleaf forests, probably because the former was more strongly affected by temperature changes. The DOM increased during the incubation period by day 55 and then sharply decreased. The absorption and fluorescence spectra showed that the DOM in the soil contained large amounts of humic substances after 97 days of incubation, which comprised organic compounds that did not readily decompose.

1. Introduction

SOM is one of the largest carbon reservoirs in the Earth's surface. The amount of carbon in the soil is estimated to be 2500 Pg C, 1550 Pg C of which is considered to be organic (Dungait et al., 2012); this is approximately twice the amount of atmospheric CO_2 and about three times the amount of on-land biomass (Ciais et al., 2013). SOM is therefore an important constituent of the Earth's carbon cycle. SOM contains biological substances such as lignin, cellulose, and polypeptides. Furthermore, since humic substances are also included, the composition of SOM is complicated and heterogeneous. The residence time of SOM differs depending on the constituting organic materials, and it varies from several hours to several hundreds of years (Dungait et al., 2012; Van Hees et al., 2005). Since the rate at which SOM decomposes is significantly influenced by temperature, even slight changes in temperature can greatly impact the carbon cycle in a given biosphere (Davidson and Janssens, 2006).

Although the fraction of DOM contained in SOM is usually extremely small, DOM is a major carbon source for microorganisms, strongly involved in the transport of metal elements to the hydrosphere as well as in both the supply of nutrient salts to plants and the supply of organic carbon to the deeper layers of soil (Kaiser and Kalbitz, 2012). Therefore, the chemical composition of DOM and its dynamics in the environment are important factors regarding the circulation of materials between lands and hydrospheres. It is known that the biodegradability of DOM varies according to the composition of organic substances (Pinsonneault et al., 2016; Straathof et al., 2014). Kalbitz et al. (2003) investigated the qualitative differences and mineralization rates of soil-derived DOM during constant temperature incubation. They found that a solution containing DOM that had a large amount of carbohydrates exhibited high biodegradability, whereas the biodegradability of a solution rich in aromatic substances was poor. Previous

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studies on peat soil have shown that the activity of phenol oxidase in soil increases as the temperature of the soil is increased, which causes both the concentration of phenolic compounds and the elevated amounts of dissolved organic carbon (DOC) to increase (Freeman et al., 2001). It is likely that the alternation of DOM caused by the changes in its surrounding environment may significantly influences the terrestrial carbon cycle.

In order to evaluate the rate of decomposition of SOM, constant temperature soil incubation experiments have been conducted in a number of laboratories (e.g. Benbi et al., 2014; Haddix et al., 2011; Rey and Jarvis, 2006). The laboratory incubation experiment is able to evaluate the effect of temperature on the decomposition of SOM within the limited time. According to some previous studies, the decomposition rate of SOM differs depending on the nature of the organic substances that it contains. Polycondensed compounds with aromatic rings such as humic acids are able to resist decomposition, whereas substances derived from more intact tissues, such as polypeptides and polysaccharides, are readily degradable. However, a limited number of studies have been reported on the structural alteration of soil derived-DOM during incubation (Guigue et al., 2014).

The purpose of the present study is to clarify the change in the chemical composition of SOM, as well as the quantitative and qualitative changes in DOM during a constant temperature incubation of soil. In this study, A-layer soil samples collected from coniferous and broadleaf forests located at different altitudes on the same mountain without artificial disturbance were incubated at 25 and 35 °C, respectively. In order to investigate the chemical composition of the SOM during the incubation period, the SOM was analyzed by pyrolysis–gas chromatography–mass spectrometry using tetramethylammonium hydroxide (TMAH-Py-GC/MS). Furthermore, the amount and chemical properties of DOM were examined using TMAH-Py-GC/MS analysis, spectroscopy, and fluorescence analysis.

2. Materials and methods

2.1. Study site and soil properties

Soil samples were obtained from a broad-leaved forest (*Fagus crenata* Blume, 36°47′39″ N, 137°34′17″ E, 1098 m a.s.l.) and a coniferous forest (*Abies mariesii* Mast, 36°45′47″ N, 137°33′53″ E, 1834 m a.s.l) at Mt. *Sougatake*, Toyama, Japan in September 2014. The mean annual temperature in Uozu city, Toyama (Toyama local meteorological office located 48 m a.s.l.) is 13.6 °C, and the mean annual precipitation is 2544 mm. At each site, after removing the litter, the soils were sampled at five random points at depths of between 0 and 15 cm before being mixed together. The sample soils were air dried and sieved with a 2-mm mesh prior to analysis. The soil pH and EC were measured using HM-20P and CM-31P (TOA DKK, Japan). The moisture content was measured for intact soil that had been dried at 105 °C in a drying oven (DX 302, Yamato Scientific Co., Ltd., Japan) until it reached a constant weight. The loss on ignition (LOI) was determined using a muffle furnace (KDF007Ex, DENKEN-HIGHDENTAL Co., Ltd., Japan). The air-dried soils were heated at a rate of 10 °C min^{-1} before being heated at 600 °C for 2 h. The C, H, and N contents were determined by a Micro Corder JM 10 type CHN analyzer (J-Science Lab. Co. Ltd.), and the content of oxygen was calculated to subtract the total percentage of C, H, N and ash from 100.

2.2. Soil incubation

Two replicates of each soil sample were incubated at 25 °C and 35 °C for 170 days (MIR-153, Sanyo Electric Co. Ltd., Osaka, Japan). The soils (50 g dry weight) were stored in 100 mL glass beakers and the water content was adjusted (the conifer soil had a water content of approximately 60% and the broad-leaved soil had a water content of approximately 50%). The glass beakers were placed in 1.0 L sealing glass jars with two plastic vials containing 20 mL of 1 M NaOH (used to trap respired CO_2) and 20 mL of H_2SO_4 (used to maintain humidity). These two alkali traps were replaced every day during the first two weeks, every third or fourth day during the second two weeks, and every seven to ten days thereafter. The soils were pre-incubated for seven days at 25 °C, and the respiration of the soils was measured after the pre-incubation period (Haddix et al., 2011). The soil respiration was measured by titration with 0.2–0.4 M HCl using phenolphthalein and bromocresol green. The alkali solutions were adjusted to pH 8.4 using HCl, and the amount of HCl used to reach pH 4.2 was recorded. The respired carbon was calculated using the following equation (Sugahara and Katoh, 1992):

$$\text{Respired Carbon (mg C g soil}^{-1}) = \frac{(C_{HCl}\, V_{HCl}\, f_{HCl} - C_{blank\ CO_2})\, 12.01}{W_{dry\ soil}}$$

where C_{HCl} is the concentration of the HCl used in the titration (mol L^{-1}), V_{HCl} is the amount of titrated HCl required (mL), f_{HCl} is the factor of the HCl solution determined using titrated Na_2CO_3 with the same concentration as the HCl solution, $C_{blank\ CO2}$ is the concentration of the CO_2 of the blank (mol), and $W_{dry\ soil}$ is the weight of the soil dried during the incubation. The soil was sub-sampled at 20, 55, 97, and 160 days during the incubation and analyzed.

2.3. TMAH-pyrolysis–gas chromatography–mass spectrometry

About 3 mg soil was placed on a pyrofoil (F590, Japan Analytical Industry Co., Ltd., Japan). Tetramethyl ammonium hydroxide (25 μL, Sigma-Aldrich) in methanol–acetone solution (40 mg mL^{-1}) and nonadecanoic acid in acetone (10 μL, 0.06 mg mL^{-1}) were added to the soil so as to act as an internal standard (ISTD). After removing the solvents under reduced pressure, the pyrofoils were tightly wrapped and introduced into a Curie point pyrolyzer JHP-5 (Japan Analytical Industry, Tokyo, Japan) that was connected to a Shimadzu GC/MS QP 2010 (Kyoto, Japan). The column was a DB-5 column (30 m × 0.25 mm i.d., film thickness of 0.25 μm, J&W Scientific, USA). Samples were pyrolyzed at 590 °C for 5 s on the pyrolyzer and were injected into the GC/MS with a carrier gas (helium, 99.9995% purity with a sprite ratio of 22). The GC was maintained at 50 °C for 1 min before being heated from 50 to 300 °C at a rate of 5 °C min^{-1}; a temperature of 300 °C was then maintained for 4 min. The total flow rate was 50 mL min^{-1}. All the peaks were identified using the NIST library and assigned if they bore a resemblance to data that was greater than 80%. In order to compare each peak area for each sample, we calculated the relative peak area and divided each area with that of the ISTD (retention time of 35.5 min) (Fujisawa et al., 2012; Fukushima et al., 2011, 2009; Iwai et al., 2013).

2.4. Water-soluble soil organic matter extraction and analysis

Soil samples and ultra-pure water (ratio of 1 g: 10 mL) were added to a glass vessel. After shaking for 24 h, the soil suspensions were filtrated under vacuum through a 0.45 μm membrane filter (mixed cellulose ester, ADVANTEC), and the DOM solutions were stored at 4 °C in a dark environment before analysis. An aliquot of the DOM solutions were analyzed using total organic carbon (TOC) analysis and fluorescence spectroscopy. The remaining solutions were freeze-dried and analyzed using TMAH-Py-GC/MS. About 1.5 mg freeze-dried DOM was introduced into the Py-GC/MS with ISTD and TMAH. A TOC analysis was performed using TOC-5000A (Shimadzu, Japan). Before the analysis, the sample solutions were acidified to a pH of 2 through the addition of HCl. After being purged for 10 min to remove inorganic carbon, the sample solutions were injected and the DOM concentration was determined. Three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy was performed using LS-55 (Perkin-Elmer, USA). The excitation and emission wave range was 200–600 nm. The irradiating intervals of the excitation waves were