Journal of Molecular Structure 1107 (2016) 7–13

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Photodegradation of major soil microbial biomolecules is comparable to biodegradation: Insights from infrared and diffusion editing NMR spectroscopies

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ARTICLE INFO

Article history:
Received 21 June 2015
Received in revised form
24 September 2015
Accepted 11 November 2015
Available online 17 November 2015

Keywords:
Biodegradation
Leachates
Soil microbial biomass
Soil organic matter
Photodegradation
Ultraviolet radiation

ABSTRACT

As a primary decomposition process in terrestrial biosystems, biodegradation has been extensively studied with regard to its impact on soil organic matter transformation. However, the biotransformation of soil microbial biomass (a primary source of soil organic carbon) remains poorly understood, and even less is known about the fate of microbial-derived carbon under photodegradation. Here, we combine infrared and diffusion editing NMR spectroscopies to provide molecular-level information on the photodegradation of major biochemical components in soil microbial biomass and leachates over time. Results indicate a considerable enrichment in aliphatic components, presumably polymethylenic-C [$(C-H_2)n$] and the simultaneous loss of carbohydrate and protein structures in the biomass. An immediate conclusion is that photodegradation increased the conversion of macromolecular carbohydrates and proteins to smaller components. However, further analysis reveals that macromolecular carbohydrates and proteins may be more resistant to photodegradation than initially thought and are found in the leachates. Although attenuated, there is also evidence to suggest that some aliphatic structures persist in the leachates. Overall, the photodegradation pathway reported here is remarkably similar to that of biodegradation, suggesting that under rapidly expanding anthropogenic land disturbances, photodegradation could be an important driver of the transformation of microbial-derived organic matter in terrestrial biosystems.

1. Introduction

Soil microbes are a primary source of soil organic matter (SOM) and therefore play a crucial role in the biogeochemical cycling of carbon, soil quality and numerous ecosystem services [1]. Therefore, even small changes to this prolific microbiome could profoundly influence the rate and extent of changes in atmospheric CO_2 concentrations [2], as well as, ecosystem stability and fertility. Despite these fundamental roles and potentials, accurate determination of the contribution of microbes to the labile and stable SOM pools remains an immense biogeochemical challenge. Nevertheless, it is generally accepted that the composition and dynamics of SOM, in particular the rapid cycling of soil C are controlled not only by molecular structure but are also a function of various mechanisms of protection offered by soil minerals, photochemical decomposition, and microbial-mediated biodegradation [3,4]. As a primary decomposition process, biodegradation has been extensively studied for its impact on the dynamics of terrigenous organic matter (OM) [5] and OM in aquatic systems [6]. However, the biotransformation of microbial biomass in soil remains poorly understood, and even less is known about its sensitivity to ultraviolet radiation (UVR). Therefore, it follows that if we are to take maximum advantage of the role of soils to act as a buffer against rising atmospheric CO_2 concentrations and as a potential sink for additional carbon [7], such endeavours should be coupled to an empirical understanding of the biodegradation and photodegradation pathways of major microbial biomolecules in soils.

In recent decades, the amount of UVR (UV-B; 280–315 nm and UV-A; 315–400 nm) reaching the Earth's surface has increased considerably, and this trend is expected to continue until the mid twenty-first century [8–10]. UVR-induced photodegradation (in particular UVB radiation) has been shown to be an important factor in litter decomposition in arid, semi-arid, and peatland systems

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http://dx.doi.org/10.1016/j.molstruc.2015.11.025
0022-2860/

[8,9,11–13], which, despite their disproportionate size, store up to one-third of global soil carbon [14,15]. There is also growing empirical evidence to suggest that UVR-induced photodegradation is an important driver of global environmental change with profound implications for the structure, function and processes of tropical, subtropical [16] and aquatic ecosystems [17]. For example, photodegradation has been shown to prime the conversion of large resistant biopolymers to smaller compounds that are more amenable to degradation by native microbes in soil [12] and aquatic environments [18]. Additionally, changes in the chemical composition of litter and the abundance and composition of microbial community have been attributed to the indirect effects of UVB radiation [8,19]. It has also been suggested that soil microbial biomass is much more susceptible to elevated levels of UVR than plant material, and that photodegradation of soil microbial biomass may influence the potential for soils to sequester carbon and nitrogen [13,19].

Moreover, it now appears to be a certainty that such impacts, especially on tropical and subtropical ecosystems will be exacerbated by constant and rapidly expanding anthropogenic land disturbances that are increasing the risk of exposing a highly reactive SOM pool to photodegradation and removal as CO_2 to the atmosphere. Allied to this, disruptions in the amount and distribution of precipitation and increasing aridity associated with climate variability [20] are likely to enhance the effects of photodegradation in terrestrial biosystems. Critically, significant amounts of carbon stored in paddy soils, the largest anthropogenic wet lands on Earth [21], could be threatened by photodegradation. Despite the likelihood of UVR to destabilize the massive stock of potentially volatile carbon in soils, our knowledge of the role of photodegradation in SOM dynamics remains constricted by a lack of adequate observational data and models of sufficiently fine resolution. Therefore, in attempting to address these knowledge gaps, we employ infrared and diffusion edited NMR spectroscopies to provide molecular-level information on the biodegradation and photodegradation of soil microbial biomass and equivalent leachates over time. Infrared and NMR spectroscopies are powerful complementary tools for describing the molecular characteristics and chemical transformation of microbial biomass and other environmental matrices (e.g. [22–24]) but remain significantly underutilized in degradation and related biogeochemical studies.

2. Materials and method

2.1. Media and growth conditions

The growth medium was a nutrient broth, containing: yeast extract (2.0 g L^{-1}); peptone (5.0 g L^{-1}); and NaCl (5.0 g L^{-1}), in distilled water. The pH of the medium was adjusted to 7.0 before introducing the inoculum, which was prepared from an Irish field site (Oakpark soil). The origin, agricultural management history and textural characteristics of the field site are summarized elsewhere [25]. Duplicate starter cultures were prepared by adding approximately 100 mg of sieved, homogenized soil to 100 mL of growth medium, and the mixture incubated at room temperature for seven days with reciprocal shaking (125 strokes min^{-1}) [26]. The initial cultures were allowed to stand overnight and a 1 mL aliquot was removed from each flask and used to inoculate a new flask containing pure growth solution. This was done to prevent any possible carryover of soil particles from the initial cultures. Cultures were incubated for an additional 14 days and the resulting microbial biomass harvested and washed repeatedly (three washes) with a large excess of distilled water by centrifugation at 4025 rpm for 30 min to remove any trace of the growth solution. The biomass was then freeze-dried and stored at −20 °C before analysis. Freeze-drying is lethal to most living systems [27] but differences in the survival rates of Gram-negative bacteria compared with those of Gram-positive may be attributed to the thinner peptidoglycan layer and the presence of lipopolysaccharides on the cell wall in the former species [28]. It is not known what influence centrifugation and washing has on cell survival rates. Note, all growth solutions and glassware were autoclaved and allowed to cool at room temperature. Identical controls were prepared without the addition of soil. In these controls, no microbial growth was observed.

2.2. Decomposition experiment

Duplicate decomposition experiments were conducted under laboratory conditions (January–July, 2010). It is fully acknowledged here, that artificial UVR does not completely represent natural light and the experimental outcome may have differed *in situ*. Freeze-dried microbial biomass was evenly distributed (~2 mm thick) in glass funnels (65 mm top diameter and 121 mm in length) with borosilicate sintered discs (20 mm in diameter) of grade 4 porosity and the funnels placed vertically in a photoreactor (Model LZC-1, Luzchem Research Inc., Canada) equipped with two overhead UVB lamps (peak, 313 nm). The incident UVB radiation was filtered through cellulose acetate films (Kodak) to eliminate any residual UVC (<290 nm) given off. The mean irradiance of the lamps was monitored with a SPR-01 spectroradiometer (Luzchem Research Inc., Canada) and determined to be ~85 μW/cm^2. Samples were exposed to 24 h UVR every day for 26 weeks to enhance photodegradation. The biomass was sprinkled with filter sterilized water (~2 mL) every second day to simulate water percolation and the runoff was collected in a vial attached to the end of the funnel. Note, the porosity of the sintered disc ensure that only dissolved organic matter was leached from the biomass. Moisture levels were kept constant throughout the experiment, and the mean chamber temperature over the period was at ~25 °C. Preliminary analysis of phospholipid fatty acids revealed no microbial growth (data not shown) on the photodegraded biomass. This would suggest that chemical alteration of the samples was primarily due to photodegradation. A second set of funnels (identical) containing microbial biomass was placed in an unheated area with windows (ambient conditions) to facilitate biodegradation [24]. Microbial biomass and leachates were collected after 0 h (initial biomass) and 26 weeks of the degradation experiments and freeze-dried before analysis. Back calculations of the degraded samples suggest that, on average, ~50% of all the starting material had been degraded during the study.

2.3. FT-IR analysis

The infrared spectra of the initial biomass, degraded biomass and leachates were recorded on a Perkin Elmer FT-IR Spectrum GX Spectrometer. Approximately 1.0 mg of dried sample (biomass or leachate) was homogenized in 100 mg of spectroscopic-grade KBr with a refractive index of 1.559 and a particle size of 5–20 μm (Sigma) and used to create a disc-like KBr pellet for analysis. Background KBr spectra were obtained and spectra ratioed to the background. Spectra were recorded by accumulating 256 scans (to increase the signal-to-noise ratio) in the 4000 to 400 cm^{-1} mid-infrared spectral range in the absorbance mode with a resolution of 4 cm^{-1}. Baseline corrections for all spectra were done using the automatic baseline correction method. Samples were analyzed immediately after preparation to minimize the suppression of key microbial signals by KBr-adsorbed atmospheric water [29].