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No photodegradation of litter and humus exposed to UV-B radiation under laboratory conditions: No effect of leaf senescence or drying temperature

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ABSTRACT

We investigated the effect of UV radiation on photo-oxidation and microbial facilitation in humus and 3 litter types (pine, mānuka, and grass). We collected litter material as naturally senesced or fresh material, to determine whether senescence stage influenced the response to UV radiation. Further, the materials were either air dried or oven dried to assess whether drying temperature also altered the effect of UV radiation on photo-oxidation and microbial facilitation in these litters. Each sample was exposed continuously for 59 days at one of five levels of UV radiation using fluorescent lamps, with the higher levels of exposure being equivalent to mid-day summer values. The samples were then subsequently weighed to assess the extent of any photo-oxidation. The materials were then inoculated with microbial extracts and incubated in darkness for 35 days (25 °C) to determine if prior UV exposure induced microbial facilitation. However, despite the intensity of continuous UV exposure, we found neither photo-oxidation nor microbial facilitation in any of our litters. Neither leaf senescence stage nor drying temperature affected the response of the materials to UV, and all were equally non-responsive. Unexpectedly, in the pine and mānuka samples, higher UV exposure was even correlated with a slight, but consistent and statistically significant weight gain during UV incubation. There was also a slight decrease of subsequent CO_2 production during microbial incubations in the pine and mānuka litters, which would indicate that these materials had become less biodegradable with increased exposure to UV radiation. These results are the opposite of those observed in most field studies, and we have been unable to find any explanation for these unexpected patterns.

1. Introduction

Photodegradation has been shown to be an important contributor to litter cycling in arid, semi-arid, and peatland systems (Pancotto et al., 2005; Austin and Vivanco, 2006; Brandt et al., 2007; Day et al., 2007; Rutledge et al., 2010). Photodegradation can be divided into two main processes: first, photo-oxidation, whereby solar radiation degrades organic matter to carbon dioxide (CO_2) (Anesio et al., 1999; Brandt et al., 2009); second, microbial facilitation, or the conversion of large resistant compounds to smaller compounds that are more readily degradable by soil microbes (Day et al., 2007; Henry et al., 2008; Gallo et al., 2009).

Weight loss from litter has been observed in many field experiments, with reported weight losses of 14–60%, depending on length of exposure, amount of radiation, and species of plant litter (Pancotto et al., 2005; Brandt et al., 2007; Day et al., 2007). A smaller number of field trials, however, have also reported no observed photodegradation in response to UV exposure (e.g. Verhoef et al., 2000; Uselman et al., 2011). Attempts to replicate the large litter-weight losses seen in the majority of field studies under laboratory conditions have produced mixed results. Foereid et al. (2010) assessed photo-oxidation of a perennial grass litter over 289 days and found average weight losses of less than 4%, with no relationship between weight losses and increasing length of UV-B exposure. They did, however, observe a more significant effect on microbial facilitation through UV exposure, with carbon loss from the litter material exposed for 289 days being about twice that of the control. Brandt et al. (2009) found a small weight loss of about 0.5% over 70 days of UV exposure, and no indication of microbial facilitation, and Feng et al. (2011) exposed corn leaves and pine needles to UV radiation for 90 days and measured organic carbon losses of 5–7% from photo-oxidation. This was comparable with

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losses due to biodegradation of the corn leaves and pine needles, which had not been exposed to UV, but incubated with field-moist soil for the same period of time at 23 °C (Feng et al., 2011).

In our previous work, we incubated dried samples of fresh pine and grass material under continuous 24-h exposure, at six radiation levels receiving a total of 0.16, 2.1, 4.8, 7.0, 8.5 and 11.6 MJ m^{-2} (290–320 nm) over a 60 day period. Despite this long and continuous UV treatment, we found no effect of increasing UV exposure on either photo-oxidation or microbial facilitation (Kirschbaum et al., 2011). There were several potential reasons for the absence of any UV effect in this previous laboratory experiment compared with field observations. First, we used fresh leaves instead of naturally senesced material. The concentration of UV-absorbing compounds may decrease in senescing leaves (Hunt and McSeveny, 2002) as protective pigments are removed by the plant as part of normal senescence. We therefore hypothesised that fresh leaves might be more protected against the effects of UV radiation due to greater concentrations of UV-absorbing compounds that might be less in naturally senesced material.

Also, leaf matter in our previous work was dried at 80 °C before UV exposure. Oven drying of leaf material can alter the surface coating of leaves (Tope, 2003), which could potentially provide greater protection against photo-oxidation. Conversely, exposure to higher temperatures may degrade compounds before UV exposure that might otherwise be photo-oxidised.

The objectives of our work were to investigate the effect of UV radiation on photo-oxidation and microbial facilitation in humus and a range of litter samples. We also investigated whether there were any differences in the UV response of fresh and senescent litter material, and litter and humus dried at different temperatures. The work aimed to explore whether leaf senescence stage or drying temperature could be possible reasons for explaining the absence of a UV response under controlled laboratory conditions that we found in our previous work.

2. Materials and methods

2.1. Litter and humus collection

To assess the effect of photodegradation on different litter types, we focused on three plant types commonly found in New Zealand. New Zealand's predominant land cover is exotic grassland, covering 33.1% of the land surface (Ministry for the Environment, 2009). Further, *Pinus radiata* plantations cover 5.4%, and mānuka (*Leptospermum scoparium J.R. Forst & G. Forst*) and/or kanuka (*Kunzea ericoides* var. *ericoides* (A. Rich) J. Thompson) shrubland contributes a further 4.4% of land cover (Ministry for the Environment, 2009).

Senescent and fresh foliage of mānuka shrubs, Yorkshire Fog grass (*Holcus lanatus* L.), pine (*P. radiata*) needles and humus from a pine stand, were collected from the Manawatu region, New Zealand. Pine trees are an introduced conifer used for production forestry, Yorkshire Fog grass is an introduced grass, and mānuka is a native shrub. Needles were collected from 20-year-old pine trees (175.63° latitude, 40.39° longitude) in November 2011. Humus was collected at the same stand of pine trees in February 2012 from underneath the litter layer, where it was unlikely to have been exposed to high amounts of UV radiation. Mānuka leaves were collected during November 2011 from 12-year-old shrubs (175.65°, 40.44°). Grass samples were collected from unused and ungrazed land on the Massey University campus during December 2011 (175.62°, 40.39°).

The pine needles had their basal sheaths removed and both the grass and pine needles were cut to 2-cm lengths following drying. Mānuka leaves are typically between 5 and 10 mm in length and did not require further size reduction. The humus was sieved at field moisture to <2 mm, and then dried. The humus and senesced and fresh leaves, were dried separately in steel trays at either 35 ± 1 °C (air-dried) or 60 ± 1 °C (oven-dried) for 2–3 days. Pine needles (~2.5 g), mānuka leaves (~1 g), grass (~1 g), and humus (~15 g) were weighed to 0.001 g into 10 × 10 cm clear polystyrene petri dishes (Thermo Fisher Scientific, Auckland, New Zealand) for UV exposure. There were three replicates in each treatment for leaf material and six replicates for humus. There was minimal self-shading in the litter samples during UV exposure, but there was likely a self-shading effect in the humus samples.

We assessed the extent of any photo-oxidation by weighing the samples before and after UV exposure. After 59 days of UV exposure, the material was re-dried at the same temperature, either 35 °C or 60 °C, in the petri dishes. While in the oven, samples were partially covered by the lid of the petri dish to avoid contamination from samples placed on racks above. Each sample was removed from the oven individually, once the lid had been placed on the petri dish, and weighed within 5 s of leaving the oven. The same balance was used throughout the experiment, the calibration of which was checked before weighing began.

We investigated several possible methods for weighing the litter samples, bearing in mind that our previous work found that the petri dishes and the litter material both readily adsorbed atmospheric moisture (Kirschbaum et al., 2011). We assessed several weighing protocols, including transferring the litter material to non-moisture absorbing vessels and the use of a desiccator. Transfer of litter to non-absorbing vessels lead to concerns of losses of sample material during transfer, and while this method would have overcome the effects of changing humidity it was ruled out as an option because any loss of sample material was considered a greater risk.

After unsuccessfully trialling various possibilities, we reverted to the measurement protocol we used previously (Kirschbaum et al., 2011). We had found that this minimised, but not eliminated, weight changes that still varied by about ±1% between measurement periods in line with changes in atmospheric moisture. The key focus of our work, however, were the differences in weight changes shown by samples exposed to different amounts of UV radiation. As their weights were all measured on the same day and under the same measurement conditions, any weight changes due to moisture adsorption would have equally affected all samples, providing unconfounded estimates of weight changes as a result of UV exposure.

At each stage of the experiment, we ensured complete randomisation of samples, including their placement for UV exposure (see below), their placement in the oven before weighing, and the order in which they were handled. This therefore avoided any possible systematic inaccuracies resulting from the experimental procedure.

2.2. UV radiation exposure

A fully enclosed exposure area consisting of metal framing and aluminium-coated reflective building paper was established on top of a bench. The bench was covered with black material to prevent back reflection of radiation. As only 120 samples could be exposed at one time, the grass and pine needles were exposed simultaneously; and the mānuka leaves and humus samples were exposed subsequently. Six UV-emitting fluorescent lights (Phillips TL 40W/12RS) were mounted within the frame about 0.9 m above the bench. The radiation output from the lights was assessed using a UV-B Biometer Model 501 Radiometer (Solar Light Company, Pennsylvania, USA). For more information on the spectral outputs from the lamps and the calibration procedure, see Kirschbaum et al. (2011).