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UV-B radiation induced changes in litter quality affects earthworm growth and cast characteristics as determined by metabolic fingerprinting

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Summary

We investigated whether exposure to UV-B results in biochemical changes in leaf litter, affecting growth rates of earthworms feeding on this litter and cast chemistry. Seedlings of *Betula pubescens* L. were grown under zero and ambient UV-B (at 52 °N) regimes under optimal conditions. Following three months exposure, plants were allowed to senesce and leaf litter was collected.

Lumbricus terrestris and *Lumbricus rubellus* were offered three substrates – processed oat grain, zero UV-B leaf litter and ambient UV-B leaf litter. Fourier transform infrared spectroscopy (FT-IR) analyses indicated that these substrates differed markedly in their composition, showing separation according to UV-B treatment and oat grain.

There were no significant effects of UV-B treatment on the mass of earthworms feeding on litter, although trends suggested that *L. rubellus* benefited from the UV-B treated litter whilst *L. terrestris* showed the converse response. FT-IR analyses of *L. terrestris* casts showed more consistent differences in their biochemistry than was the case for litter.

UV-B induced changes in living leaf material introduced to soil, even after earthworm processing, may have implications for cast microbial communities and nutrient cycling.

Key words: UV-B, birch litter, earthworms, FT-IR spectroscopy

Introduction

UV-B radiation is an important factor in terrestrial ecosystems influencing trophic interactions, particularly decomposition and biogeochemical cycles (Zepp et al. 1998). Exposure of vegetation to UV-B radiation, and resulting changes in leaf litter quality, may slow litter incorporation and decomposition (Gehrke et al. 1995) and might influence belowground microbial diversity (Johnson et al. 2002). Earthworm activity is fundamental to the dynamics of forest ecosystems, incorporating leaf litter and accelerating its decomposition (Cortez 1998). By preventing the accumulation of a permanent litter layer, their activity influences physiochemical conditions where seeds germinate. Fine comminution of litter by earthworms (Schulmann & Tiunov

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1999) modifies its microbial community, accelerates nutrient mineralization and enhances plant nutrient uptake (Brown et al. 1998; Tiunov & Scheu 1999).

The current study investigated whether the growth of birch seedlings with differing UV-B exposure results in changes to the chemical composition of leaf litter that affects its food value to *Lumbricus terrestris* and L*umbricus rubellus* and the metabolic profiles of casts as described by FT-IR spectroscopy.

Materials and Methods

Seedlings of *Betula pubescens* L. were grown under zero and ambient UV-B (at 52 °N) regimes with a photoperiod of 16 hours day and 8 hours night (20 °C and 12 °C) respectively. The biologically effective UV-B (UV-B_{BE}) dose in the UV-B cabinet was 5.3 $kJm^{-2}day^{-1}$. The daily irradiation was estimated according to Björn & Murphy (1985) and Björn & Teramura (1993). The control treatment represented zero UV-B. PAR (photosynthetically active radiation 400–700 nm) was maintained at 150 µmoles m^{-2} s^{-1}. Following 12 weeks exposure, plants were placed in a polytunnel for three weeks to initiate autumn senescence. Leaf litter was collected and stored in a dessicator for further experimentation.

Earthworms (*L. terrestris* or *L. rubellus*) were incubated for 5 weeks in 1 litre pots (20 °C and optimum moisture) containing an artificial soil mix (sand, moss peat and kaolin – OECD 1984) with 7 replicates of each food X species combination. Earthworms were weighed prior to commencement of the experiment and at its conclusion having voided soil from their gut. Similar live weights, though different numbers (3 *L. terrestris* or 12 *L. rubellus*), of adults of the two species were offered processed oat grain (reference food type), zero UV-B leaf litter or ambient UV-B leaf litter, applied to the surface. Fresh casts were collected at the end of the experiment.

Sample treatment for FT-IR analysis

Litter and cast samples were frozen at –80 °C until analysed. Litter samples were mixed with 30 ml liquid nitrogen, ground and suspended in 0.6 ml sterile 0.9 % NaCl. Final concentrations of each litter sample were 75 mg litter/ml saline. Equal numbers (7) of casts from *L. terrestris* were analysed for each litter treatment; there were problems with storage of *L. rubellus* casts that prevented their analysis. The final concentration was 125 mg cast/ml saline.

Aliquots (µ10 ml) of litter or cast suspensions (in triplicate) were added to each well of an aluminum plate and dried at 50 °C for 30 min. Using Fourier transform infrared spectroscopy (FT-IR) (Goodacre et al. 1998), spectra were monitored and collected by an IBM computer with Opus software (version 2.1). The scan range of spectra was 4,000 to 600 cm^{-1} and resolution was 4 cm^{-1}; each spectrum was represented by 882 points.

Data manipulation and statistical analysis

To minimize baseline shift problems, the FT-IR spectra were normalised for absorbance from 0 to +1 and the smoothed first derivatives of these spectra were calculated using the Savitzky-Golay algorithm. Spectra absorbance data were then analysed by principal components analysis (PCA) to reduce the dimensionality of the data from 882 to 20 PCs. Next discriminant function analysis (DFA) differentiated between groups based on these retained PCs and the *a priori* knowledge of which spectra were replicates, a process that does not bias the analysis. Only DFA analyses are presented here. Statistical analysis was performed using Matlab version 5 (MathWork Inc., Natick, MA, USA). Earthworm and food type means were compared by two-way analysis of variance.

Results

The total dry weight of birch was 12 % lower ($P = 0.052$) and stem dry weight 20 % lower ($P < 0.005$) with UV-B. However, all other parameters including leaf area, leaf dry weight, root dry weight and associated ratios were unaffected ($P > 0.05$).

Litter removal rates were not significantly affected by earthworm species or by differences in UVB exposure (data not shown). Both species lost biomass relative to oat grain (Fig. 1) when fed litter, particularly *L. rubellus*; there was a statistically significant ($P < 0.01$) food X species interaction. Comparing litters, *L. terrestris* tended to grow less well when feeding on UV-B litter whilst the reduction in biomass of *L. rubellus* was less pronounced when fed on this litter.

FT-IR analyses

The main litter and cast differences in absorbance occurred in the fatty acid, peptide and phosphate regions of the spectra. FT-IR spectra (Figs. 2a and 3a) are complex and thus need to be analysed by DFA to show separation within litters and casts (Figs. 2b and 3b). Whilst, separation of the litter substrates was less consistent, the analysis of earthworm casts showed highly significant changes in their metabolic fingerprints according to food type (Fig. 3b).

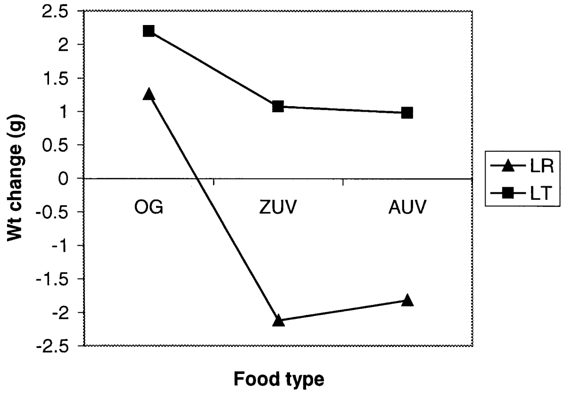

Fig. 1. Changes in earthworm weights over incubation period (LR – *L. rubellus*; LT – *L. terrestris*) with differing food sources (OG – oat grain; ZUV – zero UVB litter: AUV – ambient UVB litter)

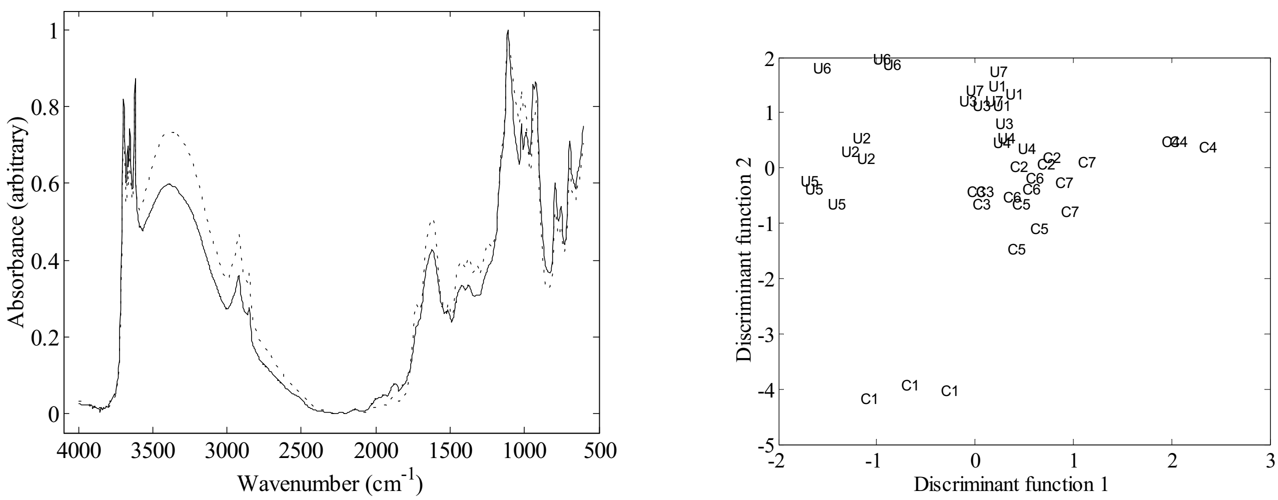

Fig. 2. a) FT-IR spectra of litter (dotted line: zero ZUV-B litter and solid line: UV-B litter) and b) discriminant analysis based on FTIR data of litters (c = zero UVB; U = ambient UVB)

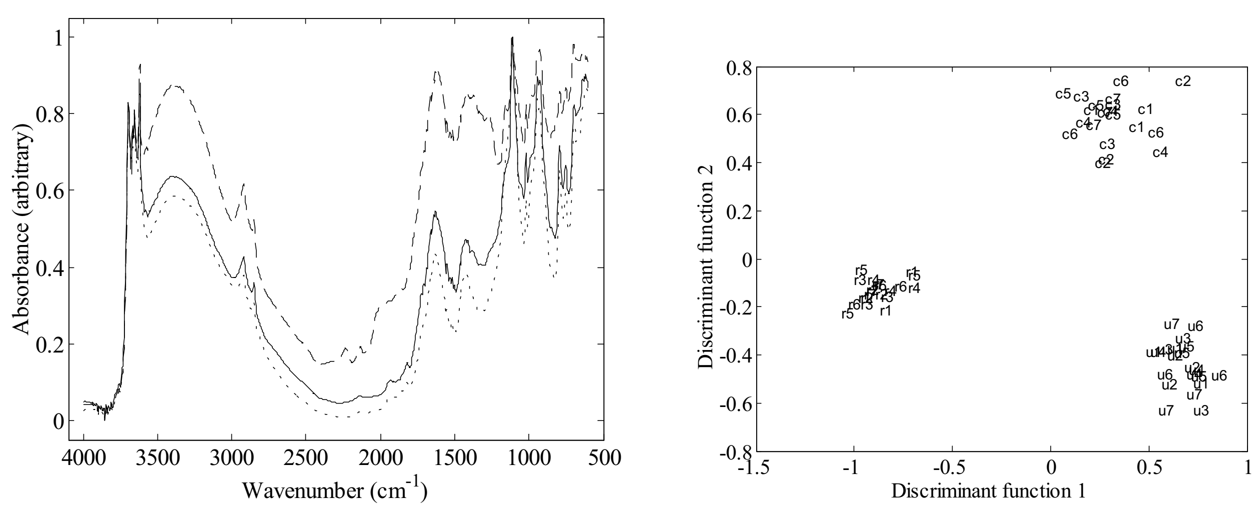

Fig. 3. a) FT-IR spectra of casts (dashed line: oat grain: dotted line: zero UV-B litter and :solid line : UV-B litter).and b) discriminant analysis based on FTIR data of casts (c = zero UVB; U = ambient UVB; r = oat grain))

Discussion

The diet x species interaction (Fig. 1) indicated that UV-B mediated effects on litter caused differential responses in the biomass of *L. terrestris* compared with that of *L. rubellus*. If these trends were repeated in field communities, they would influence competitiveness between these species. Resulting changes in population composition could have implications for removal of surface litter and its distribution within soil. Since there was no apparent difference in food selection, as indicated by the rate of litter removal from the surface, it is likely that the two species vary in their ability to derive nutrition from litter differing in its chemistry (Fig. 2). In this case, UV-B induced changes in lignin content (Paul et al. 1999) may be important.

The plant growth responses to varying UVB exposure obtained in this investigation were typical of those found in other studies (e.g. Searles et al. 2001). FT-IR spectra (Fig. 2) indicated differences in litter composition, but did not confirm that these resulted from changes in leaf secondary metabolites and lignin as reported in a review by Paul et al. (1999). Johnson et al. (2002) showed dramatic impacts of plant responses to enhanced UV-B on the belowground microbial biomass and structure in sub-artic heath. In temperate systems, UV-B may also induce changes in the chemical composition of plant litter that affect earthworm populations and microbial activity in their faeces. FT-IR analyses of indicated that UV-B induced changes in litter resulted in shifts in the metabolic fingerprint of earthworm casts (Fig. 3).

We conclude that UV-B induced changes in living leaves are manifest in leaf litter and introduced to soil after earthworm processing. Changes in litter chemistry could have implications for earthworm and soil microbial communities. Further studies will identify specific chemical responses to UVB in plant leaves and resulting litter. Additional investigations of earthworm casts will elucidate the implications of such changes for decomposition processes in soil.

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