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# Arctic microorganisms respond more to elevated UV-B radiation than CO<sub>2</sub>

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Surface ultraviolet-B radiation and atmospheric CO<sub>2</sub> concentrations have increased as a result of ozone depletion and burning of fossil fuels<sup>1,2</sup>. The effects are likely to be most apparent in polar regions<sup>3</sup> where ozone holes have developed and ecosystems are particularly sensitive to disturbance<sup>4</sup>. Polar plant communities are dependent on nutrient cycling by soil microorganisms, which represent a significant and highly labile portion of soil carbon (C) and nitrogen (N). It was thought<sup>5</sup> that the soil microbial biomass was unlikely to be affected by exposure of their associated plant communities to increased UV-B. In contrast, increasing atmospheric CO<sub>2</sub> concentrations were thought to have a strong effect as a result of greater below-ground C allocation<sup>6</sup>. In addition, there is a growing belief that ozone depletion is of only minor environmental concern because the impacts of UV-B radiation on plant communities are often very subtle<sup>7</sup>. Here we show that 5 years of exposure of a subarctic heath to enhanced UV-B radiation both alone and in combination with elevated CO<sub>2</sub> resulted in significant changes in the C:N ratio and in the bacterial community structure of the soil microbial biomass.

We used an established experiment situated close to the Abisko Scientific Research Station, Swedish Lapland (68.35° N, 18.82° E, 360 m above sea level) in a subarctic heath. The site has an open canopy of *Betula pubescens* ssp. *czerepanovii* and a dense dwarf shrub layer with scattered herbs and grasses. Plots were exposed to factorial combinations of UV-B radiation (simulating 15% ozone depletion under clear sky conditions) and elevated CO<sub>2</sub> (600 ± 50 p.p.m.). We determined soil microbial biomass C ( $C_{mic}$ ) and N ( $N_{mic}$ ) using the fumigation-extraction procedure<sup>8,9</sup> and the patterns of C source utilization using extractable bacteria in

customized Biolog plates containing a range of ecologically relevant C sources<sup>10</sup>.

$C_{mic}$  decreased significantly from 3.1 mg C per g soil dry weight in the control to 1.5 mg C per g soil dry weight in the UV-B treatment (Table 1) but remained unaffected by the application of elevated CO<sub>2</sub> in combination with increased UV-B. This apparent ameliorative effect may reflect differences in the quality and quantity of C entering the soil in the UV-B only and UV-B with CO<sub>2</sub> treatments. In contrast,  $N_{mic}$  increased from approximately 0.1 mg N per g soil dry weight in the control to 0.3 mg N per g soil dry weight in the CO<sub>2</sub> + UV-B treatment (Table 1). The overall main effect of the UV-B treatment was to significantly increase  $N_{mic}$  by over 100% from 0.12 to 0.27 mg N per g soil dry weight (Table 1). These changes were reflected in the microbial biomass C:N ratio ( $C_{mic}:N_{mic}$ ) which decreased significantly by 320% in the plots receiving enhanced UV-B, but was not influenced by exposure to elevated CO<sub>2</sub> (Table 1).

The average well colour development (AWCD; see Methods) of all C sources in the Biolog plates was significantly lower ( $P < 0.05$ ) in all of the treatments compared to the controls (Table 1), suggesting either a lower or less active population of bacteria. Multivariate analysis of the sole C source utilization tests showed significant discrimination ( $P = 0.019$ ) between the treatments (Fig. 1) indicating that the soil microbial community structure had also been affected by the treatments. The plots receiving either ambient or elevated CO<sub>2</sub>, regardless of UV-B, tended to be separated primarily by the first canonical variate, while the combined addition of increased UV-B, resulted in separation owing to both the first and second canonical variates (Fig. 1). Thus, the combination of elevated CO<sub>2</sub> and UV-B resulted in a population structure different to that from the individual treatments. These substantial shifts in patterns of C utilization among the four treatments will almost certainly have resulted from a change in the dominant bacterial species extracted from this soil. These observations are independent of changes in the abundance of microorganisms, as reflected by AWCD and  $C_{mic}$  (Table 1), and further demonstrate the sensitivity of the microbial component of the ecosystem.

The results from our experiments demonstrate that, unlike the plant community, the soil microbial biomass is highly sensitive to elevated UV-B radiation and CO<sub>2</sub> concentrations. More importantly, the impacts of the UV-B treatment on the accumulation of N in the microbial biomass may have far-reaching implications for the supply of N to plants, because the productivity of many semi-natural ecosystems is limited by N (ref. 11). We are uncertain whether these changes reflect either increased microbial

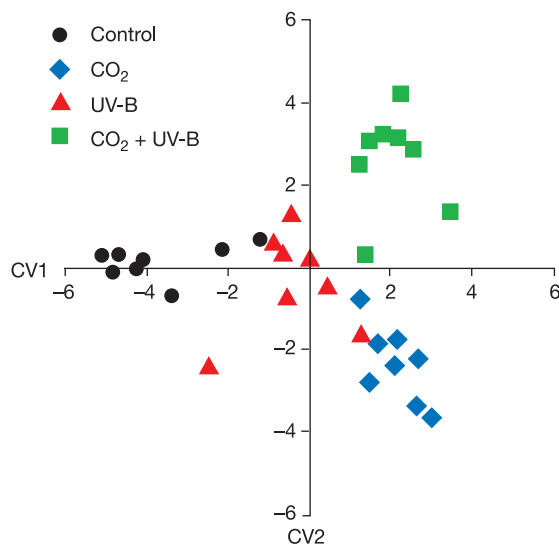


Figure 1 Ordination plot of canonical variates 1 and 2 (CV1, CV2) for sole carbon source data obtained from customized Biolog MT plates.

**Table 1 Microbial responses to elevated UV-B and CO<sub>2</sub>**

Treatment	C <sub>mic</sub>	N <sub>mic</sub>	C <sub>mic</sub> :N <sub>mic</sub>	AWCD
Control	2.7 <sup>a</sup> ± 0.38	0.13 <sup>a</sup> ± 0.26	35.7 <sup>a</sup> ± 10.5	0.20 <sup>a</sup> ± 0.07
CO <sub>2</sub>	3.1 <sup>a</sup> ± 0.56	0.10 <sup>a</sup> ± 0.28	36.7 <sup>a</sup> ± 7.6	0.12 <sup>b</sup> ± 0.06
UV-B	1.0 <sup>b</sup> ± 0.24	0.22 <sup>a</sup> ± 0.42	4.8 <sup>a</sup> ± 1.4	0.15 <sup>b</sup> ± 0.06
CO <sub>2</sub> + UV-B	3.7 <sup>a</sup> ± 0.32	0.31 <sup>a</sup> ± 0.21	11.3 <sup>b</sup> ± 1.6	0.14 <sup>b</sup> ± 0.05
Main effect of ambient and increased UV-B				
Ambient UV-B	2.9 <sup>a</sup> ± 0.47	0.12 <sup>a</sup> ± 0.27	36.2 <sup>a</sup> ± 9.1	0.16 <sup>a</sup> ± 0.07
Increased UV-B	2.4 <sup>a</sup> ± 0.28	0.27 <sup>a</sup> ± 0.32	8.2 <sup>b</sup> ± 1.5	0.14 <sup>a</sup> ± 0.06

Microbial biomass (mg per g soil dry weight) of carbon (C<sub>mic</sub>) and nitrogen (N<sub>mic</sub>), C<sub>mic</sub>:N<sub>mic</sub> ratio and average well colour development (AWCD; A<sub>590</sub>) for combined carbon sources in customized Biolog plates (mean ± s.e.). Values sharing a letter within columns are not significantly different (*P* > 0.05). Individual treatments and main effects are compared separately.

accumulation of N or a change in the species composition of the microbial biomass to one with an overall lower C:N ratio. The shifts in C source utilization (Fig. 1) lend support to the second hypothesis. The capacity for subarctic semi-natural heaths to act as major sinks for fossil fuel-derived carbon dioxide is likely to be critically dependent on the supply of N, as recently reported in loblolly pine stands<sup>12,13</sup>. Exposure of arctic plant communities to long-term UV-B radiation may thus alter the amount of N held within the soil microbial biomass. A full understanding of the impacts of global climate change, and in particular increased UV-B radiation, on polar plant communities can only be achieved by increasing our studies of the key soil organisms and processes that drive these ecosystems. □

**Methods**

**Site description**

The vegetation contains a layer of mosses (such as *Hylocomium splendens* (Hedw.) Br. Eur.) and lichens (such as *Peltigera aphthosa* (L.) Willd.). The major dwarf shrub species are *Empetrum hermaphroditum* Hagerup, *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. and *Vaccinium vitis-idaea* L. (ref. 14). The soil is a nutrient-poor podzol on well-drained acid moraine and has a pH (H<sub>2</sub>O) of 3.4 to 5.6 (ref. 15).

**Experimental design**

We erected 16 (eight controls and eight simulating enhanced UV-B radiation) metal frames (2.5 × 1.3 × 1.5 m high) each holding six UV-B fluorescent tubes during the spring of 1993. Fluorescent tubes were used to simulate 15% ozone depletion under clear sky conditions. This represents a 25% increase above ambient levels. The actual ozone depletion under typical cloud conditions at Abisko in 1994 has been calculated to be 19% (ref. 16). Timers controlled the UV-B lamps in a stepwise 'square wave' manner to account for midday increases in photosynthetically active radiation (PAR) and ambient UV-B (maximum 5.5 kJ day<sup>-1</sup>)<sup>16</sup>. The daily exposure time was changed every two weeks to follow the seasonal levels of UV-B radiation at Abisko. Before use, the lamps were pre-burnt to ensure a stable UV-B output. The lamps in the control frames had glass panels to filter out UV-B and UV-C radiation. The enhanced UV-B radiation plots had ultraviolet-transmitting Plexiglas (Röhms 2458, Röhms GmbH) and cellulose diacetate (Courtaulds) to remove UV-C radiation. This was regularly replaced throughout the growing season. All plots received ambient levels of UV-A and UV-B radiation. The mean daily air temperature inside the chambers was typically 1 °C higher than that outside<sup>17</sup>.

**Treatment application**

Elevated CO<sub>2</sub> was introduced into the plots using open top chambers (0.5 m high, 0.73 m<sup>2</sup> area) constructed from Plexiglas (Röhms 2458, Röhms GmbH) placed beneath each of the frames. Air was supplied to the base of each chamber from an independent fan system (Soler and Palau). Elevated CO<sub>2</sub> was achieved by bleeding pure CO<sub>2</sub> into the air supply of half of eight of the chambers in order to maintain concentrations at 600 ± 50 μl l<sup>-1</sup>. Concentrations of CO<sub>2</sub> within the chambers were monitored using an infrared gas analyser (Series 2000, ADC Bioscientific)<sup>17</sup>. Treatments were applied each year (from 1993 onwards) during the snow-free months of the subarctic summer from late May to early September.

**Soil microbial biomass carbon and nitrogen**

Duplicate cores (4 cm diameter, 6 cm deep) were extracted from each plot, the surface litter removed and the bulk soil sieved (2 mm). Microbial biomass C (C<sub>mic</sub>) and N (N<sub>mic</sub>) were determined in 5-g subsamples using the fumigation-extraction procedure<sup>8,9</sup>. Total organic C (TOC) in the fumigated (24-h exposure to ethanol-free CHCl<sub>3</sub>, previously washed six times with distilled water) and unfumigated extracts (0.5M K<sub>2</sub>SO<sub>4</sub>) was determined by sodium persulphate/ultraviolet absorption (LabTOC, Pollution and Process Monitoring). For N<sub>mic</sub>, 2-ml subsamples of the extracts were acid-digested<sup>18</sup> and the total N determined<sup>19</sup>. Both C<sub>mic</sub> and N<sub>mic</sub> were calculated using the formula: C<sub>mic</sub> or

N<sub>mic</sub> = (X<sub>f</sub> - X<sub>uf</sub>)/K<sub>c</sub>, where X<sub>f</sub> and X<sub>uf</sub> are the C or N concentrations in fumigated and unfumigated extracts, respectively, and K<sub>c</sub> is the proportion of microbial C (0.38; ref. 8) or N (0.54; ref. 9) extracted from soil. The C<sub>mic</sub> and N<sub>mic</sub> data sets were combined to produce a microbial biomass C:N ratio.

**Sole carbon source utilization**

Direct incubation of soil suspensions in Biolog microtitre plates containing different carbon sources in individual wells was used to determine (1) changes in the potential rate of C-source utilization, and (2) changes in relative and absolute rates of utilization of individual substrates to discriminate between soil microbial communities<sup>10,20</sup>. We tested 30 ecologically relevant C sources using customized Biolog MT type plates in which the wells contained six amino acids, two carbohydrates, eight carboxylic acids, nine phenolic acids and four long-chain aliphatic acids<sup>10</sup>.

We analysed data using two different approaches. In the first, absolute rates of colour development measured as absorbance at 590 nm (A<sub>590</sub>) were compared for individual C sources. The average well colour development (AWCD) during the initial 96 h of incubation was compared using two-way analysis of variance (ANOVA) and least significant difference (LSD) multiple comparison tests. In the second, multivariate analysis of the A<sub>590</sub> values at equivalent AWCD from different times of incubation were compared. They were also transformed by dividing by the AWCD to avoid bias between samples with different inoculum density<sup>10</sup>. The absorbance data were analysed by canonical variate analysis, after first reducing the dimensionality by principal component analysis and by comparison of mean intergroup Mahalanobis distances with simulated confidence limits and Monte-Carlo testing of significance<sup>10</sup>. Simulated confidence limits for four groups (treatments) with eight replicates were 2.26 and 2.57 at the 95% and 99% confidence limits, respectively. All ANOVA, regression and multivariate analyses were conducted using Genstat 5.4 (NAG).

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**Competing interests statement**

The authors declare that they have no competing financial interests.

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