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Changes in soil microbial community structure under elevated tropospheric O_3 and CO_2

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ABSTRACT

We studied the effects of O_3 and CO_2 alone and in combination on soil microbial communities by assessing the changes in total PLFA biomass, profiles and specific subgroups. Meadow mesocosms were exposed to slightly elevated O_3 (40–50 ppb) and CO_2 (+100 ppm) in open-top chambers for three subsequent growing seasons (2002–2004). Decreased total, bacterial, actinobacterial, fungal PLFA biomass values as well as fungal:bacterial PLFA biomass ratio were measured after three growing seasons of fumigations with elevated O_3. There were significant differences in the relative proportions of individual PLFAs between the control and elevated O_3 treatments. Moreover, enhanced O_3 alone and in combination with CO_2 modified the structure of the microbial community. The effects of elevated CO_2 given alone on PLFA profiles were negligible. Our results show that elevated O_3 alone and in combination with CO_2 even at moderate levels may cause changes in the biomass and composition of the microbial community in meadow soils, which may lead to functional changes in soil ecosystem processes.

1. Introduction

The concentrations of tropospheric ozone (O_3) and carbon dioxide (CO_2) have been rising due to human activity since the Industrial Revolution (IPCC, 2007; Vingarzan, 2004). The increase of O_3 is expected to continue at the level of 1–2% per year, which may result in triplication of the O_3 concentration within the next 30–40 years (Chameides et al., 1994; Marenco et al., 1994). Concurrent with the O_3 rise, global CO_2 concentrations have progressively increased up to today's level of 360 ppm and are predicted to increase to 550 ppm by the mid-21st century (IPCC, 2007).

O_3 is a phytotoxic compound with a potential to suppress crop yields and to reduce the above-ground growth of trees and herbs (Ashmore et al., 2004; Davison and Barnes, 1998; Fuhrer et al., 1997; Rämö et al., 2006). Even more significantly, the harmful effects of O_3 may be extended to reductions in below-ground processes, such as root growth and carbon (C) allocation (Andersen, 2003; Bender et al., 2002). So far, research on the effects of O_3 on below-ground processes has mainly focused on mycorrhiza (e.g. Edwards and Kelly, 1992; Kainulainen et al., 2000; Kytöviita et al., 2001; Rantanen et al., 1994), soil microbial biomass (Islam et al., 2000), and root production (Andersen et al., 1997; Olszyk et al., 2001) of forest trees. The impacts of O_3 on the structure and functioning of soil microbial communities at the ecosystem level in natural vegetation, however, remain poorly understood (Andersen, 2003; Bender et al., 2002) and somewhat controversial. In a recent study, Dohrmann and Tebbe (2005) found that herbaceous plants did not select for structurally different bacterial communities in their rhizospheres, as revealed by genetic profiling of PCR amplified 16S rRNA gene sequences, when exposed to double the ambient O_3 concentrations for 5–6 weeks. Also, Yoshida et al. (2001) found that, in response to O_3 stress for 16 weeks, the soil bacterial biomass decreased, but the fungal biomass increased in blue wild rye.

Contrary to O_3, the effects of CO_2 are mainly considered beneficial for plants, since plant ecosystems respond to rising CO_2 concentrations with increased photosynthesis, growth and resource allocation (Bazzaz, 1990; Jablonski et al., 2002). Thus, an increase in the atmospheric CO_2 concentration may increase the production of organic compounds by plants and alter resource allocation, and this may affect biomass, community structure, and the activity of soil microbes through quantitative alterations in C, nutrients, and water availability (Hu et al., 1999). Since the plant carbohydrate concentration is higher, the C input into soil generally increases in response to elevated CO_2, even when there is no significant CO_2 stimulation of above-ground growth (Körner and Arnone, 1992). Although the general trend in CO_2 enrichment is toward increased mycorrhizal colonization (Hodge, 1996; Klironomos et al., 1996; Wiemken et al., 2001) and increased bacterial counts (Schortemeyer et al., 1996), not all studies are consistent with these findings (Kasurinen et al., 1999; Zak et al., 2000). The contradictory results may be due to the wide

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variety of experimental designs applied to different species. However, the microbial community structure under correspondingly elevated levels of CO_2 (500–600 ppm) in grasslands and forest tree ecosystems has been reported both to change (Montealegre et al., 2002; Ringelberg et al., 1997) and to show no response (Ebersberger et al., 2004; Kandeler et al., 1998).

It has been suggested that CO_2 could ameliorate the negative effects of O_3 in above-ground processes (Volin et al., 1998) by lowering the O_3 flux into leaves (Allen, 1990; McKee et al., 1997, 2000) and by increasing the availability of photosynthates that can be used for detoxification and repair processes (Allen, 1990; McKee et al., 1997). However, only a few studies on the possible effects of both gases on below-ground processes have been conducted so far, and variation between species and experiments is quite vast. For instance, Larson et al. (2002) found that microbial biomass and respiration were not significantly altered by elevated CO_2 and O_3; while Holmes et al. (2003), contrawise, discovered that gross N mineralization and gross ammonium immobilization were more abundant under elevated CO_2 than in the presence of both CO_2 and O_3. Phillips et al. (2002) detected changes in microbial community composition under elevated CO_2 and O_3 separately, but not when the two gases were combined. Kasurinen et al. (2005) concluded that CO_2 and O_3 treatment effects on the soil PLFA profiles of silver birch rhizospheres were negligible.

Responses in microbial communities induced by elevated O_3 and CO_2 can cause alterations in the whole soil ecosystem. For instance, fungi might come to dominate over bacteria (Klironomos et al., 1996), bacterial nutritional groups may change (Elhottová et al., 1997), and mycorrhizal fungi can be altered (Hodge, 1996). Structural changes, in turn, may have significant effects on the functioning (including emission of greenhouse gases and nutrient cycling) of the microbial community and its interaction with the plant community (Grayston and Campbell, 1996; Grayston et al., 1998; Griffiths et al., 1998).

Previous studies indicate that plants are especially sensitive to O_3 in the Northern European climatic conditions (De Temmerman et al., 2002; Lövblad et al., 1996; Ojanperä et al., 1998; Pleijel et al., 2000; Vorne et al., 2002), probably due to the long summer days and the high relative humidity, which may promote O_3 uptake. Therefore, the absorbed O_3 dose may be high, even though ambient O_3 levels are lower in Finland than e.g. in Central Europe (Benton et al., 2000; De Temmerman et al., 2002; Pleijel et al., 1999). Daytime averages in May–July in Southern Finland vary around 40 ppb (Laurila et al., 2004) and are influenced by the long-range transport of O_3 and precursors from Central Europe (Laurila et al., 2004). In view of this, we wanted to assess the possible effects of elevated but realistic O_3 and/or CO_2 concentrations (up to 1.8× O_3 and 1.3× CO_2) on semi-natural vegetation, since there is scant experimental data on the effects of moderately elevated O_3 and/or CO_2 concentrations on soil–plant dynamics. Other experiments with elevated O_3 and/or CO_2 levels have used considerably higher levels; in a few cases the concentrations were even doubled (e.g. Islam et al., 2000; Holmes et al., 2003; Kasurinen et al., 1999).

In this study, we exposed mesocosms that mimicked the semi-natural vegetation typical for Northern European lowland hay meadows to elevated O_3 and/or CO_2 in open-top chambers (OTCs) for three consecutive growing seasons (2002–2004). Plant species typical of fens and meadows may be particularly sensitive to O_3 (Power and Ashmore, 2002), and previous studies have revealed that considerable variation exists among and even within wild species (e.g. Bassin et al., 2004; Franzaring et al., 2000; Nebel and Fuhrer, 1994). Our specific objectives were to examine (i) the changes in the composition and microbial biomass of a soil microbial community in response to elevated O_3 and CO_2, and (ii) whether the harmful effects of elevated O_3 could be counteracted by elevated CO_2. We explored these aims by using PLFA fingerprints, which have shown to be reliable for soil microbial community characterization (Zelles, 1999; Palojärvi, 2006), and the microbial biomass values of meadow mesocosms exposed to supplemental CO_2 and O_3 alone and in combination in southern Finland. The above-ground and root growth of the mesocosms has been reported in a separate paper (Rämö et al., 2006).

2. Material and methods

2.1. Experimental design and set-up

In June 2002, we constructed ground-planted mesocosms that simulated hay meadows by planting seedlings of perennial species (approx. 3 months old) as follows: 25 seedlings of each of the following perennial species; *Agrostis capillaris* (L.), *Anthoxanthum odoratum* (L.), *Fragaria vesca* (L.), *Campanula rotundifolia* (L.), and *Ranunculus acris* (L.), and five seedlings of *Trifolium medium* (L.), and eight seedlings of *Vicia cracca* (L.) in a 2.25 m^2 area. We exposed the planted mesocosms to elevated O_3 and/or CO_2 in open-top chambers (OTCs) for three growing seasons (2002–2004) in Jokioinen (60°49′N, 23°28′E) in south-western Finland. The mesocosms were harvested once a year in early September, except in the first year, 2002, when the plant coverage was inadequately developed. The soil consisted of coarse sand (sand 86.5%/silt 11.9%/clay 1.6%) with an average pH of 6.7–7.0. The soil physical and chemical properties have been summarized in Kanerva et al. (2005, 2007).

The OTCs were placed on the experimental field in a completely randomized design. The treatments included unchambered open-field plots (AA) and the following OTC treatments: (i) non-filtered ambient air (NF), (ii) non-filtered air + elevated O_3 (NF + O_3), (iii) non-filtered air + elevated CO_2 (NF + CO_2), and (iv) non-filtered air + elevated O_3 + CO_2 combined (NF + O_3 + CO_2). Each treatment was repeated three times. All OTCs were equipped with blowers to exchange three air volumes per minute. The chambers were removed for the winter, and the mesocosms were left to overwinter naturally. More detailed information about the mesocosm design, the chambers, and the fumigation system has been given in an earlier paper (Kanerva et al., 2005). The exposure lasted from July 1 till August 28 in 2002, from June 3 till August 31 in 2003 and from May 19 till August 23 in 2004. Daily exposure time was 9 h, from 10.00 to 19.00 h, during each growing season. The seasonal 9-h average O_3 concentrations were 1.5, 1.6, and 1.8× ambient in 2002, 2003, and 2004, respectively, in the elevated O_3 treatments, and those of CO_2 were 1.3× ambient in each growing season in the elevated treatments. The seasonal 24-h average O_3 concentrations were 1.3, 1.2, and 1.4× ambient in 2002, 2003, and 2004, respectively, in the elevated O_3 treatments, and those of CO_2 1.1× ambient in each growing season in the elevated treatments. There were no statistical differences in the O_3 and CO_2 concentrations between the replicates in each treatment ($P > 0.05$) (data not shown). More detailed information about the exposure concentrations has been given by Kanerva et al. (2006, 2007).

2.2. Soil sampling and laboratory analyses

Soil sampling was done twice; after the first exposure period in mid-September 2002 and at the end of the experiment in mid-September 2004. Twenty bulk soil samples were taken from the depth of 0–20 cm in the central area of each mesocosm, pooled, and stored in polyethylene bags at −18 °C. The dry weight of the soil samples was determined by drying 40 g of fresh soil at 105 °C overnight.

PLFAs were analyzed with the method proposed by Frostegård et al. (1993), Stoeck et al. (2002) and Palojärvi (2006) with slight modifications. Briefly, 3 g of fresh soil sample was extracted with a chloroform/methanol/citrate buffer mixture (1:2:0.8; "Bligh&Dyer