Influence of elevated CO_2 and GM barley on a soil mesofauna community in a mesocosm test system

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ABSTRACT

We hypothesized that the combined effect of rising levels of atmospheric carbon dioxide (CO_2) and increasing use of genetically modified (GM) crops in agriculture may affect soil food-webs. So we designed a study for the assessment of the effects of elevated CO_2 (eCO_2) concentrations and GM barley on a soil-mesofauna community employing a 2nd tier mesocosm test system. The GM barley, *Hordeum vulgare* cv. Golden Promise, had a modified content of amino acids and it was compared with three non-GM barley cultivated varieties including the isogenic line. Our mesocosm experiment was conducted in a greenhouse at ambient (aCO_2) and eCO_2 (+80 ppm) levels and included a multispecies assemblage of Collembola, Acari and Enchytraeidae with either a GM or conventional spring barley varieties. To detect food-web changes we added dried maize leaves naturally enriched in $\delta^{13}C$ and $\delta^{15}N$ relative to the soil substrate. Soil, plants and animals were collected after five and eleven weeks. We found that the eCO_2 concentration did not affect the plant biomass, but the predatory mite and two collembolan species showed significantly lower abundances at eCO_2. The densities of three collembolan species (*Folsomia fimetaria*, *Proisotoma minuta* and juveniles of *Mesaphorura macrochaeta*) was significantly lower in the GM treatment compared to some of the non-GM varieties. *F. fimetaria* was less abundant in presence of GM barley compared to the cultivated barley variety "Netto" at both CO_2 levels, while the density of *P. minuta* was significantly reduced with the GM barley compared to variety "Netto" at aCO_2 and the isogenic variety at eCO_2. Maize litter acted as a food source for the community, as it was revealed by $\delta^{13}C$ values in microarthropods. Microarthropod $\delta^{13}C$ decreased over time, which indicates a diet change of the species towards carbon derived from barley, due to maize litter decomposition. The industrially produced CO_2 gas also had a role as an isotopic marker, as the different $\delta^{13}C$ values were reflected in the barley and in the collembolan species. GM barley did not affect $\delta^{13}C$ and $\delta^{15}N$ values of soil animals indicating that the overall trophic structure of the mesofauna community was not changed compared to the non-GM cultivated varieties. The mesocosm methodology integrating stable isotope analysis demonstrates the potential of the multi-species mesocosm as a tool to detect and track changes in the soil trophic interactions in response to environmental pressures, climate and novel agricultural crops.

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1. Introduction

The increase in atmospheric CO_2 levels and the increasing use of genetically modified (GM) plants have been identified as possible agents of alterations in soil communities, processes, and nutrient cycling (Turbé et al., 2010). How the soil mesofauna may respond to GM plants and rising CO_2 is of particular interest because the mesofauna play a key role in stimulating decomposition processes and nutrient cycling (Brussaard, 1998; Coleman et al., 2004), through their interactions with the microbial community.

The effects of GM plants on non-target belowground organisms and processes have been investigated extensively for *Bt*-crops (Icoz and Stotzky, 2008). The majority of these studies have been performed on single species and non-target soil fauna (Romeis et al., 2003; Zwahlen et al., 2003; Heckmann et al., 2006; Hönemann and Nentwig, 2009; Lindfeld et al., 2011; Yuan et al., 2011), and on soil microbial communities (Griffiths et al., 2007; see Bruinsma et al., 2003; Icoz and Stotzky, 2008 for reviews). However, contrary to recent ecotoxicological studies that were based on multispecies assemblages (Edwards, 2002; Cortet et al., 2006; Scott-Fordsmand et al., 2008; Schnug et al., 2014; Sechi et al., 2014), only few GM plant studies have considered trophic interactions in the soil community, e.g. as advocated for by Birch et al. (2007). To provide a quantitative underpinning of the analysis of the trophic structure of the soil fauna, we employed measurements of the natural abundance of stable isotopes. This technique has been used in soil ecological studies for two decades now, and has provided new insights into the soil invertebrate food web (Spain et al., 1990; Schmidt et al., 1997; Scheu, 2002). Here we advocate for applying it to ecotoxicological studies in order to promote the integration of more ecology into ecotoxicology.

To adopt established effects assessment approaches EFSA (2006, 2010) and Garcia-Alonso et al. (2006) suggested to use the tiered approach similar to risk assessment of pesticides (European Commission, 2002), which involves a three tier testing approach, from the laboratory scale (1st tier) to the field scale (3rd tier). This elicits a growing request for soil multispecies 2nd tier tests (EFSA, 2010), in which a range of soil fauna species are involved and exposed to conditions intermediate between laboratory and field, e.g. model ecosystems or mesocosms under greenhouse or semi-field conditions (Birch et al., 2007; Scholz-Starke et al., 2011). Through this approach, it is possible to gain information about effects on artificially assembled soil communities with constrained species richness and composition, while manipulating experimental conditions and biotic components to focus on specific ecotoxicological research and risk assessment questions.

Interactions of GM plants with future predicted climate are an as yet unstudied research topic and is needed as this combination may affect ecological functioning of agroecosystems and associated ecosystem services. Rising levels of atmospheric CO_2 are expected to increase plant productivity and hence produce changes in belowground carbon (C) allocation, litter composition and altering C fluxes in the soil. Even though soil fauna would not be directly affected by higher CO_2 concentrations due to their adaptation to elevated CO_2 (eCO_2) in the soil, indirect effects on soil communities could arise due to changes in quantity and quality of below-ground plant biomass and litter (Couteaux and Bolger, 2000; Wardle et al., 2004; Haimi et al., 2005; Sticht et al., 2006). Investigations of eCO_2 level effects on soil fauna have involved studies at various scales, from considering single-species responses (e.g. Sticht et al., 2008) up to whole model communities (e.g. Lawton, 1996), but so far no studies have to our knowledge investigated the interaction of eCO_2 and GM plants. Chang et al. (2011) found by examining the interactive effect of ground-level ozone (O_3) and *Bt* cotton on the abundance, diversity and community structure of Collembola, that *Bt* cotton can buffer the negative ozone effect on Collembola through different allocation of carbohydrates in the roots.

In order to test the individual and combined effects of GM plants and eCO_2 on a model mesofauna community, we set up a mesocosm experiment in a greenhouse. Each experimental unit contained species of Collembola, Acari and Enchytraeidae representative of a typical North European agro-ecosystem. We used the GM *Hordeum vulgare* cv. Golden Promise spring-barley C-hordein line (line 5, see Lange et al., 2007), which is characterized by a modified amino acid composition of the grains, and tested it against three different cultivated varieties of barley including the near-isogenic parental line, in order to include the variability of cultivated varieties in the experiment (Griffiths et al., 2007; Duc et al., 2011). The mesocosms were exposed to ambient CO_2 and an elevated level of CO_2 of minimum 450 ppm according to a mitigation scenario developed by the ENSEMBLES project and labelled E1, where atmospheric CO_2 stabilises at a concentration of 450 ppm by 2140, corresponding to the European climate policy (Van der Linden and Mitchell, 2009). We amended the soil with dried maize leaves with isotopically distinct values of $\delta^{13}C$ and $\delta^{15}N$ compared to barley, in order to detect food-web changes among the different treatments. As maize litter and the barley rhizosphere are C and N sources for detritivores, their contrasting isotopic signatures enables detection of shifts in C and N usage by the community, which may change according to the rhizosphere environment created by the different barley varieties. We hypothesized that (i) the soil fauna community and trophic structure would be unaffected by the GM compared to the non-GM cultivars since the intended transgenic modification alters the amino acid composition of the barley grains only, (ii) any unintentionally changes of the barley would be below detection levels of the mesocosm test system, and (iii) an increase of CO_2 would boost overall plant growth and therefore (iv) stimulate collembolan densities since the rhizosphere based energy channel is important for Collembola.

2. Methods

2.1. Experimental design

The experiment was running from September to December 2010 in our greenhouse at Department of Bioscience, Silkeborg, Denmark, at minimum 15 °C and a photoperiod of minimum 16 h by supplying day-light with artificial light (model OSRAM PLANTASTAR® 400 W, light intensity at plant level 10,000 lx, wavelength 400–700 nm). Two separate greenhouse cells were used to simulate two levels of CO_2, while aiming for the local ambient CO_2 (aCO_2) level and an elevated level (eCO_2) of minimum 450 ppm according to the E1 mitigation scenario (Van der Linden and Mitchell, 2009). The CO_2 injection system was programmed to ensure an average difference of 80 ppm between the eCO_2 and aCO_2 cells and the CO_2 air concentration was measured every 4th minute in each greenhouse cell.

Each mesocosm unit consisted of a Plexiglas transparent tube of 9 cm inner diameter × 33 cm high, filled with approximately 2100 g dry weight (dw) of soil (water content adjusted to 20%, WHC 28.34%) collected in Askov Experimental Station, Denmark (55° 28′ 20.16″, 9° 6′ 36.77″). The soil was a sandy loam with the following characteristics: 37.3% coarse sand, 41.7% fine sand, 9.8% silt, 9.1% clay, 2.1% humus, pH 6.4, 1.24% carbon (C) total, 0.27% nitrogen (N) total, C:N 4.71, phosphorous (P) total 0.33 mg/kg, potassium (K) total 1.11 mg/kg, magnesium (Mg) total 0.67 mg/kg, nitrates (NO_3^-) 19.9 mg/kg, ammonium (NH_4^+) 1.53 mg/kg.

The soil was sieved through an 8 mm mesh and then defaunated by two freeze−thaw cycles at −18 °C and +20 °C altogether lasting approximately eight days. Each cylinder consisted of three soil