Interactions between above- and belowground organisms modified in climate change experiments

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Climate change has been shown to affect ecosystem process rates[1] and community composition[2], with direct and indirect effects on belowground food webs[3]. In particular, altered rates of herbivory under future climate[4] can be expected to influence above–belowground interactions[5]. Here, we use a multifactor, field-scale climate change experiment and independently manipulate atmospheric CO_2 concentration, air and soil temperature and drought in all combinations since 2005. We show that changes in these factors modify the interaction between above- and belowground organisms. We use an insect herbivore to experimentally increase aboveground herbivory in grass phytometers exposed to all eight combinations of climate change factors for three years. Aboveground herbivory increased the abundance of belowground protozoans, microbial growth and microbial nitrogen availability. Increased CO_2 modified these links through a reduction in herbivory and cascading effects through the soil food web. Interactions between CO_2, drought and warming can affect belowground protozoan abundance. Our findings imply that climate change affects aboveground–belowground interactions through changes in nutrient availability.

Plant species composition and community structure in terrestrial environments have been predicted to shift in response to climate change[6]. Recent climate change experiments have shown the effects of drought, warming and increased CO_2 on plant productivity[7], nitrogen cycling[8] and species interactions[4]. However, multifactor experiments on climate change are scarce[9] and climate change effects on interactions between the above- and the belowground subsystem[5,10,11] are rarely considered.

Most terrestrial plant species control or mediate the interaction between above- and belowground subsystems, for example through altered litter quality[5] or root exudates[12], suggesting that changes in the aboveground compartment will cascade between the aboveground- and the belowground compartment[13]. For example, herbivores feeding on aboveground plant parts have been shown to induce changes in a wide range of processes in the root zone, affecting rhizodeposition[12] and soil decomposer organisms[14]. Furthermore, indirect pathways from herbivores through greenfall, frass or litter input to the belowground subsystem have been reported[5].

Increased CO_2 (ref. 15), increased temperature[16] or drought[17] have been shown to affect aboveground herbivory. Owing to the strong link between aboveground herbivory and belowground processes, it is likely that such climatic or atmospheric changes will lead to herbivory-induced changes in belowground processes
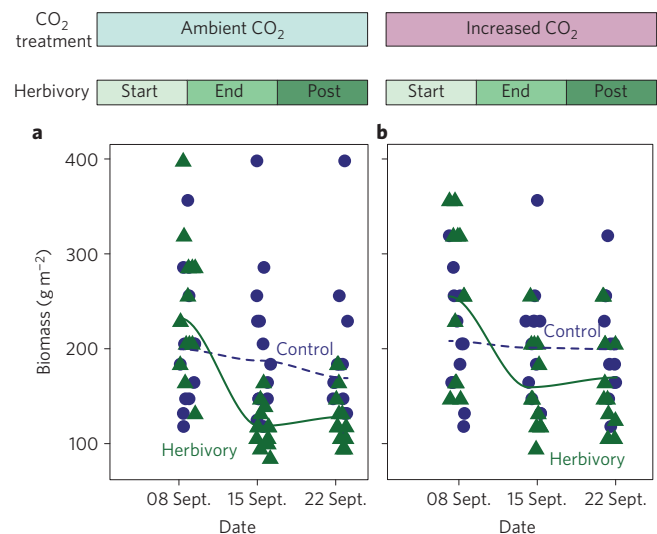

Figure 1 | Effects of increased CO_2 and herbivory on plant aboveground biomass. The y axes show the biomass of *Deschampsia flexuosa* (g m^{-2}) in each cage as a function of herbivory (triangles) or control treatment (no herbivory; circles), herbivory time and CO_2 exposure at ambient CO_2 (**a**) and increased CO_2 (**b**). Dates shown are in 2008. Solid and dashed lines show average grass biomass in cages with and without grasshoppers respectively, fitted as non-parametric smoothing functions.

such as rhizodeposition, in addition to any direct effects of climate change drivers.

However, despite the importance of rhizodeposits for the growth of soil microbes and many other groups of soil organisms feeding on these, at present it is not known how different climate change drivers will interact with herbivory to affect rhizodeposition. It is therefore crucial to improve our understanding of these interactions, using well-replicated factorial field experiments.

Here, we analyse how combined atmospheric and climate change (referred to as climate change, for brevity) affects aboveground herbivory and how this effect is transferred to the belowground subsystem. We independently manipulate ambient air and soil temperature by passive night-time warming (resulting in about +0.3 °C average day and night), precipitation by rainout shelters (four-week summer drought) and atmospheric CO_2 concentration by a free-air carbon enrichment (FACE, 510 ppm) system in all combinations in 48 field plots of 7 m^2 in a shrubland ecosystem

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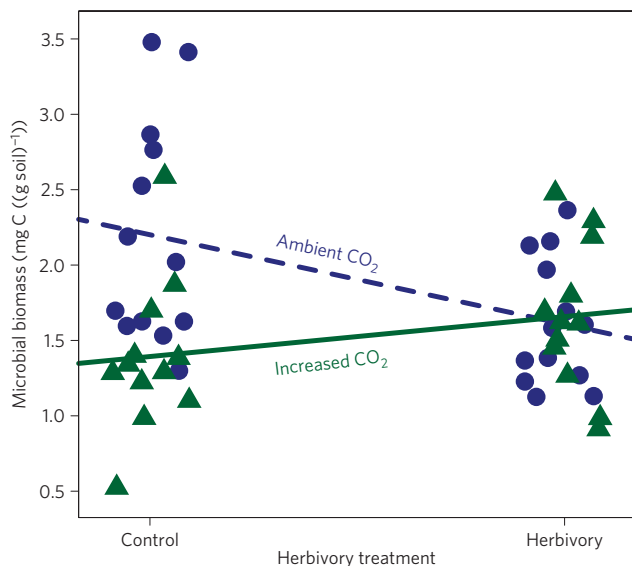
Figure 2 | Effects of aboveground herbivory and increased CO_2 on belowground microbial biomass. Microbial biomass was reduced by increased CO_2; herbivory increased microbial biomass, but only under increased CO_2.

in Denmark[18] (see Methods). We installed 25 herbivory cages (containing locally occurring grasshoppers) and 25 control cages on the plots (Supplementary Fig. S1) and measured vegetation parameters and abundance of belowground organisms and carried out a microbial growth assay (see Methods). Cages were dominated by a grass species that had been exposed to the treatments for more than two years.

Grasshoppers removed roughly 20% of the grass biomass inside the cages under ambient conditions (Supplementary Table S1; herbivory effect at harvest: $F_{1,40} = 27.81$, $P < 0.0001$). Under increased CO_2, herbivory was significantly reduced relative to ambient conditions (date: herbivory: CO_2 interaction: $F_{1,68} = 4.55$, $P = 0.036$; Fig. 1; Supplementary Table S2). None of the climate change factors affected leaf structural compounds or morphology (Supplementary Table S4) and increased CO_2 did not affect silica content (Supplementary Table S5), indicating that treatments did not influence these components of plant antiherbivore defence.

Moreover, root mass was not affected by temperature, drought, or CO_2 treatments (Supplementary Fig. S4), in contrast to previous studies showing increased root mass at increased CO_2 (ref. 19). Aboveground herbivory had strong and significant effects on all measured components of the belowground subsystem, except root mass: microbial biomass, protozoan abundance, as well as microbial growth were highly significantly affected by aboveground herbivory (Supplementary Tables S1, S3; Figs 2, 3; Supplementary Figs S2a, S3). Herbivory reduced microbial biomass, but greatly increased protozoan abundance and microbial growth on carbon in the microbial growth assay (Supplementary Table S3), indicating that microbial activity was stimulated by herbivory. Furthermore, microbial growth correlated strongly with protozoan abundances (Supplementary Fig. S2b), showing that herbivory effects were passed on in the belowground food web. Belowground nematode abundance was not significantly affected by aboveground herbivory. Root mass was not affected by herbivory (Supplementary Fig. S4), indicating that root productivity was also unaffected by herbivory.

Under experimental climate change, the response of belowground microbial biomass to aboveground herbivory was clearly modified (Supplementary Table S3; Fig. 2), indicating that climate change drivers strongly affected aboveground–belowground links. In a similar study[20], microbial biomass also increased under increased CO_2, but only if enough soil nitrogen was available. Notably, belowground grass-root biomass and soil organic matter contents were not significantly affected by our climate change treatments, indicating that the observed effects on microbes were not caused by differences in root production or decomposition. Arbuscular mycorrhizal fungi were present at only 5–10% of the root length, independent of the treatments, and there was no indication of fungal endophyte presence. These findings make it unlikely that plant–fungal interactions were important in our study.

Grasshopper herbivory also had a stimulating effect on microbivorous protozoa under increased CO_2 in interaction with drought (Supplementary Table S3 and Fig. S3). This indicates that CO_2 and drought also modulated above–belowground effects on microbivorous soil organisms. The cascading effect of foliar herbivory on the soil food web being stronger at increased CO_2 (Fig. 4) is also in support of increased nitrogen limitation under these conditions.

Because our study system was increasingly nitrogen-limited at increased CO_2 (ref. 21), it is likely that soil microbes experienced
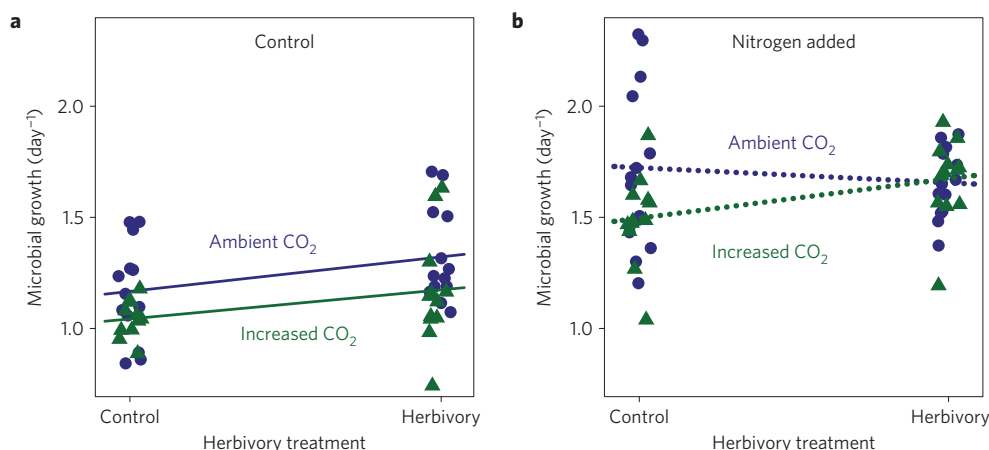

Figure 3 | Limitation of microbial growth. Results from microbial growth assays on soil samples from plants grown with or without grasshopper herbivory and exposed to ambient CO_2 (circles) or increased CO_2 (triangles) and with addition of carbon (**a**) and carbon and nitrogen (**b**) sources. Microbes tended to grow less under increased CO_2 ($P = 0.077$, Supplementary Table S3); whereas aboveground herbivory increased belowground microbial growth when only carbon was added (**a**; $P = 0.02$, Supplementary Table S3), approaching growth rates observed when both carbon and nitrogen were added (**b**). Dashed lines in **b** were not significant, indicating that herbivory effects are owing to relieved nitrogen limitation.
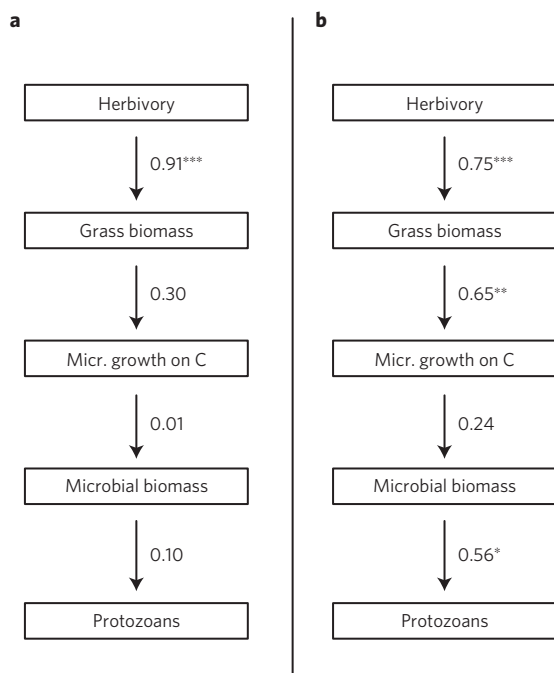
Figure 4 | Effects of increased CO_2 on above–belowground interactions. Shown are the pairwise correlations between individual variables, with significance indicated by asterisks (*$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$). Corresponding structural equation models produced essentially similar results but are not included here because the number of replicates precludes the use of structural equation models in this case. **a**, Ambient CO_2; **b**, increased CO_2.

progressive nitrogen limitation[8,21]. It is possible that aboveground herbivory counteracted nitrogen limitation in microbes by inducing plant nitrogen transfer to the root zone. We tested this hypothesis by providing microbial community assays with extra sources of nitrogen (NH_4NO_3).

The assay showed that microbial growth was nitrogen-limited even under ambient conditions (Fig. 3a versus b). This nitrogen limitation was further amplified under increased CO_2, as predicted by the progressive nitrogen-limitation hypothesis[22]. When we added grasshopper herbivores to the system, microbial growth was consistently stimulated (Fig. 3a). Finally, when we experimentally added nitrogen again, nutrient limitation disappeared and grasshoppers did not stimulate microbial growth (Fig. 3b).

Taken together, these findings strongly suggest that the competition between plants and microbes for nitrogen being intensified at increased CO_2 was reduced owing to aboveground herbivores, because the demand for nitrogen from the plant was diminished at a reduced biomass. Note, however, that longer-term dynamics may differ, for example because of plant compensatory responses to herbivory.

Recently, long-term experiments[7] have shown that terrestrial net primary production under increased CO_2 may be limited by nitrogen availability. Our study has shown that nitrogen limitation affects not only aboveground plant biomass, but also the belowground subsystem. The relieved nitrogen limitation owing to herbivory at increased CO_2 reported here may be compromised by the general reduction in herbivory under future CO_2 levels suggested in a meta-analysis[23]. This means that the increase in terrestrial nitrogen limitation under increased CO_2 will not be compensated by herbivore effects on the belowground subsystem. Overall, these processes may alter components of the global nitrogen and carbon cycle and reduce terrestrial carbon sequestration.

Methods

Experiments were conducted in a FACE facility (Supplementary Fig. S1) in a sand dune area near Brandbjerg (55°53′ N, 11°58′ E) approximately 50 km northwest of Copenhagen, Denmark, where drought, warming and atmospheric CO_2 concentration are experimentally manipulated since 2005 (ref. 18). The experimental treatments are increased temperature (+1 °C in the upper 5 cm of soil), increased CO_2 (ambient 380 ppm, increased 510 ppm) and summer drought (soil moisture decreasing to 5% (vol/vol) during about one month). The experiment is fully factorial, giving eight treatments with six replicates, in total 48 plots, arranged in a randomized blocks design[19]. In December 2005, two soil cores (10 cm diameter, 20 cm deep) were established in all plots and filled with sieved and well-mixed soil from the area. In March 2006, cores were planted with *Deschampsia flexuosa*, the locally dominant grass species. On 3 September 2008, grass height was assessed in the cores and 25 of the 48 plots had a pair of cores with sufficient grass growth, that is, plant height 16.0 cm ± 0.5 cm (average ± standard error). Grass survival during the 2.5 years before the experiment was completely random among the treatments, with no indication of the grass survival being influenced by drought, CO_2, or warming (P values > 0.69, that is, there was a 75% chance that grasses died at random; see Supplementary Table S6 for numbers of replicates per treatment combination). A nylon net bag was mounted on top of the 50 cores. About 100 females of a locally dominant grasshopper species (*Chorthippus brunneus Thunb*) were collected in the area. The following day (4 September 2008) two adult female grasshoppers were selected at random and added to one of the cores in each plot (Supplementary Fig. S1). Effects of drought in this study are legacy effects (Supplementary Fig. S7), because the drought treatment terminated two months earlier and soil water at the time of soil sampling (8.8 wt%) did not differ significantly between moisture treatments. Measurements of temperature, soil moisture and CO_2 concentration before and during our study are presented in Supplementary Figs S5–S10. Dead grasshoppers (four in total) were replaced every two to three days during the following eight days. On 12 September grasshoppers were removed and grass height measured in all cores. Ten days later (22 September) grass height was measured again and the 50 soil cores were brought to the laboratory. Rhizosphere soil was analysed for microbial biomass (substrate-induced respiration) during the first four hours of incubation[24] but using soil slurries amended with carbon or carbon and nitrogen[25]; the carbon-amended slurries were used for microbial biomass determination. Microbial growth was assayed as fractional increase in respiration rate[26], in this case between 0–4 h and 4–20 h incubation of agitated soil slurries (respiration rate 4–20 h/respiration rate 0–4 h) in the differently amended soil slurries. The number of bacterivorous protozoa (most probable number method[27]), and number of nematodes[28] were also assessed. Grass material from cores without grasshoppers was oven dried at 65 °C, weighed and analysed for silicon and crude fibre[29]. The grass biomass before harvest was estimated backwards from height data using a nonlinear generalized least squares model. A subsample of the roots was boiled for three minutes in a 10% KOH solution, washed several times in tap water and then boiled for two minutes in a 5% ink/vinegar solution. Subsequently, root colonization by arbuscular mycorrhiza and the presence of fungal endophytes was measured by a line intersect method, using a stereo microscope (×40 magnification). Endophytes were also assessed in leaf material. Data were analysed using R 2.14.1 (ref. 30) using mixed-effects models fit by restricted maximum likelihood with six blocks and two CO_2 rings within each block as random effects and herbivory, CO_2, warming and drought as fixed effects (including interactions). Random effects for drought and warming nested within CO_2 were not supported by the data. Variance functions were used to model heteroscedasticity. Models were simplified using Akaike's information criterion, corrected for small sample sizes.

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Author contributions

K.S. and C.S. contributed equally to this manuscript. C.S., S.C. and C.B wrote the manuscript. K.S. and S.C. planned and initiated the study. K.S., D.G., C.S. and S.C. collected the data and had initial discussions of their implication. C.S. and S.C. carried out all statistical analyses. T.N.M. was in charge of the field study. All authors discussed the analysis and results and commented on the manuscript text.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.S.