Impacts of Rising Atmospheric Carbon Dioxide on Model Terrestrial Ecosystems

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In model terrestrial ecosystems maintained for three plant generations at elevated concentrations of atmospheric carbon dioxide, increases in photosynthetically fixed carbon were allocated below ground, raising concentrations of dissolved organic carbon in soil. These effects were then transmitted up the decomposer food chain. Soil microbial biomass was unaffected, but the composition of soil fungal species changed, with increases in rates of cellulose decomposition. There were also changes in the abundance and species composition of Collembola, fungal-feeding arthropods. These results have implications for long-term feedback processes in soil ecosystems that are subject to rising global atmospheric carbon dioxide concentrations.

Above-ground plant and ecosystem responses to elevated atmospheric carbon dioxide (CO_2) are varied (*1–7*). However, all these potential responses may be constrained by below-ground processes and mediated by responses of soil biota to both direct and indirect effects of CO_2 enrichment (*8–12*). Roots, mycorrhizal fungi, and other rhizosphere organisms may be substantially affected by changes in CO_2 concentration, yet comparatively little attention has been paid to the effects of increasing atmospheric CO_2 on these below-ground organisms and their functioning (*13*). In addition, reliable predictions about the ecological effects of elevated CO_2 at the community, ecosystem, and biosphere levels are difficult to make; most available information is based on experiments conducted at lower levels of organization, such as leaves or individual plants (*1, 3, 14*). Only relatively recently have longer term experiments on populations and communities been initiated. Current experimental evidence questions the justification for predicting community and ecosystem responses from results obtained with isolated plants growing under controlled (mostly optimal) conditions (*1, 3, 14, 15*). One possible solution is to use model laboratory systems of intermediate complexity. Here, we used the Ecotron controlled environment facility at Silwood Park (*16*) to provide evidence, from direct experimental studies, of changes in soil biota as a consequence of elevated atmospheric CO_2 concentrations.

The experiment used 16 terrestrial microcosms, each 1 m^2, maintained in the Ecotron (*16, 17*). Conditions were the same for all chambers (*18*), except that eight were maintained at ambient external atmospheric CO_2 concentrations, which fluctuated naturally between 350 and 400 μmol mol^{-1}, and eight were dynamically maintained at 200 μmol mol^{-1} above ambient (*19*). The community, established in soil that was relatively poor in nutrients (*18*), consisted of primary producers, herbivores, secondary consumers (parasitoids), and soil micro- and macroorganisms (Table 1). All chambers were initiated with the same community, and several ecosystem processes were measured over three plant generations. The results discussed below, from as many as four independent experimental runs (*20, 21*), primarily concern the soil; not all parameters were measured in every run.

The communities growing in elevated CO_2 fixed more carbon for most of the experimental period (*22*). Changes in the above-ground community were relatively small (*23*) and broadly in line with other whole-ecosystem studies (*1–4, 6, 7, 24*). More marked effects, previously unreported, were observed in soil biota. Total numbers (all species pooled) of Collembola per kilogram of Ecotron soil were significantly higher at the end of run 1 in elevated CO_2 [density (± SE) = 252 ± 35 (elevated), 166 ± 54 (ambient); $P < 0.05$]. Species composition also changed (Fig. 1). *Proisotoma minuta* dominated communities in ambient CO_2, whereas *Folsomia candida* dominated in elevated CO_2. *Pseudosinella alba* was also present in significantly higher proportions in elevated CO_2 in run 1 but not in other runs. It is well known that key environmental variables influence soil microarthropods (*25*). Of these, temperature, water content, and pH of soil showed no significant differences between treatments in any run. Nor can change in the collembolan community be attributed to changes in root biomass (*26*) or in root "quality" (as assessed by C:N ratios) (*27*).

Soil microbial biomass (*26*) was unaffected by elevated CO_2; similar results have been obtained in most (*18, 28, 29*) but not all (*9*) other studies. Enzymes involved in carbon and nitrogen cycling in the soil also showed no major significant treatment effects (*30*). All ecosystems were initiated with standard samples drawn from a filtered soil-wash microbial pool (*31*) (Table 1). Bacterial assemblages in the topmost 10 cm

Table 1. Composition of the Ecotron community (cf., species very similar, but not exactly like type-specimen).

Plant species	*Cardamine hirsuta* *Poa annua* *Senecio vulgaris* *Spergula arvensis*
Herbivore and parasitoid species	Mollusk (*Helix aspersa*) Aphids (*Brevicoryne brassicae*, *Myzus persicae*) Whitefly (*Trialeurodes vaporariorum*) Leaf miner [*Phytomyza (Chromatomyia) syngenesiae*] Parasitoids (*Aphidius matricariae*, *Dacnusa sibirica*, *Encarsia formosa*)
Soil biota	*Earthworm* (*Lumbricus terrestris*) Wood louse (*Porcellio scaber*) Collembola (*Folsomia candida*, *Proisotoma minuta*, *Protaphorura* cf. *armata*, *Pseudosinella alba*, *Sphaeridia* cf. *pumilis*) Plus soil bacteria, fungi, protists, and nematodes seeded into each chamber by means of a filtered soil leachate (*31*)

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were assayed with DNA profiles (*32*); at the end of run 1, we found only minor differences in bacterial taxonomic composition between chambers, and no consistent difference between treatments.

Fungi, in contrast, showed differences between ambient and elevated atmospheric CO_2 treatments. One functional group, cellulose decomposers, had higher biomass in elevated CO_2 (*33*), probably accounting for the increased decomposition rates of cotton strips placed in the soil ($P < 0.05$) (*34*). Moreover, fungal taxonomic composition differed between treatments: 14 of the 33 species isolated were common to both treatments, whereas 9 species were restricted to ambient CO_2 and 10 to elevated CO_2, a pattern extremely unlikely to occur by chance (*35*).

These results imply that enhanced atmospheric CO_2 concentrations will have major impacts on soil food chains. A substantial proportion of photosynthetically fixed carbon is allocated below ground (*8*, *9*, *36*); after release, much of this carbon becomes available to rhizosphere microorganisms (*10*, *37*). At the end of run 1, soil concentrations of dissolved organic carbon (DOC) were significantly higher in elevated CO_2 (Fig. 2) [analysis of variance (ANOVA), $P < 0.05$], and soil-water dissolved organic nitrogen (DON) concentrations were higher, almost reaching statistical significance ($P = 0.06$). These changes are probably sufficient to drive observed differences in soil fungi. Collembola are major consumers of, and selective grazers on, different species of fungi (*38*). We suggest that differences in the collembolan community were driven by differences in the soil fungal assemblage, which in turn were driven by differences in organic substrates derived from higher plants.

Thus, we hypothesize that at elevated atmospheric CO_2 concentrations over three plant generations, a relatively long pathway of alterations occurs: increased plant photosynthesis → below-ground transport of carbon → increased DOC → changes in soil fungal assemblages → changes in Collembola species abundance and composition. Possible long-term feedbacks remain unknown: Collembola are selective fungal grazers, and hence it is possible that they not only respond to but also drive changes in soil fungal species composition, with unknown consequences for the long-term decomposition of soil organic matter (*8*, *36*).

Despite these differences, other soil biota and processes (root biomass and C:N ratio, bacterial taxa, enzymatic activity) remained unchanged. Microbial biomass may have remained unchanged despite increases in soil DOC because microbial populations were regulated by grazing from components of the ecosystem that we did not monitor, for example, protozoa or nematodes. These apparent differences in, and lack of coupling between, bacterial and fungal components of the soil food web may reflect compartmentalization of soil ecosystem processes (*39*).

We urge caution in overgeneralizing these results. The Ecotron houses model ecosystems (*16*, *17*). Published studies (*11*, *28*, *40*) provide conflicting data on soil microbial responses to elevated CO_2, with the possibility that responses are specific to particular plant species, communities, or ecosystems. Considerably more attention must be paid to the long-term impacts of increasing atmospheric CO_2 concentrations on soil ecosystem processes and soil biota.

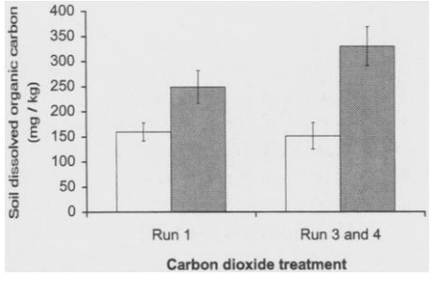

Fig. 2. Concentrations of DOC in the topmost 15 cm of soil at the end of the experiment in runs 1, 3, and 4 (run 1, both treatments; run 3, ambient CO_2; run 4, elevated CO_2). Means (± SE) are given. Open bars represent ambient CO_2; shaded bars represent elevated CO_2.

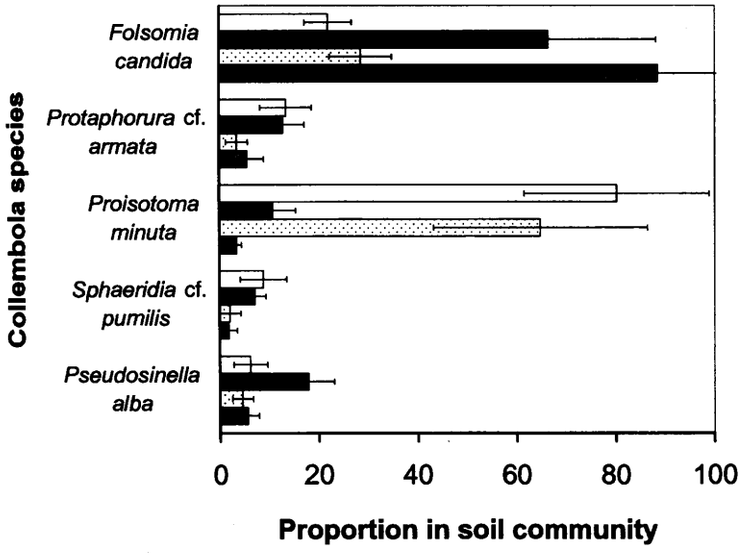

Fig. 1. Composition of Collembola community at the end (after 9 months) of Ecotron experiments. Data (mean ± SE) from three runs are presented: ambient (open bars) and elevated (solid bars) CO_2 from run 1,.ambient CO_2 (dotted bars) from run 3, and elevated CO_2 (hatched bars) from run 4. No data are presented from run 2 because it only lasted 4.5 months. For each treatment there are significant differences (ANOVA; F test, $P \ll 0.05$) in the proportion (arc sine transformed) of each of the five species.

REFERENCES AND NOTES

1. F. A. Bazzaz, *Annu. Rev. Ecol. Syst.* **21**, 167 (1990).
2. H. A. Mooney *et al.*, *Bioscience* **41**, 96 (1991).
3. W. C. Oechel *et al.*, *Nature* **371**, 500 (1994).
4. P. Vitousek, *Ecology* **75**, 1861 (1994).
5. G. Bowes, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**, 309 (1993); H. H. Rogers, G. E. Runion, S. V. Krupa, *Environ. Pollut.* **83**, 155 (1994).
6. G. W. Koch and H. A. Mooney, Eds., *Carbon Dioxide and Terrestrial Ecosystems* (Academic Press, San Diego, CA, 1996).
7. C. Körner and F. A. Bazzaz, Eds., *Carbon Dioxide, Populations, and Communities* (Academic Press, San Diego, CA, 1996).
8. G. I. Ågren *et al.*, *Ecol. Appl.* **1**, 118 (1991).
9. B. A. Hungate *et al.*, *Nature* **388**, 576 (1997).
10. E. G. O'Neill, *Plant Soil* **165**, 55 (1994).
11. M. J. Sadowsky and M. Schortemeyer, *Global Change Biol.* **3**, 217 (1997).
12. P. Barbosa, V. A. Krischik, C. G. Jones, Eds., *Microbial Mediation of Plant-Herbivore Interactions* (Wiley, New York, 1991); J. C. Moore, H. W. Hunt, E. T. Elliott, *ibid.*, chapter 4; S. C. Rabatin and B. R. Stinner, *ibid.*, chapter 5; E. R. Ingham and R. Molina, *ibid.*, chapter 6; W. H. Schlesinger, *Biogeochemistry: An Analysis of Global Change* (Academic Press, San Diego, CA, 1991).
13. B. Griffiths and R. D. Bardgett, in *Modern Soil Microbiology*, J. D. van Elasas, E. Wellington, J. T. Trevors, Eds. (Dekker, New York, 1997), pp. 165–182; D. C. Coleman, in *Microfloral and Faunal Interactions in Natural and Agroecosystems*, M. J. Mitchell and J. P. Nakas, Eds. (Nijhoff/Junk, Dordrecht, Netherlands, 1986), pp. 317–348; J. Lussenhop, *Adv. Ecol. Res.* **23**, 1 (1992); S. Visser, in *Ecological Interactions in Soils*, A. Fitter *et al.*, Eds. (Blackwell Scientific, Oxford, 1985), pp. 297–317.
14. J. Weiner, in (*7*), pp. 431–441; F. I. Woodward, G. B. Thompson, I. F. McKee, *Ann. Bot.* **67** (suppl. 1), 23 (1991).
15. C. Körner, *Plant Cell Environ.* **18**, 1101 (1995).
16. J. H. Lawton *et al.*, *Philos. Trans. R. Soc. London Ser. B* **341**, 181 (1993); J. H. Lawton, *Ecology* **77**, 665 (1996).
17. S. Naeem *et al.*, *Nature* **368**, 734 (1994).
18. The experiment was set up with a photoperiod of 18 hours (2:00–20:00), including a gradual dusk and

lawn of 2 hours. Average light intensity at canopy (1 m from lights) was 294 $\mu m\ s^{-1}\ m^{-2}$. Pot volume was 0.4 m^3; the initial soil, 0.1 m^3 of gravel, was topped with 0.3 m^3 of 40:60 sand–Surrey loam mix (41.61 ppm nitrogen, 17.63 ppm phosphorus, 12.45 ppm potassium). Temperature varied smoothly between a maximum of 20°C during the day and a minimum of 12°C at night. Relative humidity varied smoothly between a maximum of 70% after watering and a minimum of 58%.

19. Following the "moderate" Intergovernmental Panel for Climate Change scenario for 2060 [J. T. Houghton *et al.*, Eds., *Climate Change 1995. The Science of Climate Change* (Cambridge Univ. Press, Cambridge, 1996)].
20. The experimental chambers were in two banks of eight (all statistical analyses have $n = 8$ for each treatment). The design of the Ecotron is such that the eight chambers in one bank are not statistically independent replicates. However, because each chamber receives air from only the air-handling unit associated with that bank and is physically sealed and separated from adjacent chambers in the bank, the chambers are effectively independent. So that we could be certain that CO_2 effects were not confounded by unexpected bank effects, the main experiment (run 1, 9 months, three plant generations) was repeated for 4.5 months (run 2, 1.5 plant generations) with the position of the experimental treatments interchanged between consecutive runs. No bank effects were detected. In addition, in later experiments (*21*) we also repeated both the ambient (run 3) and the elevated (run 4) CO_2 in one bank while manipulating temperature in the other bank, always for 9 months. In all runs, results obtained with a particular CO_2 treatment were similar. For simplicity, we concentrate here on the results from run 1 with corroborating information only from later runs. An artificial "winter" was imposed by cutting most of the above-ground vegetation at the end of each generation and replacing it immediately as litter.
21. L. J. Thompson, K. Sanbrooke, S. E. Hartley, unpublished data; T. M. Bezemer, T. H. Jones, K. J. Knight, unpublished data; S. E. Hartley *et al.*, unpublished data.
22. Infrared gas analyzer measurements were taken as flux (in ppm CO_2) over 48-hour periods with an airflow of 0.25 m^3 per second per chamber.
23. Three of the plant species (*Cardamine hirsuta*, *Senecio vulgaris*, and *Spergula arvensis*) showed increased rates of photosynthesis during the course of the experiment; there was no significant change in the rate of photosynthesis of *Poa annua*. Elevated CO_2 also resulted in significant differences in the plant populations that were both species and generation dependent. Herbivores showed species-specific changes: For example, in run 1, *Brevicoryne brassicae* populations at the end of the second plant generation were higher in ambient CO_2 than in elevated CO_2. The reverse was true for *Myzus persicae* at the end of the third plant generation.
24. C. Körner and J. A. Arnone III, *Science* **257**, 1672 (1992).
25. J. N. Klironomos and B. Kendrick, *Plant Soil* **170**, 183 (1995).
26. C. Kampichler *et al.*, *Global Change Biol.* **4**, 335 (1998).
27. Root carbon and nitrogen contents were determined in a Carlo Erba NA 1500 elemental analyzer. Mean (± SE) C:N ratios in run 1 were as follows: 0 to 10 cm, 34.5 (±1.1):1 (ambient), 36.1 (±1.2):1 (elevated), $P = 0.351$; 10 to 20 cm, 41.9 (±3.5):1 (ambient), 35.0 (±1.0):1 (elevated), $P = 0.068$.
28. C. W. Rice *et al.*, *Plant Soil* **165**, 67 (1994).
29. J. N. Klironomos, M. C. Rilling, M. F. Allen, *Funct. Ecol.* **10**, 527 (1996).
30. Enzyme activities measured were urease, xylanase, trehalase, and arginine deaminase [F. Schinner *et al.*, *Methods in Soil Biology* (Springer, Heidelberg, 1996)]. See (*18*) for details.
31. Soil was initially sterilized by methyl bromylation. Each replicate then received 120 ml of a microbial inoculum prepared from 20- to 25-μm pore filtrate (Whatman number 4) of Silwood Park soil. This treatment also introduced nematodes and protists to all chambers [see (*16, 17*)].
32. R. I. Amann, W. Ludwig, K. H. Schleifer, *Microbiol. Rev.* **59**, 143 (1995). DNA was extracted directly from 1 g of each soil sample [K. D. Bruce *et al.*, *Mol. Ecol.* **4**, 605 (1995)]. The polymerase chain reaction (PCR) was used to amplify eubacteria 16S ribosomal RNA genes present in the extracted DNA with fluorescently labeled universal eubacterial oligonucleotide primers pA and pH′ [U. Edwards *et al.*, *Nucleic Acids Res.* **17**, 7843 (1989)]. PCR products were digested with the restriction endonuclease Hae III, separated with an ABI 373 A automated DNA sequencer, and analyzed with Genotyper software (version 1.1). The resulting profiles, each peak of which identifies a different variant, were compared visually.
33. Soil samples from all chambers were inoculated onto agar plates, using 10 different protocols. Colony numbers and identities were ascertained after varying periods of incubation. Only the cellulose agar (for cellulolytic fungi) showed statistically significant differences [mean (± SE) number of colonies ($\times 10^2$ g^{-1} soil) recovered, 17.2 (±9.4) (ambient), 35.0 (±14.8) (elevated); $P < 0.05$] between the ambient and elevated CO_2 treatments.
34. Measured as cotton rotting rate [M. O. Hill, P. M. Latter, G. Bancroft, *Can. J. Soil Sci.* **65**, 609 (1985)]. For run 1, this value was 36.5 cotton strips per year ± 3.53 (elevated) and 26.2 cotton strips per year ± 4.20 (ambient); $P < 0.05$.
35. We used a randomization test on the observed distribution of fungal species against distributions from a null model in which each of the 33 species was randomly assigned to one of the two treatments a total of n times (n = the number of chambers it occupied in the experiment). The probability that each occurrence of a species was assigned to each treatment was 0.5 and did not depend on the number of times the species had already been assigned to that treatment. A maximum of eight occurrences per species per treatment was allowed. The null model was run for a total of 5000 iterations. Not once did the null model yield as few as 14 species common to both treatments (the minimum was 23), or as many as 9 restricted to a single treatment (the maximum was 6) (two-tailed $P < 0.001$).
36. G. I. Ågren and E. Bosatta, *Ecology* **68**, 1181 (1987); G. I. Ågren *et al.*, in *Effects of Climate Change on Grasslands and Coniferous Forests*, J. M. Melillo and A. Breymeyer, Eds. (Wiley, Chichester, UK, 1996), pp. 207–228.
37. E. G. O'Neill, R. J. Luxmoore, R. J. Norby, *Can. J. For. Res.* **17**, 878 (1987); K. Ineichen, V. Wiemken, A. Wiemken, *Plant Cell Environ.* **18**, 703 (1995); G. M. Bernston and F. A. Bazzaz, *Plant Soil* **187**, 119 (1996); D. L. Godbold and G. M. Bernston, *Tree Physiol.* **17**, 347 (1997).
38. S. P. Hopkins, *Biology of the Springtails. Insecta: Collembola* (Oxford Univ. Press, Oxford, 1997). *Folsomia candida* is also reported to feed on nematodes [Q. Lee and P. Widden, *Soil Biol. Biochem.* **28**, 689 (1996); S. Visser and J. B. Whittaker, *Oikos* **29**, 320 (1977); D. Parkinson, S. Visser, J. B. Whittaker, *Soil Biol. Biochem.* **11**, 529 (1979); R. D. G. Hanlon, *Oikos* **36**, 362 (1981); R. D. Bardgett, J. B. Whittaker, J. C. Frankland, *Biol. Fertil. Soils* **16**, 296 (1993); (*25*)].
39. J. C. Moore and P. C. De Ruiter, in *Multitrophic Interactions in Terrestrial Systems*, A. C. Gange and V. K. Brown, Eds. (Blackwell Scientific, Oxford, 1977), pp. 375–393.
40. H. H. Rogers, S. A. Prior, E. G. O'Neill, *Crit. Rev. Plant Sci.* **11**, 251 (1992); H. A. Torbert, S. A. Prior, H. H. Rogers, *Soil Sci. Soc. Am. J.* **59**, 1321 (1995); A. S. Ball, *Global Change Biol.* **3**, 379 (1997); E. Paterson *et al.*, *ibid.*, p. 363; J. N. Klironomos *et al.*, *ibid.*, p. 473.
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Control of Alternative Splicing of Potassium Channels by Stress Hormones

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Many molecular mechanisms for neural adaptation to stress remain unknown. Expression of alternative splice variants of *Slo*, a gene encoding calcium- and voltage-activated potassium channels, was measured in rat adrenal chromaffin tissue from normal and hypophysectomized animals. Hypophysectomy triggered an abrupt decrease in the proportion of Slo transcripts containing a "STREX" exon. The decrease was prevented by adrenocorticotropic hormone injections. In *Xenopus* oocytes, STREX variants produced channels with functional properties associated with enhanced repetitive firing. Thus, the hormonal stress axis is likely to control the excitable properties of epinephrine-secreting cells by regulating alternative splicing of Slo messenger RNA.

Stressors including cold exposure, hypoglycemia, and physical constraint trigger adaptive changes in catecholamine- and peptide-secreting chromaffin cells of the adrenal medulla. Rapid stress-induced increases in transcription of the epinephrine-synthesizing enzyme phenylethanolamine-*N*-methyltransferase (PNMT) result from direct interaction of receptor-bound glucocorticoid stress hormones with glucocorticoid response elements in the promoter (*1*). Glucocorticoids also regulate transcription of voltage-gated K channel genes in cardiac and pituitary cells (*2*). In chromaffin cells, large-conductance "BK" calcium- and voltage-gated K channels are particularly prominent, participating in action po-

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