Ecol Res (2006) 21:483–487
DOI 10.1007/s11284-005-0139-5

NOTE AND COMMENT

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The Earthtron facility for below-ground manipulation study

Received: 25 July 2005 / Accepted: 24 October 2005 / Published online: 8 December 2005

Abstract A controlled environmental facility is necessary for investigation of the interaction between above-ground and below-ground components in microcosm experiments. The Earthtron is a simple computer-controlled chamber simulating natural environments: diurnal light/dark cycles, and separately controlled soil and air temperature, humidity and CO_2 concentration. In soil core incubation experiments, the Earthtron was able to simulate the dynamics of soil temperature field conditions. Environmental control also affects the dynamics of soil water and distribution patterns of nutrients in the microcosm, linking to the distribution of plant roots and soil biota. The Earthtron can not only reproduce field conditions but also predict the effects of global climatic change on terrestrial ecosystems.

Keywords Earthtron · Environmental control · Microcosm · Soil temperature · N mineralization

Introduction

There is increasing recognition of the influence of above-ground and below-ground components on one another. A combined above-ground/below-ground approach to community and ecosystem ecology has enhanced our understanding of the regulation and functional significance of biodiversity and of the environmental impact of human-induced global change phenomena (Wardle et al. 2004). Since field manipulation experiments cannot control environmental conditions, e.g. temperature and moisture variations, resulting in a low repeatability and leading to difficulties with interpretation of the results, simplified laboratory 'microcosms' have been constructed as surrogates for the complicated field situation (Beyer and Odum 1993; Verhoef 1996). Although several experiments have been conducted to investigate the effects of a decomposer on above-ground plant productivity with microcosms in climate chambers, not so much attention has been paid to controlling soil temperature (e.g. Laasko and Setälä 1999; Liiri et al. 2002). Recently, a type of soil microcosm called the terrestrial model ecosystem (TME) has been reported to be a valid method for use by soil ecologists and especially soil ecotoxicologists (van Straalen 2002; Knacker et al. 2004). The TME approach uses undisturbed soil columns taken from the field and includes living vegetation growing on the soil to allow interactions between living soil organisms and plant roots. Soil organisms are so small that the soil column can contain multiple soil biota within a small volume. Such a miniature ecosystem should be manipulated under environmentally controlled conditions to take the changes in above-ground and below-ground conditions in the field into consideration.

An environmental chamber, the 'Earthtron', was built at Yokohama National University in 2004. It allows us to construct replicate terrestrial compartments at ecosystem level under controlled environmental conditions. We describe here the facility emphasizing its unique features and report the effects of environmental control on the dynamics of temperature, water and nutrients in the soil core.

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Materials and methods

The Earthtron (Dalton Company, Tokyo, Japan) is a computer-controlled chamber simulating natural environments. It consists of a large environmental chamber (12.4 m^2) with two cabinets in it (Fig. 1). The chamber can provide diurnal light/dark cycles, and control of air

Fig. 1 The Earthtron facility. Two cabinets are fixed to opposite sides of the chamber. The cabinets can be assigned with experimental pots (the experiment shown in this photograph is not the present study)

temperature (10–25°C), humidity (40–95%) and CO_2 concentration. Controlled air is blown from the side faces into the chamber with low wind speed to minimize the effects on plant growth. Each cabinet can accommodate 30 soil cores (maximum 80 cm in height and 20 cm in diameter), exposing the soil surface to the controlled air. The cabinets can also be connected to another temperature control unit which allows the "soil temperature" around the soil cores to be controlled (10–25°C). The environmental control is a feed-back system: the chambers are supplied with air to adjust to a set temperature, humidity and CO_2 concentration near the air conditioning system in the chamber. The cabinets also have the same system. In contrast, the Ecotron facility, which contains 16 physically and electronically integrated environmental chambers (1 m^2 terrestrial ecosystem in each) in the Centre for Population Biology at Silwood Park (Ascot, UK), is a feed-forward system: chambers are supplied with air at a known temperature and humidity (Lawton 1996). The Ecotron is a more macroscale facility than the Earthtron, but the way the air is controlled in the Earthtron is closer to natural macroclimatic conditions. Then, what is the advantage of such environmental control for microcosm experiments? We conducted an experiment to investigate the dynamics of temperature, moisture and nutrients in soil cores set in the Earthtron.

We used white high-impact styrol pots (14 cm height and 11.5 cm diameter). Each pot was filled with fresh soil to 11 cm height (from Watarase retarding basin, central Japan, sieved through a 2-mm mesh; pH 5.89, organic matter 11%, clay content 23%, dry bulk density 0.52 g cm^{-3}, 47% total water holding capacity). Soil cores were divided into five treatments: EA1, EA2, ES, IS1 and IS2. Treatment pots EA1 and EA2 were placed on the top of the cabinet in the Earthtron. The temperature over the cabinet (air temperature) was controlled at 25°C during the day (14 h) and at 16°C during the night (10 h). Relative humidity and CO_2 concentration were controlled at 65% and 380 ppm during the day, and 80% and 400 ppm during the night, respectively. Treatment pots EA2 were wrapped with aluminum foil to prevent radiant heating from the fluorescent lighting. Treatment pots ES were set in the cabinet. The temperature in the cabinet (soil temperature) was set at 22°C during the day and at 19°C during the night with the soil surface exposed to controlled air over the cabinet (air temperature over the cabinet was controlled at 25°C during the day and 16°C during the night). Treatment pots IS1 and IS2 were placed in an incubator (Eylatron FLI-161, Tokyo Rikakikai Company, Tokyo) which was controlled at 22°C during the day and 19°C during the night, the same as the Earthtron cabinet temperature (soil temperature). Treatment pots IS2 were also wrapped with aluminum foil. The incubator used in this study had no capacity to control humidity or CO_2 level. The illumination was 4500 lux during the day in both the Earthtron and incubator. There were six replicates of each treatment. The environmental conditions and management for each treatment are summarized in Table 1. The pots were incubated for 28 days. The soil was wetted manually with distilled water every other day to maintain the initial water content during the experiment, and loss of water per day was calculated for each pot.

The soil temperatures at depths of 5 and 10 cm in the center of the soil cores were measured during the experiment with thermal probes in one pot per treat-

Table 1 Summary of the conditions of each treatment in the pot experiment

Instrument with controlled factors (day/night)	Temperature (day/night)	Treatment	Management	Replicates
Earthtron illumination 4500 lux; CO_2 380/400 ppm; relative humidity 65/80%	Air 25/16°C	EA1	On top of the cabinet	6
		EA2	On top of the cabinet wrapped with aluminum foil	6
	Soil 22/19°C	ES	In the cabinet with soil surface exposed to air temperature	6
Incubator illumination 4500 lux; no control of CO_2 or humidity	Soil 22/19°C	IS1	In the incubator	6
		IS2	In the incubator wrapped with aluminum foil	6

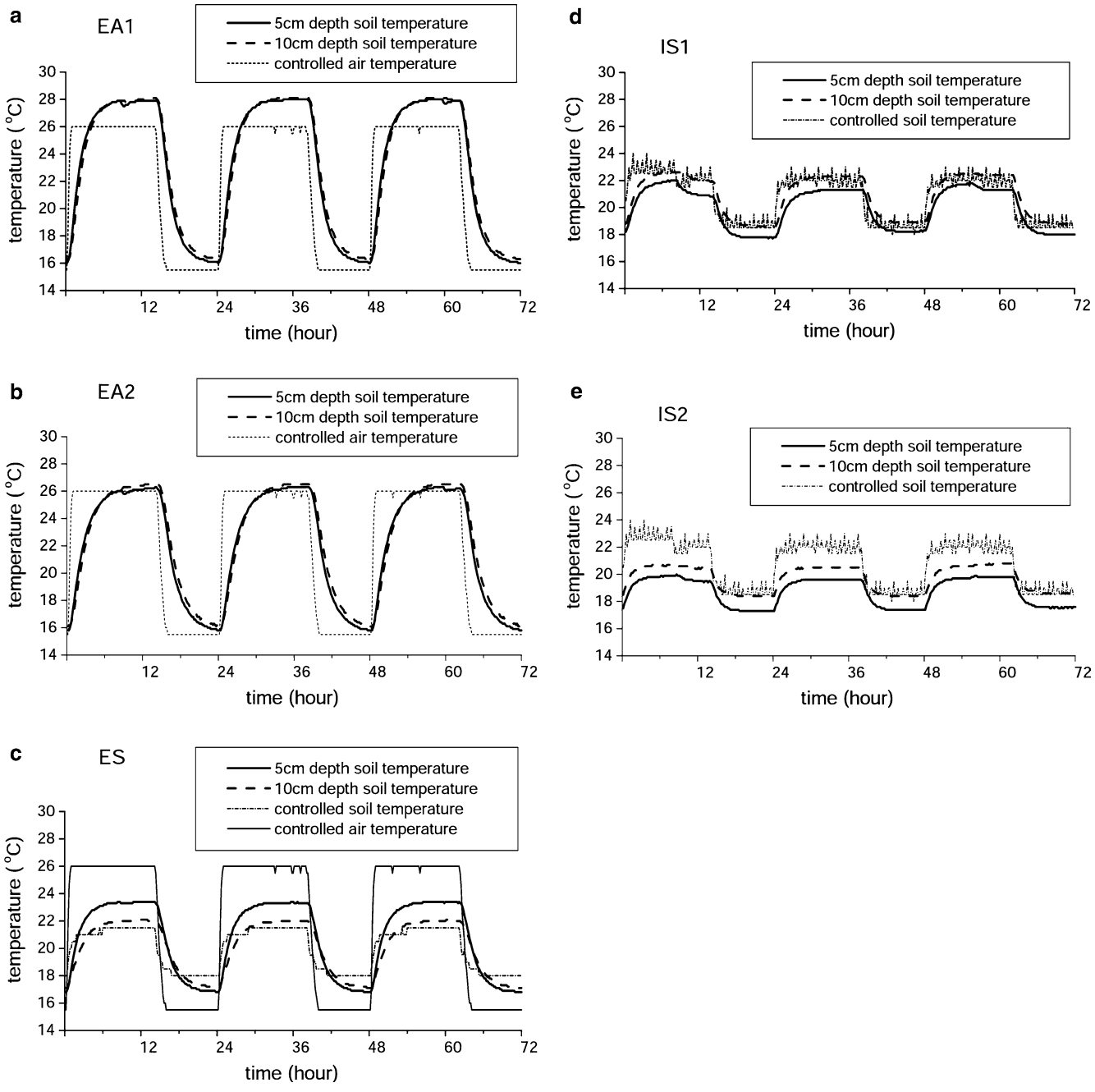

Fig. 2 Time-course of changes in soil core temperature at 5 and 10 cm depth over 72 h during incubation with the surrounding temperature controlled in each treatment

ment. Relative humidity was also measured near the pots in each facility. The soil solution was sampled from every pot on days 7 and 21 of the incubation with a Rhizon soil moisture sampler (Rhizon MOM, Rhizosphere Research Products, Wageningen, The Netherlands) from the top of the soil. About 10 ml of soil solution was extracted by vacuum applied using a syringe for analysis of cations (Na, K, Mg and Ca) and anions (Cl, SO_4 and NO_3) with an ion chromatograph (IC-20, DIONEX, Calif.). After 28 days of incubation, extractable mineralized N ($N–NO_3$ and $N–NH_4$) of the soil at depths of 5 and 10 cm was determined in 1 *M* KCl extracts using an autoanalyzer (Integral Futura, Alliance Instruments, Frépillon, France). Soil pH (soil water ratio 1:2.5) was also determined at each depth.

Results and discussion

Dynamics of soil temperature and water

Relative humidity just near the pots was controlled at about 45% during the day and 70% during the night in the Earthtron. In contrast, relative humidity in the

incubator oscillated in a short cycle from 25% to 65% every day, probably because of periodical activity of the conditioning fan. The low wind speed in the Earthtron would have resulted in a steady humidity. The temperatures in both facilities were well controlled to the set values (Fig. 2). The EA1 soil cores showed a higher temperature than the controlled air temperature during the day, while the temperature of the EA2 cores coincided with the controlled values (Fig. 2a, b). The aluminum foil wrapped around the EA2 pot efficiently cut radiant heating from the fluorescent lamps. No difference in temperature was observed between the 5 cm and 10 cm depths in each treatment. The IS1 soil core adjusted well to the controlled soil temperature at 10 cm depth, but the temperature was about 1°C lower at 5 cm than at 10 cm (Fig. 2d). The IS2 soil core showed similar thermal fluctuation to the IS1 core, but was about 2°C lower than the IS1 soil core during the day (Fig. 2e). The lower soil core temperature at 5 cm depth in the incubator was the result of evaporation resulting from the saturation deficit, which took the heat of vaporization from the soil surface (Nakano 1991).

The fluctuation in temperature at the 5 cm depth in the ES soil core was smaller than that of the controlled air temperature and larger than that of the controlled soil temperature (Fig. 2c). The amplitude was damped at the 10 cm depth. Under field conditions, both the highest and lowest soil temperatures in a day appear at the surface and the soil temperature becomes constant toward the deeper layers (Miyazaki 1993). Soil cores controlled by the Earthtron showed similar temperature dynamics to those under field conditions. At both soil depths, the cumulative temperatures were higher in the EA1 and EA2 soil cores than in cores of the other treatments (Table 2). The cumulative temperature at the 5 cm depth was higher than at the 10 cm depth in the ES soil cores, while the cumulative temperature at the 10 cm depth was higher than at 5 cm depth in the IS1 and IS2 cores. The loss of water from the soil core was also different among treatments (Table 2). Treatment ES showed the lowest water loss. The soil water in the EA1 and EA2 soil cores was considered to evaporate more as a result of the higher soil temperature, while the higher saturation deficit between soil and air would result in a higher water loss in the IS1 and IS2 soil cores. Thus, the dynamics of soil temperature and water changed typically according to the environmentally controlled conditions.

Dynamics of soil nutrients

The soil pH with every treatment increased slightly after incubation from 5.89 on day 0 (Table 2). There were few differences between the 5 and 10 cm depths and the soil pH with treatment ES seemed to be slightly higher than with the other treatments. The cumulative amounts of mineralized N at the end of incubation differed significantly among treatments (Table 2). The amounts of mineralized N with treatments EA1, EA2 and ES were larger than with treatments IS1 and IS2 at the 5 cm depth. There was no difference in concentrations of mineralized N between the 5 and 10 cm depths with treatment ES, while the other treatments showed higher concentrations at the 10 cm depth. The distribution pattern of mineralized N in the profile would be related to the dynamics of temperature and water in the soil. High cumulative temperature seemed to enhance N mineralization at each depth, but water flow in the soil would complicate the distribution in the profile. The soil solution was investigated on days 7 and 21. An increase in cations and NO_3 concentrations in soil solutions was observed on day 21 compared to day 7 for every treatment (Table 3). The concentrations with treatment ES were smaller than with treatments EA1 and EA2, but larger than with treatments IS1 and IS2. The concentrations of NO_3 showed similar patterns to the amount of mineralized N in the whole core. Variations in environmental conditions would affect the solubility of cations and anions as well as the distribution patterns of mineralized N in the profile. It was notable that dispersion of all data was suppressed with treatment ES, indicating accurate control by the Earthtron system.

Advantages of using the Earthtron

In the soil incubation experiment, the dynamics of temperature and moisture in the soil cores were changed by the surrounding temperature and humidity. The Earth-

Table 2 Mean rate of water loss per pot, cumulative temperature during incubation, soil pH and mineralized N after incubation (mean ± SD, $n = 6$) for each treatment

Treatment	Water loss (g/day per pot)	Cumulative temperature (°C)		Soil pH		Mineralized N (μg/g dry soil)	
		5 cm	10 cm	5 cm	10 cm	5 cm	10 cm
EA1	23.7	648	651	5.98 ± 0.02^{a}	5.92 ± 0.02^{a}	14.3 ± 2.2^{a}	20.5 ± 1.4^{a}
EA2	19.5	616	623	6.00 ± 0.02^{a}	5.96 ± 0.01^{ab}	12.9 ± 1.5^{a}	17.4 ± 1.3^{b}
ES	15.9	581	561	6.01 ± 0.01^{b}	5.99 ± 0.01^{b}	12.4 ± 0.7^{a}	13.1 ± 0.5^{c}
IS1	38.7	559	584	5.99 ± 0.02^{ab}	5.93 ± 0.03^{a}	8.0 ± 1.2^{b}	15.1 ± 2.6^{bc}
IS2	34.7	525	551	5.98 ± 0.01^{a}	5.94 ± 0.02^{a}	9.1 ± 1.0^{b}	14.9 ± 1.5^{bc}

Different letters in each column indicate that the values are statistically significantly different in the Sheffé test ($P < 0.05$)

Table 3 Cations and anions in soil solution extracted on days 7 and 21 of incubation (mean ± SD, $n = 6$) for each treatment

Treatment	Cations (ppm)				Anions (ppm)		
	Na	K	Mg	Ca	Cl	SO_4	NO_3
7 days incubation							
EA1	1.15 ± 0.07^{a}	11.4 ± 0.5^{a}	2.66 ± 0.16^{ab}	8.9 ± 1.2^{ab}	3.10 ± 0.16	2.25 ± 0.28	58.8 ± 3.8^{a}
EA2	1.16 ± 0.07^{a}	10.6 ± 0.4^{ab}	2.75 ± 0.30^{a}	12.1 ± 3.7^{a}	3.16 ± 0.13	2.73 ± 0.35	53.8 ± 3.8^{ab}
ES	1.02 ± 0.03^{b}	9.7 ± 0.3^{bc}	2.36 ± 0.16^{abc}	7.6 ± 0.5^{b}	3.19 ± 0.22	2.32 ± 0.27	50.3 ± 3.0^{bc}
IS1	0.99 ± 0.08^{b}	9.1 ± 0.6^{c}	2.18 ± 0.27^{c}	8.3 ± 2.5^{ab}	3.38 ± 0.29	2.58 ± 0.21	45.1 ± 5.5^{c}
IS2	0.99 ± 0.07^{b}	8.9 ± 0.6^{c}	2.26 ± 0.21^{bc}	8.8 ± 2.5^{ab}	3.21 ± 0.16	2.54 ± 0.46	44.7 ± 5.5^{c}
21 days incubation							
EA1	2.01 ± 0.14^{a}	18.2 ± 1.2^{a}	6.56 ± 0.69^{a}	19.7 ± 2.1^{ab}	3.30 ± 0.05	2.05 ± 0.11	120.2 ± 11.9^{a}
EA2	2.01 ± 0.10^{a}	17.2 ± 1.0^{a}	6.21 ± 0.54^{ab}	20.7 ± 2.9^{a}	3.36 ± 0.18	2.14 ± 0.30	112.5 ± 10.3^{ab}
ES	1.76 ± 0.02^{b}	15.0 ± 0.4^{b}	5.41 ± 0.15^{b}	16.0 ± 0.5^{bc}	2.86 ± 0.18	1.81 ± 0.07	97.3 ± 2.8^{b}
IS1	1.61 ± 0.10^{b}	13.1 ± 0.8^{c}	4.18 ± 0.44^{c}	12.6 ± 1.3^{c}	3.23 ± 0.71	2.35 ± 0.18	73.5 ± 8.2^{c}
IS2	1.60 ± 0.13^{b}	12.9 ± 1.0^{c}	4.22 ± 0.56^{c}	12.9 ± 1.7^{c}	3.17 ± 0.30	2.30 ± 0.36	73.5 ± 11.5^{c}

Different letters in each day and column indicate that the values are statistically significantly different in the Sheffé test ($P < 0.05$)

tron was able to construct the characteristics of soil temperature under field conditions by means of separate temperature control systems for above-ground and below-ground conditions. Furthermore, the distribution patterns of soil nutrients in the profile reflected the dynamics of soil temperature and moisture. Nutrient mineralization in the soil profile will dictate root response (Fujimaki et al. 2004), linking to the microbial activity in relation to root surfaces and rhizospheres (Coleman et al. 2004), and microbivorous nematodes (Papatheodorou et al. 2004). The distribution of soil temperature and moisture also affects the transition of soil invertebrates (Coleman et al. 2004). Such effects on microbe and soil invertebrates lead to changes in the decay rate of litter (Salamanca et al. 1998). Thus, accurate environmental control is necessary to measure and understand the interaction between above-ground and below-ground components in microcosm experiments.

In addition, the CO_2 concentration can be controlled in the Earthtron, providing an accurate estimation of plant growth in microcosm experiments. The TME (Knacker et al. 2004) described in the introduction did not take CO_2 concentration into consideration. Moreover, the Earthtron can be used to explore the effects of enhanced CO_2 and temperature on plant, soil biota and ecosystem dynamics, simulating global environmental changes. Microcosm experiments in the Earthtron provide not only a biologically realistic bridge between simple laboratory experiments and the complicated real world, but also allow prediction of the effects of global climatic changes on terrestrial ecosystems.

Acknowledgements We are grateful to T. Ueda, Hokkaido Dalton Co. Ltd., for technical advice about the Earthtron. This work was supported by the 21st Century COE Program "Bio-Eco Environmental Risk Management" of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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