Supplementary information

Ecotron-related facilities

Here we present a non-exhaustive list of Ecotron-related controlled environment facilities that paved the way to the development of current ecotrons (e.g. the phytotrons of the 1950s and 1960s, early sunlit growth chambers, the Closed Ecological Life Support Systems, the early ecotron-like facilities), as well as facilities currently under development for aquatic ecosystems research and for plant phenotyping.

Phytotrons*:* In the 1950s and 1960s, more than 30 large controlled environment laboratories were built worldwide to enable plant scientists to experimentally study the growth and development of (Downs R.J., 1980; Munns, 2017). The term "phytotron" was given as a nickname to the first of these large complexes of air-conditioned rooms for plant growth, the Earhart Plant Research Laboratory, built by Frits Went in Pasadena at CalTech in 1949, in reference to the cyclotron that had recently been built at the University of California in Berkeley (Galston & Sharkey, 1998). The term "ecotron" was coined in 1963 for an ecophysiology infrastructure project in Montpellier, led by Frode Eckardt on his return from visiting F. Went at CalTech, and the term was independently re-coined by John Lawton's group at Imperial College London in Silwood Park in the 1990s. Some of these early phytotrons are still in use (e.g. the Duke University https://biology.duke.edu/facilities/phytotron or the North Carolina State https://phytotron.ncsu.edu/ phytotrons), while others have been repurposed (e.g. the Canberra phytotron has been transformed into the High Resolution Plant Phenomics Centre, a component of the Australian Plant Phenomics Facility https://www.plantphenomics.org.au/contact/#canberra-csiro). The term phytotron currently encompasses both single climate-controlled growth chambers and infrastructures composed of many of these; they can bear specific names (Envirotron, Biotron, etc,). They are basic equipment of many biology laboratories in universities, research institutes and agro-companies. Early versions suffered from low light intensities and unrepresentative light spectra compared with the field conditions and the currently available LEDs and plasma lamps. A typical example of these early facilities is the Canterbury New Zealand Biotron, (https://bioprotection.org.nz/Facilities/new-zealand-biotron/?sti=1), a two-storey facility with six growth chambers on the top floor each of them coupled to (likewise air-conditioned) an additional room underneath that can accommodate large lysimeters with independent control of soil temperature. This PC2 certified facility can control a plant's growing environment above and below the ground in order to study how plants and pests interact under realistic field conditions. The environmental control is close to what is provided in ecotrons, but there are no ecosystem process measurements, in accordance to the more organism-centred (bioprotection) research programmes of its institution.

Most phytotrons are designed to host short vegetation. A counterexample is the "Dasotrons" (Finer *et al.*, 2001) where trees up to 3.7 m can be exposed to various soil and atmosphere conditions, from tropical to boreal. Four chambers can hold four lysimeters each. Sensors relate primarily to soil and root biology, but sap flow sensors are also available (https://www.luke.fi/en/natural-resources/forest/silviculture/research-on-root-systems/). Multi-year experiments can be run, and typical outside annual cycles can also be accelerated to simulate two growth periods per year.

Sunlit plant growth chambers: With the exception of very early example (Thomas & Hill, 1937), these facilities, with transparent canopy chambers inserted on *in situ* crops or soil containers, were developed by field physiologists starting in 1960s. Liu *et al.* (2000) reviewed 14 of them built in the US between 1961 and 1996; some were also built in other countries (e.g. France: Eckardt *et al.* 1971, Belgium: Nijs *et al.*, 1988)). They provide light intensity and variability close to natural conditions, although some light intensity reduction (often 15-20%) and spectral changes were inevitable. Several were designed to also track the natural variation of air temperature and relative humidity. Many of them, composed of only canopy chambers, were mobile and could be installed in the field, while others included a soil containment (bin, lysimeter). In the latter, contrary to ecotrons, the soil temperature was not controlled. The Soil Plant Atmosphere Research (SPAR) facility of the Beltsville Agricultural Centre (Phene *et al.*, 1989) is one of these facilities still running (https://www.ars.usda.gov/ARSUserFiles/12755100/faciliitiespamphlet-v4.pdf). Most studies conducted in the SPAR facility concern the response of potted crop plants (rather than whole ecosystems) to environmental variables, including atmospheric CO_2. Canopy physiological data are then used to feed crop models. Another such facility is the Corvallis Teracosms (Tingey *et al.*, 1996, http://www.teraglobalchange.org/research/mesocosm-design). The 12 chambers available in this facility include soil in buried, 1 m deep, containers and can measure many canopy and soil physiological parameters. They were used lately for a warming experiment on grassland species (Phillips *et al.*, 2016) after initial experiments on seedlings of Douglas fir (CO_2 x Temperature, e.g. Hobbie *et al.*, 2004) and Ponderosa pine (CO_2 x O_3, e.g. Lee *et al.*, 2009). Closed in 2015, this facility could be reopened through the foundation TERA Global (http://www.teraglobalchange.org/home). While the facilities above were designed to study plant or ecosystem responses to global changes, there are also sunlit facilities to more specifically study soil processes, and in particular, the response to biodiversity. The Nijmegen Rootlab-Phytotron (https://www.ru.nl/bgard/research-facilities/rootlab-phytotron/) for example, is a facility with 32 x 3 containers sheltered under a transparent polyethylene tunnel acting as a rain shelter, which receive irrigation from a computer controlled watering system. Soil sensors and soil access at different depths through mini-rhizotron tubes have allowed to study root × soil × biodiversity relationships for more than a decade on a variety of communities, sometimes in relation to global change (e.g. Padilla *et al.*, 2019).

Closed Ecological Life Support Systems: In the context of human space exploration, simulating the Earth's biosphere to provide life support in space led to the development of bioregenerative life support systems based on man-made ecological systems (Escobar & Nabity, 2017). Experimental facilities, some of them including humans in their closed modules, have been developed in Russia (Bios 1 to 3, Salisbury *et al.*, 1997), USA (https://biosphere2.org still in use for ecosystem research by Arizona State University), Europe (http://www.esa.int/Enabling_Support/Space_Engineering_Technology/Melissa), Japan (Nelson *et al.*, 2009), Canada (http://www.ces.uoguelph.ca/facility.shtml) and recently in China (Lunar Palace 1: https://www.space.com/40612-china-lunar-palace-1-mock-moon-mission.html). After pioneering work in the 90s (e.g. Lamotte *et al.*, 1999; Massimino & André, 1999), links with research in Controlled Environment Facilities have been tenuous, but may develop in the future, especially within ecotrons thanks to their ecosystem process measurement capacities.

Early Ecotrons: In the beginning of the 1990s, replicated facilities with the capacity to simultaneously control the environment and to measure ecosystem processes have been developed, especially by agronomists. The Wageningen Rhizolab (van de Geijn *et al.*, 1994) is a prominent example: with 16 units (1.6 m^2) it had many soil sensors (moisture, temperature, respiration, trace gases) and four of these units were equipped with canopy enclosures for control of atmospheric CO_2 and continuous measurements of photosynthesis, respiration and transpiration. This facility ran multi-year experiments on the impact of elevated CO_2 on crops. The merging of the Wageningen research institute and the University into Wageningen UR precipitated the closure of the facility. Although with less measurement capacities, ecologists also started to build similar facilities, with the University of Basel's 4 climate-controlled chambers being among the first ones (Körner & Arnone III, 1992). Built around the same time, the Silwood Park ecotron (Lawton *et al.*, 1993), with 16 climate-controlled chambers, played a pioneering role in the development of ecotrons for ecological research, especially by successfully initiating experiments on the relation between biodiversity and ecosystem functioning (Naeem *et al.*, 1994). A lack of funding for necessary technological improvements and a change in NERC funding rules lessened the attractivity of this facility and led to its closure in 2013.

Aquatic mesocosms: Aquatic mesocosms are experimental facilities enclosing large volumes (1-1000 m^3) of natural water in situ or on shore to measure the separate and interacting effects of multiple stressors on whole ecosystems over weeks to years. They offer a realistic method to test how future climate and pollution may impact both freshwater and marine aquatic ecosystems (Stewart *et al.*, 2013). Typical measurements in these mesocosms are profiles of temperature, dissolved oxygen concentration and saturation, conductivity, underwater light (PAR), pH, turbidity, Chl-a, flow rate, light spectrum and nitrogen. In Europe, the project Aquacosm (https://www.aquacosm.eu/) coordinates 19 facilities spread over 12 countries. The LakeLab in Germany (https://www.lake-lab.de/) developed 24 large enclosures (9 m diameter) going down to the lake Stechlin sediments at 20 m depth. Each enclosure has its own profiler with sensors as well as sediment traps. Experiments on night light pollution and lake connectivity are currently conducted. The Planaqua facility near Paris (http://www.cereep.ens.fr/spip.php?article45&lang=en) has a range of experimental systems: 16 artificial lakes (750 m^3 each), 12 freshwater tanks with wave beater (10 m long, 12 m^3) (Hulot *et al.*, 2017), standard mesocosms and environmentally controlled microcosms. Examples of current experiments are interaction between lake eutrophication and fish diversity and ecological divergences of a model fish. The Silwood Park Mesocosm facility https://www.imperial.ac.uk/silwood-park/research/silwood-lte/mesocosm/) with now 234 artificial ponds (2 m diameter) for warming and pollution experiments is one of the world's largest. Some other Aquacosms platforms are running experiments on shallow coastal waters, moored to a pier (Whal *et al. 2015*)or transportable by research vessel to any coastal waters (Mostajir *et al.*, 2013). Some facilities (e.g. the Solbergstrand Experimental Facility, https://www.umu.se/en/research/infrastructure/mesocosm-facility/) can work with sea, lake or river water. Six United States facilities are part of the worldwide network Mecocosm (http://mesocosm.eu/mesocosm-country/usa/) with the aquatic Research Facility at the University of Kansas running over 1000 ponds and tanks. (https://biosurvey.ku.edu/sites/biosurvey.ku.edu/files/docs/Field%20Station%20aquatic%20resources%20brochure.pdf).

Plant Phenotyping facilities: Phenotyping aims at linking phenotypic and genetic approaches to characterize and finally improve a wide range of crops. Plant phenotyping is the quantitative description of plant traits as a response to well-defined and monitored environmental conditions (ideally) by non-invasive measurements with a capacity to screen several hundreds to thousands of plants in a short time. Phenotyping activities have typically been conducted indoors in phenotyping installations (mostly greenhouses) but are now also moving to the field (Pieruschka & Schurr, 2019). This activity is developing strongly through coordination at national, continental and global level (see https://www.plant-phenotyping.org/). The development of deep phenotyping with lower throughput (tens to hundreds of plants) and more sophisticated measurements over shorter timescales (weeks) and time steps (minutes to hours) is underway and will bring closer the technologies of ecotrons and phenotyping facilities. One direction is to run the phenotyping measurements in an array of growth chambers (phytotrons) were different environmental conditions are simulated. Bao *et al.* (2019) describe the Enviratron developed and run by the Office of Biotechnology at Iowa State University. It consists of eight growth chambers and of a roving robot with a number of sensors which travels from chamber to chamber. The growth chambers have a plant growth compartment of 1.8 m² and are controlled for temperature (10 to 44 °C), air relative humidity (down to 40 %) and CO_2 (from 150 to 5000 ppm). Another approach was selected at the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany. They developed a unique plant cultivation hall with fully automated phenotyping systems including a so-called rhizotron-system, that allows tracking of shoot and root phenotypic features (https://www.ipk-gatersleben.de/en/phenotyping/organism/). On ca. 500 m², plants can be cultivated under field-like precisely adjustable environmental conditions allowing repeating experiments under highly reproducible conditions. Newly developing phenotyping facilities will further propel the combination of environmental simulation with phenotyping measurements, for example the Netherlands Plant Eco-phenotyping Centre (https://www.dtls.nl/2018/07/04/npec-will-a-tiny-country-lead-the-next-green-revolution/). A second, more advanced direction, is through the type of phenotype traits to be measured. So far, all phenotyping measurements refer to structural and spectral characteristic of plants, with the major exception of chlorophyll fluorescence. Additional physiological traits measurements are starting to be integrated into plant phenotyping measurements. For example, Jud *et al.*, (2018) have developed a plant phenotyping platform to measure the photosynthetic gas exchange and transpiration as well as the emission of volatile organic compounds (VOCs), under ambient or abiotic and biotic stress conditions. These measurements are conducted in the ExpoSCREEN facility described in our ecotron review, with some adaptations (https://www.helmholtz-muenchen.de/eus/facilities/voc-screen-platform/index.html).

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