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Ecotrons: powerful and versatile ecosystem analysers for ecology, agronomy and environmental science

Running head: **Ecotrons to analyse ecosystem functioning**

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Abstract

Ecosystems integrity and services are threatened by anthropogenic global changes. Mitigating and adapting to these changes requires knowledge of ecosystem functioning in the expected novel environments, informed in large part through experimentation and modelling.

This paper describes 13 advanced controlled environment facilities for experimental ecosystem studies, herein termed ecotrons, open to the international community. Ecotrons enable simulation of a wide range of natural environmental conditions in replicated and independent experimental units whilst simultaneously measuring various ecosystem processes.

This capacity to realistically control ecosystem environments is used to emulate a variety of climatic scenarios and soil conditions, in natural sunlight or through broad spectrum lighting. The use of large ecosystem samples, intact or reconstructed, minimises border effects and increases biological and physical complexity. Measurements of concentrations of greenhouse trace gases as well as their net exchange between the ecosystem and the atmosphere are performed in most ecotrons, often quasi continuously. The flow of matter is often tracked with the use of stable isotope tracers of carbon and other elements. Equipment is available for measurements of soil water status as well as root and canopy growth.

The experiments run so far emphasize the diversity of the hosted research. Half of them concern global changes, often with a manipulation of more than one driver. About a quarter deal with the impact of biodiversity loss on ecosystem functioning and one quarter with ecosystem or plant physiology.

We discuss how the methodology for environmental simulation and process measurements, especially in soil, can be improved and stress the need to establish stronger links with modelling in future projects. These developments will enable further improvements in mechanistic understanding and predictive capacity of ecotron research which will play, in complementarity with field experimentation and monitoring, a crucial role in exploring the ecosystem consequences of environmental changes.

Introduction

In the face of rapid climate change and biodiversity loss, the goods and services provided by ecosystems are under increasing threat (Scheffers *et al.*, 2016; Pecl *et al.*, 2017), and securing their future delivery is one of today's most pressing challenges (Wheeler & Von Braun, 2013; Challinor *et al.*, 2017; Arneth *et al.*, 2019; Díaz *et al.*, 2019). To do so, we need a better understanding of the fundamental processes underpinning ecosystem functions and services and of how these processes will be altered in novel environments of the future. This understanding will foster the development of mitigating management strategies through innovation and adaptation. Ecosystem science is developing at a fast rate, taking advantage of progress in other scientific disciplines (*e.g.* genomics, metabolomics, phenomics, spectronomics, etc.) and of development of new technologies (*e.g.* metabarcoding, new laser gas/isotope analysers, high resolution proximate and remote sensing, etc.). What is now expected from ecosystem science is a stronger adoption of interdisciplinary approaches connecting theory, experiments, field observations, modelling and simulation to address pressing questions on the future of ecosystems and societal welfare (Mauser *et al.*, 2013; Hanson & Walker, 2020) and the complexity of biodiversity-ecosystem feedbacks (Abiven *et al.*, 2017).

In this context, controlled environment facilities (CEFs), such as growth chambers and advanced greenhouses, have become standard tools to simulate different environmental conditions and disentangle their influences on ecosystem functioning. These have been used for example to reveal the underlying mechanisms of observed overall responses, for model parametrization and for theory testing (Kreyling *et al.*, 2014; Clobert *et al.*, 2018; Hanson & Walker, 2020). CEFs have been steadily improved through the use of better lighting systems, the regulation of additional parameters, such as CO_2 and ozone, user-friendly computerized environmental control and the possibility of remote operation and security checks. During the last three decades, however, a more innovative step forward has been achieved through the development of a more heavily instrumented type of CEF: herein termed ecotrons. We define an ecotron as an experimental facility comprising a set of replicated enclosures designed to host ecosystems samples, enabling realistic simulations of above- and belowground environmental conditions, while simultaneously and automatically measuring ecosystem processes. Therefore, ecotrons provide continuous information on ecosystem functioning (fluxes of energy and matter).

This principle of using enclosures (a lysimeter for the soil and an aerial compartment around the canopy) for simultaneous environmental control and process measurement has been pioneered, at the canopy level, by field physiologists as early as the 1930s (Thomas & Hill, 1937), but most of these sunlit facilities were developed from the 1960s (Liu *et al.*, 2000). Starting with the München ExpoSCREEN (Payer *et al.*, 1986), the Imperial College ecotron in Silwood Park (Lawton, 1993) and the Desert Research Institute EcoCELLs in Reno (Griffin *et al.*, 1996), larger permanent infrastructures, open to national and international collaboration, were constructed. Four ecotrons were built between 1985 and 2006, eight between 2010 and 2020 and two more are under construction. A more thorough historical background with the etymology of the word ecotron is provided in the supplementary information file 'Ecotron-related facilities'. These ecotrons can be seen as a new means of performing ecological research through centralized, shared and heavily instrumented research facilities mirroring practices in other disciplines such as astronomy and physics (Granjou & Walker, 2016; Rineau *et al.*, 2019).

This paper reviews the characteristics of existing ecotrons (or ecotrons under construction), focusing on their environmental control capacities and the design and technology underpinning ecosystem process measurements. Since most of them are open to national and international collaboration, we also outline the advantages and prospects of using the listed ecotrons. An analysis of the experiments conducted so far reveals the large range of research topics that can be addressed in these infrastructures, but we also address their limitations, emphasizing the necessary complementarity between ecotrons and other experimental or observational facilities for the pursuit of predicting, mitigating and adapting to ongoing global environmental changes. Finally, we discuss the perspectives on the future development of ecotrons and the need to combine their experiments with modelling efforts.

The features of ecotrons

Advantages of ecotrons are increasingly acknowledged by the scientific community and funding bodies, as indicated by the growing investment of research institutes and universities in such new facilities. However, part of the scientific community is not up to date with the more recently developed features and is not fully aware of the advantages and trade-offs of ecotron experiments relative to greenhouse or field experimental approaches. Throughout this section, we discuss the features of ecotrons showing that in addition to developing specific technologies, their strength is to cumulate many of the advantages found in some of these other experimental facilities.

More realistic experiments, across a broad range of environmental conditions

Ecosystem experiments can be conducted in settings that vary in realism, environmental control, and replication of experimental units, along previously described trade-offs (Diaz *et al.*, 2003; Stewart *et al.*, 2013; De Boeck *et al.*, 2015). Here we define realism as providing conditions as close as possible to the complexity of natural environments, whether in the past, present or predicted future. This implies the capability to simulate natural ranges, dynamics and combinations of abiotic and biotic variables. Especially in global change research, realism also includes the need to impose experimental treatments going beyond the historical record (Hanson & Walker, 2020), and even beyond the current model-predicted climate change scenarios (De Boeck *et al.*, 2020).

Generally, confined ecosystems have a reduced spatial and biological complexity, are surrounded by walls and have modified physics (*e.g.* energy exchange), compared with natural ecosystems. Ecotrons deal with these issues via a series of features that set them apart from typical growth chambers and which render the experimental conditions closer to field conditions. One such feature is using large ecosystem samples hosted in large enclosed atmospheric volumes (see details on the features in the section 'Characteristics of current ecotrons'), thus incorporating more above- and belowground biological complexity and spatial heterogeneity. Whenever possible and suitable, intact soil monoliths are extracted in situ and inserted in the ecotron enclosures, thus preserving soil physico-chemical properties, soil biota and vegetation. Getting closer to realistic outdoor conditions is important, as there is accumulating evidence that the use of small and simplified systems such as pots or small containers brings identified biases (Poorter *et al.*, 2012) or unidentified lab-specific artefacts (*e.g.* Massonnet *et al.*, 2010; Milcu *et al.*, 2018) that may generate results with less external validity (*i.e.* results which can be generalized with less confidence) (Poorter *et al.*, 2016).

Another feature that improves experimental realism is the capacity of most ecotrons to simulate a wider range of environmental parameters than is usually the case in growth chambers, or in some of the earlier ecotrons (*e.g.* Silwood Park ecotron, Lawton *et al.*, 1993). The following conditions can be reached, although not in all facilities (details in the section referred to above): freezing or near-freezing air temperatures maybe achieved through use of refrigeration, such as compressed gas expansion within the air circuit; very low air relative humidity achieved by injecting dry air; replicating the in situ soil matrix potential at the bottom of the soil column that affects evapotranspiration (Groh *et al.*, 2016) ; and replicating the in situ soil temperature gradient that affects soil respiration as well as plant growth (Füllner *et al.*, 2012). To improve realism, some ecotrons take advantage of natural sunlight, whilst only reducing light intensity to a minor extent including in the UV range, while others combine light-emitting diodes (LED) or a mixture of metal halide/quartz halogen lamps and fluorescent tubes (as cited in Ghirardo *et al.*, 2020) to achieve a radiation spectrum approaching that of the sun. Taken together, these features considerably step up the realism of environmental control and allow for the simulation of past or future environmental conditions and climatic extremes with improved accuracy and precision. As a result of the realism of the simulated environmental conditions combined with the incorporation of more above- and belowground complexity, ecotron experiments are often much closer to field experiments than most typical CEF experiments.

Disentangling ecosystem mechanisms through confinement and replication

The ecotrons' capacity to independently manipulate biotic as well as abiotic variables comes with many advantages, the most important one being the disentanglement of the ecological effects of variables that often co-vary in natural settings. For example, drought and co-varying factors such as temperature, vapour pressure deficit and sunlight (De Boeck & Verbeeck, 2011), or atmospheric CO_2 and its effect on leaf and canopy temperature (Leuzinger & Körner, 2007), can be independently controlled and their impacts untwined. Similarly, treatments that manipulate soil biota presence and diversity can be relatively easily established in ecotrons, a manipulation challenging in the field without disturbing the ecosystem and risking contamination from the surroundings. Combining soil sterilization techniques and subsequent inoculation of specific species or groups of species, ecotrons are a powerful tools for exploring the effects of specific biota on ecosystems (Bradford *et al.*, 2002). Another overlooked feature of ecotrons is their inherent capacity to incorporate environment-biotic feedbacks as well as the possibility to impose feedbacks as experimental treatments. For the first aspect, we emphasize the fact that the large size of the experimental systems will inherently incorporate more of the natural biological diversity of the model system. Hence, more key taxa that will be present and will realistically respond to the experimental treatments and feedback on soil properties or other taxa, a response likely less to occur with smaller pot-size systems. For the second aspect, while some of these feedbacks can be performed in the field and in classical CEFs (*e.g.* plant–soil biota feedbacks; Van der Putten *et al.*, 2013), other feedbacks require greater control over the environmental variables. For example, using materially closed systems in the former Silwood Park ecotron, Milcu et al. 2012 established CO_2-temperature feedback treatments in a simplified physical model of the terrestrial C cycle. The temperature of the experimental systems was continuously adjusted depending on the emerging CO_2 concentration of the units (using the most likely CO_2-temperature sensitivity) resulting from the combination of simulated anthropogenic emissions, photosynthesis and plant and soil respiration.

Disentangling the ecological effects of different variables requires multiple identical and independently controlled experimental units. Lack of replication at the unit level can lead to biased parameter estimates (Porter *et al.*, 2015), because any confounding chamber effect is not taken into account (Potvin & Tardif, 1988). Given that the high construction cost can limit the number of ecotron experimental units, several facilities opted for a minimum of 12 units, since it allows the establishment of treatments with 6, 4 and 3 replicates per treatment combination for experimental designs with 2, 3 and 4 treatment combinations, respectively. Such a series of experimental units also suits gradient experiments with many different, un-replicated treatment levels, to which a regression-type analysis is applied instead of an analysis of variance requiring replication. Gradient experiment is an underused methodology appropriate for identifying thresholds, tipping points and response functions (Kreyling *et al.*, 2018).

A major focus on measurements of ecosystem processes

Next to the capacity to simulate and measure multiple environmental conditions, the most compelling characteristic of ecotrons is their focus on non-destructive, automatic, real-time measurements of ecosystem-level processes. Some ecotrons are specifically designed to use the confinement of the ecosystem as large gas exchange chambers (*e.g.* Barton *et al.*, 2010; Milcu *et al.*, 2016a), analogous to leaf chambers in portable photosynthesis systems. This allows the measurement of the net ecosystem exchange between the terrestrial compartment and the atmosphere for various molecules (CO_2, N_2O, CH_4, H_2O, O_3, NO_x, VOCs) by using either a static non-steady state or a dynamic (flow-through) steady state approach. Both approaches are feasible but they require that one single ecosystem is hosted within each chamber, in stark contrast with CEFs where different model systems (set-ups in pots or containers) are incubated side by side. These net ecosystem exchange rates are measured at high frequency (every 10 to 20 min), capturing both the short-term and the cumulative long-term responses of elemental budgets with a high degree of confidence (*e.g.* Roy *et al.*, 2016). Ecotrons offer the possibility to balance all fluxes: the inflow, stock and outflow can be precisely quantified for most components of the soil/plant/atmosphere system, including energy. Furthermore, the recent availability of multi-gas and multi-isotopologue laser analysers enables simultaneous measurements of the molecules listed above, as well as their isotopologues (*e.g.* McManus *et al.*, 2015; Braden-Behrens *et al.*, 2017; Braendholt *et al.*, 2019). Examples of processes estimated by the measurements of isotopic fractionation and isotopomers include canopy conductance and respiration as well as the coupling of CO_2 and H_2O cycles via $\delta^{13}C$ and $\delta^{18}O$ of CO_2 (Harwood *et al.*, 1999), nitrification and denitrification processes via $\delta^{15}N$, $\delta^{18}O$ and isotopomers of ^{15}N, *i.e.* the ^{15}N site preference (SP) in N_2O (Baggs, 2008; Butterbach-Bahl *et al.*, 2013), and tracing ecosystem water fluxes and disentangling evapotranspiration via $\delta^{2}H$, $\delta^{18}O$ of H_2O (liquid and vapour) (Oerter & Bowen, 2017).

Other ecotrons focus on automatic measurements of ecosystem properties that are not related to ecosystem gas exchange. These include root growth using minirhizotrons (Möller *et al.*, 2019), invertebrate and plant community composition using novel imaging techniques like computerized trap systems, video cameras or radio frequency identification (Dell *et al.*, 2014; Dombos *et al.*, 2017), thermography to analyse the heterogeneity of transpiration, and hyperspectral reflectance for canopy biomass and chemical content (Tan *et al.*, 2018; Xie *et al.*, 2020). Similar to what is done in other experimental settings, in all ecotron experiments the automatic measurements are complemented by low-frequency samplings of soil, plants, soil solution, leachate, etc., for further analysis of fauna and microbe diversity, elemental and isotopic composition of plant material, delivering a more complete understanding of the impact of the experimental treatments. These complementary analyses are generally performed by the hosted teams and are often not the responsibility of the ecotron facility.

Experimental flexibility

While generally costly to build, run and maintain, ecotrons offer significant experimental flexibility. First, they can host many different types of treatments (climate, atmospheric composition and pressure, pollution, soil types, trophic levels, biodiversity within trophic levels, ecosystem management, etc.). For some of these treatments which are outside of the range of current environmental conditions, specific regulations have been installed, for example decreased CO_2 in air using scrubbing molecular sieves to reach pre-industrial CO_2 concentrations; ozone fumigation produced by an ozone generator plugged on pure oxygen gas bottles; low oxygen concentration through dilution with nitrogen and simultaneous readjustment of CO_2 concentration. Furthermore, unlike field facilities which are bound to a specific ecosystem, in ecotrons the targeted model system can change from one experiment to the other, ranging from agricultural systems to grasslands, peatlands, shrublands, and regenerating forest (saplings), essentially any ecosystem type where plant stature fits the height of the units. However, the flexibility to host various ecosystem types in consecutive experiments often trades off against their duration. One solution is to bring ecosystem samples extracted from long-term field experiments to the ecotron for short-term, more thorough, physiological measurements (*i.e.* use the ecotron as an ecosystem analyser, *e.g.* Milcu *et al.*, 2014) or for applying complementary treatments. Ecotrons are often also flexible in terms of dimensions. The size of the lysimeters and the height of the canopy enclosures can often be tuned to the particular experiment. In some ecotrons the main experimental unit can be divided into subunits in order to vary their connectivity, thus allowing the study of spatial and meta-population dynamics (Eisenhauer & Türke, 2018). Some ecotron platforms can work with either sunlight or artificial light (*e.g.* Resco de Dios *et al.*, 2016). Another element of flexibility is the option for hosted research teams to temporarily install supplementary costly instruments in the ecotron air circuits, such as VOC or NO_x analysers in order to bring added value by answering additional questions. The length of the experiments carried out in ecotrons so far is variable as it depends on the addressed scientific question. It can be relatively short (4 months), especially when samples from long term field experiments are used (see section complementarity between ecotron and field experiments) or can last up to 3 years. The average length of experiments run so far is 1 year.

Open access to the infrastructure

The physical sciences traditionally share their state of the art, large and costly infrastructures with hundreds of scientists from all over the world. Since ecotrons are also costly to build and run, albeit to a lesser scale, the experiments often include international teams assembled in large consortia. This arrangement facilitates the interaction among scientists with the complementary expertise needed to perform interdisciplinary research projects, for example, plant and animal ecologists, hydrologists, microbiologists, chemists, modellers, data scientists etc. Therefore, most ecotrons work with open access calls. In most cases, ecotron experiments do not require a permanent presence of personnel from the external teams running an experiment: most of the environmental controls and key process measurements are automated, and the ecotron personnel regularly check the proper functioning of the instruments. Moreover, data are accessible in quasi real-time via dedicated web interfaces. The external team is mainly involved in setting up the experiment and specific measurement campaigns. The most ambitious experiments, however, rely on a dedicated post-doc and/or technician located at the ecotron.

Although open to private companies, most ecotrons are primarily running projects involving researchers from universities and/or research institutes and supported by public funding. The facilities charters may impose an open access data policy after a short embargo period. This access is often organised at the national level (nodes coordinating sets of ecosystem experimental facilities) or international level (*e.g.* the European ESFRI infrastructure AnaEE – Analysis and Experimentation on Ecosystems – www.anaee.com, which includes several ecotrons). In Europe, the Cluster of Environmental Research Infrastructures (ENVRI) is developing a project (https://envri.eu/home-envri-fair) to feed the data from its constituent infrastructures (AnaEE and others) to the European Open Science Cloud (EOSC).

Choosing between running experiments in CEF, field or ecotron

In the context of the former Silwood Park ecotron, J. Lawton (1995) argued that 'model laboratory systems (real organisms interacting in the laboratory) are a halfway house between mathematical models and the full complexity of the field'. Current ecotron facilities, through their improved realism, are much closer to experimental field conditions. However, a comparison among experimental systems should include multiple criteria to inform choice of the facility best fit for the purpose: hypotheses that require environmental conditions technically difficult to achieve in the field and/or intensive process measurements would be better tested in ecotron experiments, as long as the number of drivers remains low. Hypotheses testing that requires a high level of realism and a large number of treatments or replicates, would be best done in field experiments, at least when the application of treatments does not demand expensive technology. CEF experiments appear to have considerably more limitations, but are certainly needed for rapid preliminary trials or when field or ecotron experiments are not available or are too costly to run.

The characteristics of current ecotrons

We identified 13 facilities having the defining features of ecotrons, including 11 facilities in Europe, 1 in North America and 1 in Australia (Table 1, Figure 1). Two were operational since the 80s and 90s, but most of them opened between 2010 and 2020, and two additional ones are currently being built. The supplementary information file 'Ecotrons description' describes each facility, with website links, pictures, contextual information, specific technical details, contacts for collaboration and a short list of key publications. Another supplementary information file ('Ecotron-related facilities'), some CEFs are described which paved the way for the development of current ecotrons (the phytotrons of the 1950s and 1960s, the sunlit growth chambers, the Closed Ecological Life Support Systems, the early ecotrons) or facilities which are now being developed for aquatic ecosystem research and plant phenotyping.

(APPROXIMATIVE POSITION OF FIGURE 1)

Design differences among ecotrons reflect the scientific and strategic objectives of the funding organizations. The average construction cost of the most recent (2010 and after) ecotron platforms is 6 M€ with a large range of variation (3 – 10 M€) revealing differences in the number or size of the experimental units or in their control and measurement capacities. For a given amount of available funds, there are unavoidable trade-offs among i) the number of controlled environmental parameters (with light quality and isotopes being the most complex/expensive ones to control), ii) the number of processes measured in real-time by automated systems (with soil respiration, trace gas emission, isotope fractionation and faunal activity being the most complex/expensive ones to measure), iii) the number of replicated units and iv) the size of these units The ecotrons planning phase took 2.5 years in average and the building phase (including tests) 3.5 years. Average annual running costs in 2019 were ≈140 k€ (with 80 k€ for the maintenance and small improvements and 60 k€ for the consumables of which electricity, with a consumption around 600 MWh, partly green, constitutes the major part). Personnel annual cost averaged ≈160 k€ (for an average 2 full-time equivalent).

(APPROXIMATIVE POSITION OF TABLE 1)

Controlled environmental parameters

(APPROXIMATIVE POSITION OF TABLE 2)

The environmental parameters controlled in these ecotrons are summarized in Table 2. Among the climatic conditions, air temperature and relative humidity are regulated, as in most CEFs, with low temperature (below 5°C) being achievable in almost all facilities, and freezing temperature and air humidity below 30% attainable in 9 out of 13 facilities, thus allowing the simulation of winter climatic conditions of most temperate regions when needed. Since light intensity and quality have been a major concern regarding the external validity of CEFs, these parameters have been given high priority in ecotron design. Hight light levels are achieved in the sunlit ecotrons, thanks to high overall transmissivity (≈ 0.9 on average) of the canopy enclosures. This transmissivity, calculated over 24h with sensors inside and outside the enclosures, is very high compared to glasshouses. This is due to a high transmissivity of the covering material, a low inter-cell shading, a dome like shape and very light supporting structures. In facilities with artificial light, when the ground-lamp distance is adjustable, the photosynthetic photon flux density (PPFD) averaged across ecotrons is 1100 µmol m^{-2} s^{-1} at 50 cm from the light sources. Otherwise, when the distance is not adjustable, the PPFD range across ecotrons is 340 – 1300 µmol m^{-2} s^{-1} at 50 cm above the ground level. Maximum PPFD outside can be significantly higher on clear summer days, but while PPFD is the most relevant variable for instantaneous photosynthesis, many plant traits at higher levels of integration are better related to the daily light integral (DLI), the PPFD integrated over a day (Poorter *et al.*, 2019 and references therein). In June the highest average DLI is 45 mol m^{-2} day^{-1} at a latitude of 40°N (Poorter *et al.*, 2019). This can be obtained in ecotrons with only 11.5 hours of constant light at a PPFD of 1100 µmol m^{-2} s^{-1}. In addition, all ecotrons running with artificial lights have dimmable lamps or step switching of the lamps to simulate typical daily light courses. Although Poorter et al. (2016) emphasized the impossibility to reach, in growth chambers, the high photothermal ratio found outside in spring at most latitudes (high light at low temperatures), photothermal ratios were found to match these field values both in a sunlit ecotron (Montpellier Macrocosms) and in an artificially lit ecotron (Gembloux Terra ecotron), for example on April days with a DLI >20.

Although rarely documented, light homogeneity across the horizontal plane is often also of concern in CEFs due to lamp positions or light interception by greenhouses structures. In the listed ecotrons, the variation coefficient of PPFD is typically below 10%. With artificial light, the vertical extinction profile of light in the canopy is much stronger than in nature due to the quadratic loss of light with increasing distance from the lamps. In tall canopies, supplemental vertical strings of LEDs can compensate for this. Surrounding the sides of the canopy with a vertical shading cloth of adjustable height and transmissivity is recommended to prevent light and turbulent air from entering the canopy sideways. Aboveground edge effects can be minimised that way, but belowground edge effects are unavoidable and are only lessened by the large area to circumferences of typical ecotron lysimeters. Light quality, which also strongly impacts the external validity of CEF studies, is considerably improved in some artificially lit ecotrons by using LED arrays providing a continuous light spectrum close to the solar spectrum with most of the physiologically active wavelength in adequate proportions (although UVB is often still missing). Given the increasing recognition of the importance of UV radiation (Ulm & Jenkins, 2015; Verdaguer *et al.*, 2017), in addition to the red : far red ratio, for the growth and development of plants (Galvão & Fankhauser, 2015) and for some trophic interactions (*e.g.* Moreno *et al.*, 2009), these light quality parameters are reported in Table 2. The average proportion of UV in the UV + PPFD spectrum, calculated from data in µmol m^{-2} s^{-1}, is 3.8% in the ecotrons with artificial light (range 0.3 – 8.6) compared with 6.6% in the standard AM1.5 solar spectrum, and the red: far red ratio is on average 1.7 (range 1 – 3.5) compared to 1.1 in the AM1.5 solar spectrum.

Despite relatively high air internal recirculation in most ecotrons (often 2 to 3 cell volumes per min), air speed at the canopy level is generally below 2 m s^{-1}. Such values are common over short statured vegetation such as agricultural crops or tree nurseries (mostly between 0.5 and 1 m s^{-1}, and rarely exceeding 3 m s^{-1}, Day & Parkinson, 1979; Barnard & Bauerle, 2016), which are the vegetation types typically studied in ecotrons.

Air CO_2 concentration is nowadays controlled in most CEFs and in all the ecotrons, except one. This control is important not only for studies simulating future CO_2 concentrations but also to prevent variation in daytime CO_2 concentration when plant photosynthesis is active (Bernier *et al.*, 1994; Romer, 2001). The one ecotron lacking routine control of air CO_2 concentration overcomes this by a high external air flux to cell air volume ratio. Pre-industrial CO_2 concentrations can be simulated in half the ecotrons by scrubbing the incoming air with a CO_2 removal system. This opens the opportunity to label the organic matter synthesized during the whole experiment by continuously injecting CO_2 with a specific delta ^{13}C signature (depleted or enriched). Ozone concentration is controlled in three ecotrons while NO_x is controlled in a single ecotron.

Due to the high content of dissolved nitrogen often found in tap water, all ecotrons, except 3, are using deionised water to simulate rainfall. Two of them have the capacity to add specific ions to the deionised water and two can alternatively use stored rainwater. Dew is generally not observed on the ecotron canopies. Snow cannot be generated within ecotrons, but can be brought in from outside.

In-house measured processes in standard operation mode

(APPROXIMATIVE POSITION OF TABLE 3)

The in-house measured processes in the ecotrons are summarized in Table 3. Overall, 18 different ecosystem processes are assessed, with an average of ten per ecotron among which five as routine high-frequency measurements. Seven of the 18 processes refer to emitted or absorbed gases at the ecosystem scale. Evaporated and transpired water as well as photosynthesized and respired CO_2 are key in understanding and measuring primary productivity. Emphasis is on the three main greenhouse gases (CO_2, CH_4, N_2O) with six ecotrons capable of measuring their fluxes, enabling calculation of metrics for the global warming potential of ecosystems (Neubauer & Megonigal, 2015). Measurements of the stable isotopes in the CO_2, H_2O and N_2O molecules are being developed. Routine or on-demand measurements of $\delta^{13}C$ and $\delta^{18}O$ of CO_2 (providing information on canopy conductance, respiration, and coupling of cycles) are possible in six and four of the ecotrons, respectively. Measurements of $\delta^{15}N$, $\delta^{18}O$ and the intramolecular site preference (isopotomers) of ^{15}N in N_2O (providing information on nitrification and denitrification) can be done in four ecotrons, and measurements of $\delta^{2}H$, $\delta^{18}O$ of H_2O (providing information on the water cycle) in two ecotrons.

Drainage fluxes and soil solution sampling are provided routinely or on demand in most ecotrons. Minirhizotrons are available in half of the ecotrons, but the root images are usually not analysed by the facility personnel. LAI meters are generally available, and one ecotron is equipped with an automatic stereoscopic measurement of LAI. Canopy temperature infrared measurement can be done in half the ecotrons (two automated at high frequency). Hyperspectral reflectance measurements are being developed for non-destructive measurement of chemical contents and biomass of canopies. Equipment for such measurements is available in half the ecotrons. Fauna activity is analysed automatically in only one ecotron through cameras and radio frequency identification, in addition to a computerized trap system for the activity of soil microarthropods.

Track record of ecotrons

Environmental control and process measurements

Examples of environmental controls achieved in ecotrons are shown in Figure 2. The sunlit ecotron of Hasselt demonstrates the capacity to track the rapidly fluctuating field conditions at the nearby ICOS station. The Gembloux ecotron, operating with artificial (LED) lights, shows the capacity to simulate ranges of light and temperature derived from a model using minimum and maximum temperatures and total solar insolation from a weather station, combined with astronomical and heat transfer data.

(APPROXIMATIVE POSITION OF FIGURE 2)

To exemplify the capabilities in terms of isotopic labelling and process measurements, we show the successive measurements in a single experimental unit while 12 units (Montpellier) or 16 units (München) are labelled or measured simultaneously with single analysers multiplexed across these units (Figure 3).

(APPROXIMATIVE POSITION OF FIGURE 3)

Scientific achievements

A cluster analysis of the published ecotron papers based on the applied experimental treatments reveals three main categories: investigating ecosystem response to abiotic global change drivers (‘novel environments’ experiments *sensu* Hanson & Walker, 2020) (55% of the papers), deepening our understanding of ecosystem processes (27%), and understanding biodiversity–ecosystem functioning relationships (18%) (Figure 4a). Since more than half of the ecotrons presented in the current paper have opened too recently to have published results, we included the experimental results papers from the Silwood Park ecotron to document more broadly the research areas. The Silwood Park data represent 27 of a total of 126 papers analysed in Figure 4a. In addition, we also analysed in the same way the running or recently completed, but not yet published, experiments in the newly opened ecotrons (Figure 4b).

(APPROXIMATIVE POSITION OF FIGURE 4)

Half of the papers on novel environments analyses the interactions between at least two environmental drivers. CO_2 and temperature were the most studied drivers (61%and 42% of 69 papers, respectively). Among the papers on ecosystem process understanding, ecotoxicology is well represented with several papers on mercury circulation in ecosystems published by one ecotron. Plant physiology and elemental cycles are also well represented. Most experiments addressing the role of biodiversity in ecosystem functioning were conducted using grassland ecosystems. Interestingly, manipulations of soil fauna and multi-trophic systems are as represented as plant manipulations. The clustering of the newly completed or running projects in the recently open ecotrons shows an increase in biodiversity experiments, especially related to fauna, as a result of the opening of the German ecotrons. Novel environment experiments are proportionally less studied in these recent ecotrons. The list of published papers and recent projects can be found in the Supplementary file ‘Ecotron published papers and recent projects’.

Below we describe selected experiments showing how the environmental control and process measurement capacities in ecotrons led to remarkable findings in each of the three main scientific areas of Figure 4.

Global change experiments:

Drake *et al.* (2018) studied how an extreme heat wave affects the physiological performance of forests. They grew whole *Eucalyptus parramattensis* trees (6 m tall) in a field setting with the Richmond Whole Tree Chambers (WTC), and crossed the heatwave with a warming treatment. The WTC controlled T_{air}, vapour pressure deficit and CO_2 concentration while measuring net CO_2 and H_2O exchange of the entire canopy every 15 min. Additional measurements were leaf temperature, fluorescence and water potential, and a leaf-level photosynthetic model was used. The heatwave reduced canopy photosynthesis more strongly than transpiration, which maintained canopy cooling. This decoupling is not captured in the standard photosynthetic models and consequently is not considered in climate models, overestimating the negative impact of heatwaves. This result, as well as an observed increase of leaf thermal tolerance during the heatwave, was identical in both the ambient and warmed treatments. Using similar high frequency ecosystem gas exchange measurements, Roy *et al.* (2016) showed in the Montpellier ecotron Macrocosms that elevated CO_2 buffered the impact of an extreme drought and heat on intact grassland monoliths, mostly owing to very strong recovery in autumn under this treatment. Also using continuous measurements of CO_2 net ecosystem exchange (NEE), Arnone III *et al.* (2008) demonstrated in the Reno EcoCells that the reduction of CO_2 uptake in intact tallgrass prairie monoliths by an anomalously warm year was carried over to the next year because soil biota respiration was stimulated. Using real-time measurements of NEE and volatile organic compounds (VOCs), Ghirardo *et al.* (2020) showed in the München ExpoSCREEN that global warming decreases carbon sequestration in subarctic tundra ecosystems via reducing NEE and increasing VOC emissions. The use of $^{13}CO_2$-labeling experiments further allowed coupling the atmospheric carbon dioxide to VOC biosynthesis and emissions.

Biodiversity–ecosystem functioning experiments:

Recent findings of an nearly 75% decline in flying insect biomass over the past 27 years (Hallmann *et al.*, 2017) motivated the investigation of the repercussions of such a decline for ecosystem processes. Using the 24 experimental EcoUnits of the Leipzig iDiv Ecotron, nine projects investigated how the decline in invertebrate biomass in grassland ecosystems affects the biotic interactions between aboveground (insects, plants, bacteria) and belowground organisms and thus the associated ecosystem functions and services. Artificial grassland communities consisting of 12 central European species were established in the EcoUnits. To mimic invertebrates decline, live aboveground invertebrates were introduced at two abundance levels (100% and 25%) in 8 EcoUnits each, while another 8 EcoUnits received no fauna. The invertebrate treatment had a large impact on the extent of an accidental aphid infestation which occurred in all 24 EcoUnits. The strongest infestation occurred in the EcoUnits without additional invertebrates and the weakest in the 100%-invertebrate EcoUnits, underlining the importance of natural pest control by predators. Invertebrate densities also shifted plant species abundances and phenology. For example, the dominant species in the grassland community, *Trifolium pratense*, declined in abundance with invertebrates present (Ulrich *et al.*, 2020).

Process understanding experiments:

In the Macrocosms platform of the Montpellier Ecotron, the effect of circadian rhythm on the diurnal gas exchange of leaves and canopies was investigated (Resco de Dios *et al.*, 2017). Such an effect is studied by maintaining all environmental parameters constant (with light or in the dark) after a few days of 'entrainment' during which light, temperature and water vapour pressure deficit follow typical outdoor conditions while gas exchange is measured throughout every 12 min. The sunlit macrocosms were planted with either bean or cotton. After one month of growth, a completely opaque cover was fitted on each macrocosm dome and light was then controlled by dimmable plasma lamps. Under these field-like conditions, circadian regulation was observed to exert control over net CO_2 exchange that was of similar magnitude to the controls exerted by direct physiological responses to temperature and vapour pressure deficit (Resco de Dios *et al.*, 2016, 2017). Circadian rhythm also induced contrasting changes in the photosynthetic pigments and photochemical efficiency in bean vs. cotton, calling into question the extrapolation of the response of model plants to other species (García-Plazaola *et al.*, 2017). Night-time dark respiration showed a circadian oscillation at both leaf and canopy level, but light-enhanced dark respiration was under circadian control only in cotton, suggesting that circadian controls may help explain temporal variability in ecosystem respiration (Gessler *et al.*, 2017).

Complementarity between ecotron experiments, field experiments and in-natura observations

The earlier-mentioned fundamental trade-off between internal and external validity dictates that no single approach can span the entire validity gradient (De Boeck *et al.*, 2015). Ecotrons can elucidate mechanisms underpinning responses to an array of potential changes in the environment, yet their inherent limitations (small spatial scale, island effect, reduced biological complexity, low number of replicates, etc.) warrant that outcomes of ecotron studies be considered in conjunction with results from other approaches.

Some ecosystems do not allow to take representative samples that could be transferred to ecotrons. This is the case for mature forests, one of the most critical ecosystems with regards to the regulation of the global climate (carbon sequestration, water cycle) and the preservation of the Earth's biodiversity. To study mature forest ecosystems, in-situ experimental platforms exist where certain features of the environment are changed, for example through FACE systems (Norby *et al.*, 2016), through the use of extended rainout shelters above (Misson *et al.*, 2011) or below (Hoover *et al.*, 2018) the canopy or through natural phenomena, such as in the FORHOT study in Iceland, where seismic activity is warming the soil under an existing Sitka spruce forest (Sigurdsson *et al.*, 2016). Furthermore, natural fluctuations in ambient weather (warm years, dry summers, etc.) enable the use of data from observational studies, including the vast array of eddy flux covariance towers (*e.g.* Schwalm *et al.*, 2010). Ecotrons studies can complement studies of the early stages of forest growth and in particular determine how trees respond to interactive factors (CO_2 and drought *e.g.* Crous *et al.*, 2012, CO_2 and temperature *e.g.* Crous *et al.*, 2013, drought and temperature *e.g.* Drake *et al.*, 2018, 2019). As the climate becomes more extreme, this type of research gains interest from both managers and conservationists interested in establishment success and growth of forests with a species or ecotype composition that is 'climate proof' (*e.g.* Coomes *et al.*, 2014).

Another strategy that combines ecotron and field studies can circumvent the fact that ecotron experiment cannot be run for many years. This strategy is illustrated by the Jena Experiment, initiated in 2002 and still running. For the main experiment in the field, herbaceous plant communities of 1-60 plant species and 1-4 plant functional groups were sown on a former arable site. A high number of plots (82 plots 20 m x 20 m each) was necessary to allow the partitioning of biodiversity effects on ecosystem functioning into the effects of species richness, functional group richness, and the contribution of particular functional groups (Weisser *et al.*, 2017). Once the diversity effects had been well established in the field, underlying physiological mechanisms were studied in the Macrocosms platform of the Montpellier ecotron. Large soil monoliths with vegetation (2 m², 2 m deep, 8 t) were sampled in plots of 4 and 16 species 9 years after sowing and inserted in the ecotron for an entire growing season. Automatic measurements of ecosystem evapotranspiration, net carbon exchange, and night time respiration together with tracer studies (^{13}C, ^{15}N, ^{2}H), modelling and a final destructive harvest enabled the determination of day respiration and photosynthesis, the partitioning of evapotranspiration into evaporation and transpiration, efficiency of water, nitrogen and light use, carbon allocation to soil compartments, patterns of root water uptake, and the relative effect sizes of biodiversity components (Milcu *et al.*, 2014, 2016b; Lange *et al.*, 2015; Mellado-Vázquez *et al.*, 2016; Guderle *et al.*, 2018; Roscher *et al.*, 2019). Only field experiments can provide such large plots for many experimental treatments and that can be used for decades, but the ecotron was necessary to answer questions arising from field observation but requiring additional measurement capacities difficult to implement in the field. Since the ecotron was used as an ecosystem analyser of treatment plots manipulated in the field for 9 years, the transient effects following the start of the treatments are considered to have faded. In this case, the length of the experiment in the ecotron could then be limited to a single growing season, another advantage of the complementarity between ecotron and field studies.

In ecotrons, the size limits also constrain the complexity of the ecosystem under study. Not all the drivers and trophic levels found in nature are typically represented in ecotrons, even though they may be relevant or even critical for the process under scrutiny. For example, it is well known that herbivores and predators can play a major role in some ecosystem responses to climate change. For example, bark beetle outbreaks often coincide with drought as trees suffering from drought stress tend to have reduced defences against pathogens and herbivores, leading to improved feeding opportunities for these insects and their offspring (Marini *et al.*, 2017; Kolb *et al.*, 2019). Such pest outbreaks can have even more profound effects on tree growth and mortality than the initial drought (Fettig *et al.*, 2019). Although these secondary effects cannot be fully explored in ecotrons, experiments where targeted herbivore species or other relevant taxonomic groups of animals are introduced in a controlled manner are possible (*e.g.* Stevnbak *et al.*, 2012; Van De Velde *et al.*, 2017). The iDiv Ecotron was specifically built to study aboveground-belowground multitrophic communities of invertebrates and their impacts on ecosystem processes (Eisenhauer & Türke, 2018). Although such studies must be supplemented by observations in the field to increase the external validity, they are valuable for testing specific hypotheses and uncovering particular mechanisms as part of a larger research strategy (e.g. Thakur *et al.*, 2020).

Ecotrons can also provide a critical contribution in verifying seedling-scale phenomena obtained in greenhouses and in testing the robustness of scaling relationships from leaf to ecosystem. For example, the Whole Tree Chambers (WTC) were used to investigate the role of heat waves on trees physiology, complementing glasshouse-based studies of potted seedlings (Aspinwall *et al.*, 2019). This provided a rare test of integrated canopy gas exchange responses to an experimental heatwave on large trees. In turn, the WTC study provided evidence to underpin ecosystem-scale assessments of the uncoupling of photosynthesis and transpiration during heat waves using eddy covariance flux data (De Kauwe *et al.*, 2019). In addition, WTC studies elucidated the mechanisms of temperature responses of tree growth and thermal acclimation of carbon exchange under field conditions (Aspinwall *et al.*, 2016; Drake *et al.*, 2016), complementing findings determined in glasshouse studies of eucalypt seedlings (Drake *et al.*, 2015, 2017). It also provided robust tests of temperature effects in response to diurnal and seasonal temperature changes under ambient and warmed treatments.

Ecotrons can also be used to complement studies in controlled laboratory environments. For example, laboratory experiments investigating patterns of gene expression and resulting physiological processes are often performed under highly constant conditions. However, models need to be developed to translate laboratory knowledge to applications in agriculture. A direct approach incorporating field fluctuations into models that predict transcriptome changes demonstrated an impact of air temperature, humidity, solar radiation and wind (Nagano *et al.*, 2012). Nevertheless, further development of such models would benefit from studying transcriptomes in ecotrons, where fluctuations in the environment can be controlled. This would enable improved parametrization of phenomenological models, such as the ones developed by Nagano et al. (2012) and to develop biophysical ones.

Further improvements

Apart from deploying additional instrumentation for environmental control and process measurements, intrinsic operational improvements of ecotrons are underway. With respect to environmental control, simulating the climate of a distant location or a preceding year remains challenging in sunlit ecotrons, as the natural correlation between temperature and light intensity is difficult to reproduce when local day-to-day light conditions are not predictable. Another challenge relates to the incorporation of feedbacks between drought and warming. When drought decreases evapotranspiration, sensible heat flux increases compared to latent heat flux, resulting in landscape-wide temperature increases (De Boeck & Verbeeck, 2011). This drought impact on temperature should be incorporated into the temperature set-points of experiments. With many regulated and measured parameters, involving hundreds to thousands of sensors, the early detection of sensor malfunction or deviation from target climatic conditions also remains challenging. This is alleviated by automated alarms on parameter thresholds or by duplicating sensors to indicate sensor drift or failure. However, the use of parameter thresholds may not detect small but deleterious deviations, especially in parameters with large daily fluctuations. Additional alarms based on algorithms taking into account the correlation between replicates and between parameters, as well as on models, need to be tested and deployed to ease experiments human supervision. In terms of improving process measurements, the investigation of soil functioning is lagging behind in ecotrons like in any other experimental facility. Much could come from non-invasive soil process measurements via automatic gas sampling in the soil. Such sampling is installed in some ecotrons to measure greenhouse gas concentrations, but this needs to be combined with soil physical properties in models calculating soil greenhouse gas emission rates. Furthermore, the analysis of volatile organic compounds (VOCs) emitted from soils via ultra-sensitive proton transfer reaction mass spectrometers is starting to reveal valuable information on function and biodiversity (*e.g.* Abis *et al.*, 2018, 2020). The chemotyping of fungi growing on medium can currently be done for some functional groups (Muller *et al.*, 2013; Guo *et al.*, 2020), but a more comprehensive soil VOCs chemotyping, requiring strong international cooperative investments, is needed to advance research on this topic.

A major improvement of ecotron research will also come from a thorough blending of experiments with modelling. Hanson & Walker (2020) wrote: “From the outset, these studies must be informed by and integrated with ecosystem models that provide quantitative predictions from their embedded mechanistic hypotheses”. Although some ecotron results have been informing model processes (Milcu *et al.*, 2016b; Guderle *et al.*, 2018; Jiang *et al.*, 2020), systematic association between models and experiments in ecotrons has not yet been implemented. In particular, recent modelling approaches based on data assimilation and forecasting based on inverse modelling and forward prediction (Luo *et al.*, 2011; Huang *et al.*, 2019) have not been explored with ecotron-produced data despite being ideal for such approaches. Quasi real time data assimilation (*e.g.* once a week or once a month) could be implemented since many ecosystem parameters are acquired at high frequency. This could be used to improve predictions of ecosystem states during the course of experiments in order, for example, to optimise the dates on which to conduct soil or gas samplings for costly and labour-intensive analyses. Real time model outputs could also be used to manage experiments. Often the experimental treatments defined at the start of an experiment (*e.g.* temperature or precipitation levels) fail to bring the ecosystem into the optimum state to uncover specific ecosystem responses. Real time modelling and data assimilation would thus allow to estimate whether or not the ecosystem will reach the target state, and if not, the treatment levels could be adjusted accordingly.

Perspectives

Hanson & Walker (2020) suggested future directions in global change biology, emphasising the need for large-scale experiments that incorporate most biochemical and biodiversity feedbacks. They also advocate that a full range of methodological approaches, including smaller spatial scales, will continue to be needed to further mechanistic understanding. This is where the ecotrons will continue to play a significant role, especially through experiments with elevated CO_2 atmospheres, anticipated warming or drought scenarios which take us beyond the historical record.

With their versatility and advanced analytical capacities, especially through isotopic approaches, ecotrons should also continue to be used to address not only global change questions, but also fundamental questions in ecology, agronomy and environmental science. Moreover, being open to the scientific community at large, we anticipate that new experiments will be created, including those not directly related to ecosystem science (*e.g.* parametrising sub models of the earth's atmospheric cycles; testing epigenetic effects in specific environments, etc.).

Extended collaborations with other disciplines will be key to take further advantage of the research capacities of ecotrons (Rineau *et al.*, 2019). This will be accomplished in particular through international infrastructure projects. So far, six of the ecotrons described in this paper participate in the Analysis and Experimentation on Ecosystems European infrastructure (ESFRI AnaEE, https://www.anaee.com/), which is providing a data and modelling centre to facilitate the blending of modelling and experiments. AnaEE, together with ICOS, LTER and LifeWatch, is a constituent infrastructure of the ENVRI consortium (https://envri.eu/), where interactions between research on the life, air, land and water components of the Earth System are developed.

This paper presents the ecotrons, a small part of the national and international efforts to serve environmental research in the context of unprecedented global changes. Its aim is to inform researchers, especially those in the ecology and agronomy fields, about the possibilities offered by these recently built experimental facilities and to encourage their cooperative use. With their high degree of environmental control and exceptional process measurement capacities, the ecotrons described here offer realistic experimental conditions that are much closer to field conditions than those of controlled environment facilities in general. Alongside field experiments and observational sites, through their complementary features, the ecotrons can play a pivotal role in uncovering mechanisms and supplying parametrisation of ecosystem processes, while fostering transnational collaboration. These infrastructures will bring key contributions to the prediction and maintenance of ecosystem services in the context of current environmental changes.

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Figures legends:

Figure 1. Left to right and top to bottom: UHasselt ecotron Hasselt, EcoCELLs Reno USA, Whole Tree Chambers Richmond Australia, iDiv Ecotron Leipzig, TUMmesa München, IleDeFrance ecotron Ecolab Nemours, ExpoSCREEN München, TERRA ecotron Gembloux, TerraXcube Bolzano, and Macrocosms and Mesocosms platforms of the European Montpellier Ecotron.

Figure 2. Examples of ecotron environmental controls: simulation of outside air relative humidity (a), CO_2 concentration (b), soil water tension (c) and air temperature (d) measured at the Maasmechelen, Belgium ICOS station and reproduced in the UHasselt sunlit Ecotron (unpublished data), and simulation of air temperature (e) and photosynthetically active radiation (f) derived from a model and reproduced in the Terra Ecotron Gembloux with artificial lights (unpublished data). Red lines: conditions to be simulated, grey area: range of variation of the parameters across 12 (a to d) or 3 (e, f) experimental units, dark grey line: average for the 12 or 3 units.

Figure 3. Examples of ecotron isotopic labelling and process measurements: Air $^{13}CO_2$ enrichment and plant ^{13}C labelling in one macrocosm of the Montpellier Ecotron (a); measurements of net ecosystem CO_2 exchange (b) and net ecosystem N_2O exchange (c) with photosynthetic photon flux density (PPFD) in one macrocosm of the same ecotron; measurements of the emission of two volatile organic compounds (isoprene and methanol) with PPFD in one of the subchambers of the München ExpoSCREEN facility (d & e).

Figure 4. Treemap diagrams showing the research areas covered by the ecotrons in the published ecotron papers (left) and in the running or recently completed projects in the newly open ecotrons (right). Blue: experiments designed to better understand specific ecosystem processes; green: global change experiments simulating 'novel' future environments; red: biodiversity experiments with manipulations at various trophic levels. Numbers indicate the number of papers/experiments in each sub-category.

Tables legends:

Table 1. Administrative and structural characteristics of the ecotrons. Additional information on each facility (including, in some cases, specific capacities of a subgroup of experimental units) are given in the supplementary information file 'Ecotrons descriptions'.

Table 2. Controlled environmental parameters in each of the ecotrons*

Table 3. In house process measurements done automatically (continuously or at high frequency) as services offered routinely by the facility to its internal or external users or done manually at a frequency to be determined after negotiation. Measurements at scales smaller than the ecosystem (leaf level for example) as well as measurements which are usually done externally on soil plant or air samples are not considered in this table.

Table 1: Administrative and structural characteristics of the ecotrons. Additional information on each facility (including, in some cases, specific capacities of a subgroup of experimental units) are given in the supplementary information file 'Ecotrons individual descriptions'.

Ecotron short name	Owner	Town, Country	Opening year	Access	Staff	No. of climate controlled cells	Area of each cell m²	No. lysimeters/cell *	Area of each lysimeter m²	Air volume ** m^3	External air flux *** m^3/min	Air internal recirculation m^3/min	Plant height max m	Soil depth max m	Soil weight / lysimeter	Biosafety level
ExpoSCREEN, München	Helmholtz Zentrum München	Neuherberg, Germany	1985	●	3	4	6	4	0.6	0.5**	1.3 to 2.7	none	0.8	≤ 0.7	0.5 t	1
EcoCELLs, Reno	Desert Research Institute	Reno, Nevada USA	1995	●	0.25	4	40.5	1	9 or 11	130	13 to 130	~660	2.4	1.8	36 t	1
Whole Tree Chambers, Richmond	Western Sydney University	Richmond, N.S.W., Australia	2006	o	1	12	8.3	1	8.3	53	0.6	180	9	1	in situ	1
Montpellier Ecotron, Macrocosms	CNRS (INEE)	Montferrier sur Lez, France	2010	●	2.7	12	20	1	2 or 4 or 5	35	2.7	70	3	0.6 to 2	3 to 15 t	1
IledeFrance Ecotron Ecolab, Nemours	CNRS (INEE)	Saint-Pierre-lès-Nemours, France	2017	●	3.5	15	4.5	1*	1.3	13	0 to 200	0 to 1.25	1.5	0.8	≤ 2 t	1
iDiv Ecotron, Leipzig	iDiv, Leipzig University	Bad Lauchstädt, Germany	2017	o	1.5	24	2	1*	0.2	3	<6	none	1.5 (1.2)	0.8	0.2 t	1
TUMmesa, München	Technical University Munich	Freising, Germany	2017	o	1	8	8	4*	0.38	36	1.9	83	1.5	0.8	238 kg	1
UHasselt Ecotron, Hasselt	Hasselt University	Maasmechelen, Belgium	2018	o	2.7	12	19	1	3.14	222	0	60	2.5	1.4	5 to 12 t	1
TERRA Ecotron, Gembloux	Liège University	Gembloux, Belgium	2018	●	2	6	20.3	1*	2	65	45	1 to 3.2	1.5	1.5	6 t	2
Montpellier Ecotron, Mesocosms	CNRS (INEE)	Montferrier sur lez, France	2018	●	2.7	18	1	1	1	4	0 to 0.5	10	1.8	1	0,3 to 2 t	1
TerraXcube, Bolzano	Eurac Research	Bolzano/Bozen, Italy	2020	●	2	4	9	4	0.13	27	1.7	60	2,5	0,4	60 kg	2
AGRASIM, Jülich	Forschungszentrum Jülich	Jülich, Germany	2021-2022	o	3	4	2.6	1	1	6.9	0.02 to 1	≤ 27	2.5	1.4	≤ 3 t	1
Antwerp Ecotron	University of Antwerp	Antwerp, Belgium	2021 -2022	●	1	not set	1	1	1	7	0.3 - 2.5	25	1	1	≤ 2 t	1

Notes:		
	Access	● open calls (see supplementary file 'Ecotrons individual descriptions' for links to these calls) with occasional in-house collaborative projects; o in-house projects with external collaborations
	Staff	Number of persons/year (permanent or on temporary contract) fully dedicated to the functioning of the ecotron. (e.g. 1 means that there is the equivalent of one technical person working 12 months full time for the facility).
	Number of cells	The cells provide (independently from each others) the climate control over a single lysimeter (they are then called enclosures) or over several lysimeters (in that case they are then called chambers)
	*	indicates that a higher number of smaller lysimeters can also be used. When available (*) this option is detailed in the supplementary file 'Ecotrons individual descriptions'
	Area cell	Area of each enclosure or chamber allowing the climate control. It is the lysimeter(s) area plus, if present, a walking area around the lysimeter(s)
	**	indicates the volume of the chamber permanently enclosing the canopy above each lysimeter, otherwise indicates the volume of the whole cell
	***	The facilities with 0 external air flux works (or can work) in a close system mode while the other facilities work in an open system mode (cf. principles of gas exchange measurements)
	Biosafety	Level 1: washing hands upon entering and exiting the lab; potentially infectious material decontaminated before disposal; lab must have a door which can be loocked to limit access.
		Level 2: items of level 1 plus: advanced training for personnel and scientists; limited access to the laboratory; extreme precautions to be taken with contaminated items; use of physical containment equipment when aerosols or splashes may occur.

Table 2: Controlled environmental parameters in each of the ecotrons*

Ecotron short name	Opening date	**Ta °C**	**Ta °C < 5**	**Ta °C < 0**	**air RH %**	**air RH < 30%**	**soil boundary condition T°C**	*number of sensors T°C soil VxH*	**type of watering S, D, M**	**SWC %**	*number of sensors SWC VxH*	**soil boundary condition ψm**	*number of sensors soil ψm VxH*	*number of sensors soil EC*	*sunlight transmission T_{vis}, T_{UV}*	*PPFD 50 cm µmol/m²/s*	*CV PPFD %*	**adjustable lamps intensity**	*% UV radiation 280-400 / 280-700*	*red / far red 600-700/700-*	*canopy air speed m/s*	**CO_2 >400 ppm**	**CO_2 pre-industrial**	**$\delta^{13}C$ CO_2**	**O_3**	**NOx**
ExpoSCREEN, München	1985	●	●	●	●	●		□	D,M	□	□				–	600	≤10	●	4 - 6.5	1.3 - 1.7	< 2	●	●	●	●	●
EcoCELLs, Reno	1995	●	●	●	●	●	●	5x15	S,D,M	●	5x6		□	□	0.8 - 0.9	s.l.	≤15	–	s.l.	s.l.	< 2	●		●		
Whole Tree Chambers, Ri	2006	●			●			3x1	S,D,M		3x3	–			0.93, 0.93	s.l.	variable	–	s.l.	s.l.	0.3	●		●		
Montpellier Ecotron, Macrocosms	2010	●	●	●	●		●	4x3	S,D,M	●	4x3	○	1x1		0.92, 0.8	s.l.	10	–	s.l.	s.l.	1	●		●		
Ile de France Ecotron EcoLab, Nemours	2014	●	●	●	●	●	●	1x1	S,D,M	●	4x10	□	1x1	□	-	1000	< 10	●	2	1.2	0.1 to 20	●	●	○	□	□
iDiv Ecotron, Leipzig	2017	●			●			3x4	S,M	●	3x4		3x4		–	340	10	●	0.3%	1.0	< 0.7	□		□		
TUMmesa, München	2017	●			●		●	4x1	S,D,M	●	4x1		□		–	1070	4	●	8.6	3.5	< 0.25	●	□	●	●	
UHasselt Ecotron, Hasselt	2018	●	●	●	●	●	●	5x3	S,M	●	5x3	●	5x3	5x3	0.95, 0.95	s.l.	< 10	–	s.l.	s.l.	1.52	●	●			
TERRA Ecotron, Gembloux	2018	●	●		●	●	●	5x1	S, M	●	5x1	●	3x1		-	1200	7	●	4.7	1.2	0.2	●			●	
Montpellier Ecotron, Mesocosms	2018	●	●	●	●	●	●	4x2	S,D	●	4x2	○	1x1		0.9, 0.8	s.l.	< 10	–	s.l.	s.l.	< 0.7	●	●	●		
TerraXcube, Bolzano	2020	●	●	●	●	●	○	3x1	S,D,M	●	3x1	●	3x1		–	1300	< 10	●	tbd	tbd	2	●		○	○	
AGRASIM, Jülich	2021-2022	X	X	X	X	X	X	7x2	D,M	X	7x2	X	7x1		–	1200	tbd	X	tbd	tbd	0 to 10	X	X	X	X	
Antwerp Ecotron	2021-2022	X	X	X	X	X	X	3x1	S,D,M	X	3x1	X	1x1		0.9, 0.8	s.l.	tbd	–	s.l.	s.l.	tbd	X				

Notes:	*	controlled parameters have their name in bold
	●	existing automatic control and/or measurement
	○	automatic control and/or measurement being installed
	□	measurement / sampling done manually at frequencies to be determined (optional, upon negociation)
	X	planned regulation and measurements (in facilities being built)
	–	indicates that this parameter is not relevant (sunlight transmission for indoors facilities, ajustable lamp intensity for sunlit facilities)
	s.l.; tbd	s.l.: sunlit facility; tbd: to be determined
	T °C, RH%	temperatures and relative humidity refers to day time or when the light are on. Some performances can only be reached under adequate outside climatic conditions (e.g. negative air temperatures not reachable in summer)
	T°C, ψm	control of the soil temperature or matrix potential at the bottom of the lysimeter. It recreates near natural soil temperature and matrix potential profiles.
	VxH	V is the number of positions of sensors vertically, H is the average number of sensors placed horizontally at each vertical position
	S, D, M	watering can be done by spray (S) with nozzle(s) above the canopy or by drip (D) with drippers on the soil surface, or manually (M)
	SWC %	soil water content controlled via watering after measurements of water loss (weighing the lysimeters) or via soil humidity sensors in the soil profile
	T_{vis}, T_{UV}	sun light transmission by the containment structure in the visible range T_{vis} and in the UV range T_{UV}
	PPFD	maximum photosynthetic photon flux density at 50 cm below lamps when soil-lamp distance is adjustable, or 50 cm above soil level when this distance is not adjustable
	CV PPFD	light homogeneity: variation coefficient of PPFD measured at several points uniformly distributed over the canopy area
	UV	(ratio radiation photons 280-400 nm / radiation photons 280-700 nm) x 100 . This percentage is 6.6 for solar radiation (based on the standard solar spectrum AM1.5 expressed in photons)
	red:far red	ratio radiation photons 600-700 nm / radiation photons 700-800 nm. This percentage is 1.1 for solar radiation (based on the standard solar spectrum AM1.5 expressed in photons)
	δ	(delta): ratio of stable isotopes in a given molecule (here ^{13}C / ^{12}C in CO_2) in reference to a standard (Pee Dee Belemnite)

Table 3: In house process measurements done automatically (continuously or at high frequency) as services offered routinely by the facility to its internal or external users or done manually at a frequency to be determined after negociation. Measurements at scales smaller than the ecosystem (leaf level for example) as well as measurements which are usually done externally on soil plant or air samples are not considered in this table.

Ecotron short name	Opening year	ET (H_2O)	NEE (CO_2)	δ ^{13}C in CO_2	Δ ^{18}O in CO_2	Soil respiration	CH_4 emission	N_2O emission	O_3 emission	VOC fluxes	δ ^{15}N, δ ^{18}O in N_2O	δ ^{2}H, δ ^{18}O in H_2O	Drainage flux	Soil solution sampling **	Root growth ***	LAI***	Canopy leaf T °C ***	Hyper-spectral reflectance ***	Fauna tracking ***
ExpoSCREEN, München	1985	●ge	●	○					●	□						□	□		
EcoCELLs, Reno	1995	●	●	□	□	●	□	□		□			●	□	□	□	□	□	
Whole Tree Chambers, Richmond	2006	●ge	●	□	□	●	□	□			□	□		□	□	□	●	□	□
Montpellier Ecotron, Macrocosms	2010	●	●	●	●	□	●	●			●		□	□		□			
Ile de France Ecotron, EcoLab	2014	●	●	○		●	□	□	●	□	□	□	●	●	□	□	□		
iDiv Ecotron, Leipzig	2017	□	□			□				□			○	□	□	□	□	□	○ed, rf
TUMmesa, München	2017	●				□							○	□	□	□		□	
UHasselt Ecotron, Hasselt	2018	●	○				○	○					●	●					
TERRA ecotron, Gembloux	2018	●	●			●	●						●	□	□	●st	□	□	
Montpellier Ecotron, Mesocosms	2018	●	●	●	●	□	●	●			●		□	□		□			
TerraXcube, Bolzano	2020	●	○			□							●	□		○	○	□	
AGRASIM, Jülich	2021-2022	X	X	X	X	X	X	X			X	X	X	X		X	X	X	
Antwerp Ecotron	2021 - 2022	X	X			X	X	X					X	X		X	X		

Notes:		
	●	existing automatic continuous (or at high frequency) measurement provided to the hosted teams
	○	automatic continuous (or at high frequency) measurement being installed
	□	measurement / sampling done manually (by the facility staff or by the hosted team), often using available portable devices, at frequencies to be determined (optional, upon negotiation)
	ET	Evapotranspiration, measured by weighing (●) or by gas exchange (●ge)
	NEE	Net Ecosystem Exchange of CO_2 (balance between canopy photosynthesis and canopy and soil respiration)
	δ	isotopic difference resulting from fractionation within the ecosystem (δ value difference of a given gas before and after going through an ecosystem in an ecotron unit)
	Root growth	measured with minirhizotrons (ingrowth cores measurements are not indicated here since they do not require instrument investment by the facility and are usually done by the hosted team).
	LAI	Leaf Area Index (it often gives also canopy transmittance, but this transmittance can also be obtained with simpler light sensors); ●st LAI measured by stereoscopic cameras
	Hypersp ...	Canopy hyperspectral reflectance
	Fauna	tracking done by real time detection number and size measurement of catched soil microarthropods (Edaphologsystem) (○ed) or/and RFID monitoring of beetle movements (○rf)
	**	indicates sampling, but not measurements on these samples (measurements often done externally)
	***	The facilities own the (often portable) equipement to run the raw initial measurements but since finalising results requires a lot of man power and/or very specific skills (i.e. analysis of the roots pictures, parametrization of the hyperspectral models), these final results are often not offered routinely to external users of the facility.

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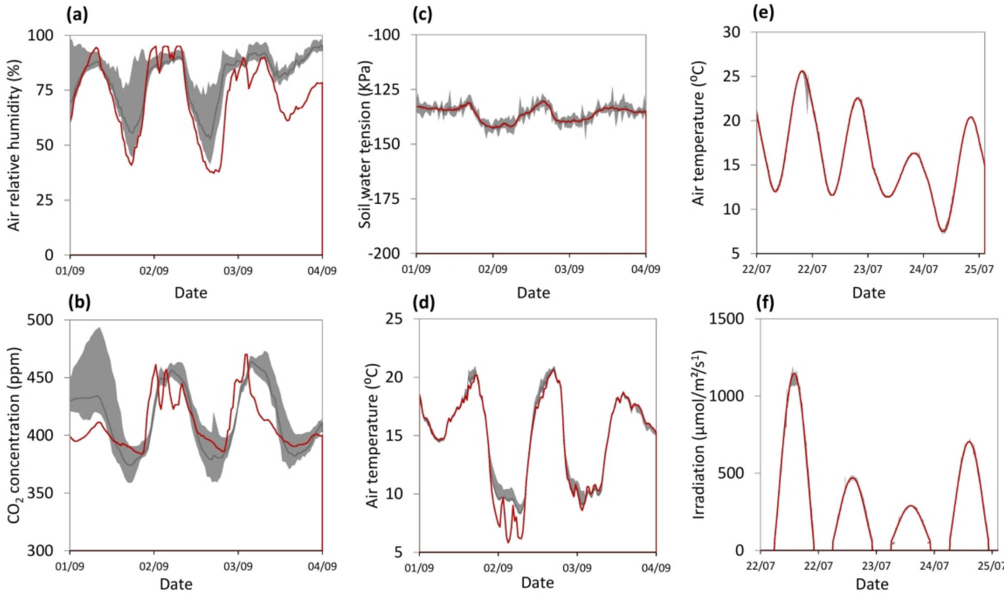

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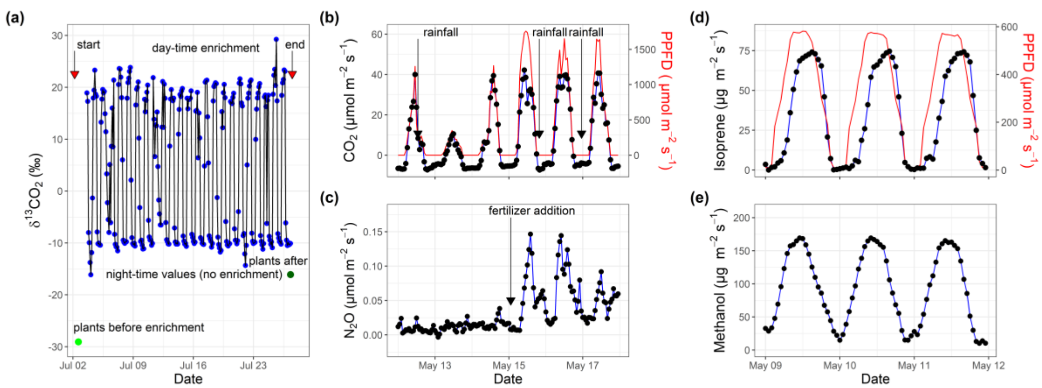

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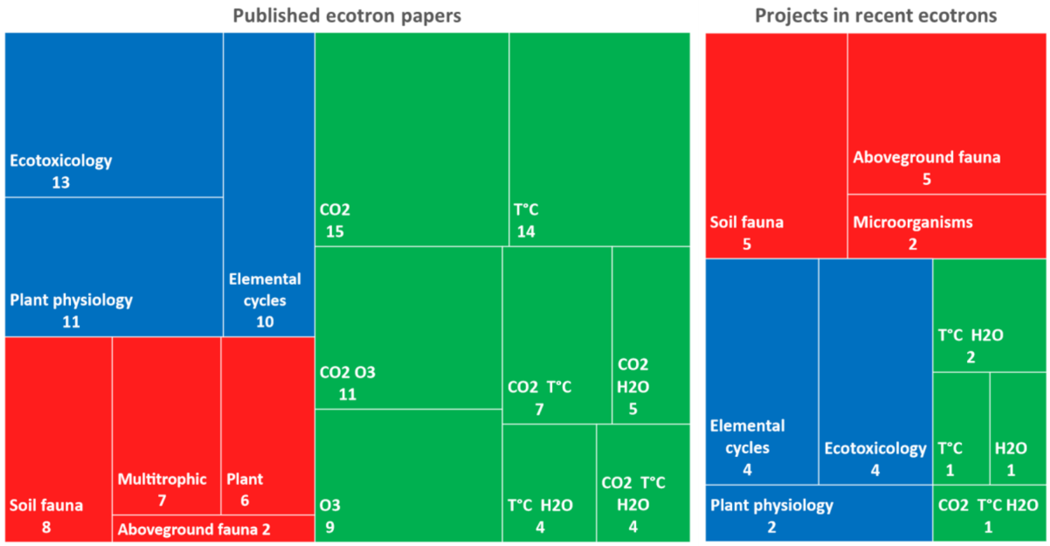

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