TUTORIAL REVIEW

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Biosphere 2 Center as a unique tool for environmental studies

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The Biosphere 2 Laboratory of Biosphere 2 Center, Arizona, is a unique, self-contained glasshouse fostering several mesocosms of tropical and subtropical regions on an area of 12 700 m^2. It was constructed around 1990 to test whether human life is possible in this completely sealed, self-sustaining artificial ecosystem. Mainly due to overly rich organic soils, the initial mission failed in a spectacular manner that raised enormous disbelief in the scientific seriousness of the project. From 1995 to 2003, the facility had been operated by Columbia University under a completely new scientific management. The aim of the project had then been to conduct research in the field of 'experimental climate change science'. Climatic conditions within the mesocosms can be precisely controlled. In studies with elevated CO_2, altered temperature and irrigation regimes performed in the rainforest, coral reef and agriforestry mesocosm, the facility had proven to be a valuable tool for global climate change research. Upon submission of this manuscript, Columbia University is relinquishing the management of this facility now although there was a contract to operate the facility until 2010, leaving it with an unclear destiny that might bring about anything from complete abandonment to a new flowering phase with a new destination.

1. History: Biosphere 2 as a holistic, observational project

The Biosphere 2 Center (B2C), located between Phoenix and Tucson, Arizona, is a research campus harboring a unique building, the Biosphere 2 Laboratory (B2L, Fig. 1). The building is a model of earth's biosphere (Biosphere 1) with tropical and subtropical ecosystems, including a rainforest, desert, thornscrub, savanna, marsh, mangrove, ocean, an agricultural, and a human habitat area, which were initially connected to each other.[1,2] The synthetic communities of plants and soils were designed along the guidelines of different scientific advisors and were enclosed in a shell of glass and stainless steel, encompassing an area of 12 700 m^2 and a volume of 180 000 m^3. The impressive structure was planned and built between 1983 and 1991 at costs of around US $150 million. The aim of the venture was to provide a prototype station for future space missions and to test whether life inside the structure would regulate itself and form conditions that are favorable for human life in a similar way as happened in the evolution of life on earth.[1–3] The building was designed to stand for about 100 years as a materially closed system maintaining equilibrium and sustaining life support for eight human beings in an inspiring environment over periods of several years.

Attempts to build materially enclosed artificial ecosystems for human life support began in the early 20th century.[4,5] Especially in Russia, this idea flourished hand in hand with the race for the stars. It culminated in the 1970s in the construction of Bios-3, a 300 m^3 module, designed to be sent to outer space, in which 2–3 men survived in experiments of up to four months, producing about 50% of their food, which was grown under artificial light, and regenerating about 90% of their air and water.[6] The Biosphere 2 test module, a prototype construction designed along the same principles as the B2L, exceeded this volumetric record mark upon its completion in 1987 by about 50% and proved that the most important technical features of B2L could also work on the large scale.[7]

The initial 2-year 'human experiment' (Mission 1) in B2L was conducted between 1991 and 1993 and revealed the full complexity of this approach. Due to a very high soil respiration, O_2 concentration decreased dramatically, reaching threatening levels after 16 months. Liquid oxygen had to be pumped into the system to sustain the experiment. A second mission with seven Biospherians was launched some months after the end of Mission 1, but was terminated after six months, because the principal problems of the system had not been overcome. The ultimate reason for shut-down was a noxious concentration of N_2O (around 80 ppm[8]) that had built up in the course of the microbial reduction processes that were still going on extensively in the soil. Furthermore, the water quality decreased[9] and waste materials ranging from organic matter to large quantities of calcium carbonate coming from scrubbed excess CO_2[10] were piling up in the basement of the building. The spiritual rather than scientific background of the privately funded group that envisioned, designed and constructed the building was often criticized after termination of the human

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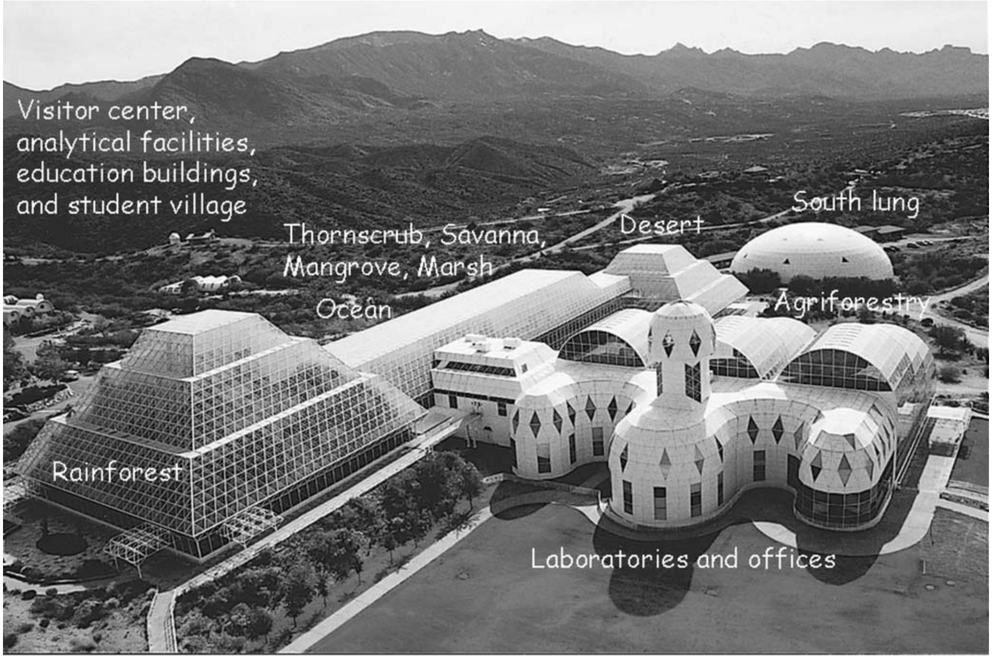

Fig. 1 Biosphere 2 Center. The central facility, Biosphere 2 Laboratory, is comprised of eight mesocosms, some laboratory and office space as well as of the south and west lung. In addition, there are facilities for maintenance of the central facility, for student life, education and public outreach on campus.

missions.[11] This and the way in which the human experiments and their throwbacks were managed led to the ambivalent perception with which B2C is still seen by both the public and the scientific community.[8,12]

Most scientific results from Missions 1 and 2 are related to the physiology of the enclosed Biospherians, who were supplied with a calorically restricted but well-balanced nutrition,[13–18] and who had to deal with low amounts of O_2.[19] The results suggested that humans react to such a nutritional regime similarly to other vertebrates.[17] Further scientific reflections on the initial experiments comprise the engineering design of the building[10,20,21], the design of the mesocosms,[9,22–27] their agronomical output,[9,15] population dynamics,[28,29] self-organization criticality,[30] and monitoring of trace gases in the atmosphere.[6] Only little was learned about the evolution of such a self-organizing system. Processes going on in the soil of the ecosystems were not investigated in appropriate detail. Performing manipulative experiments within the overall experiment was not in the scope of those first missions; too many technical features had never been tested before at the large scale that B2L provides.

Drinking water in B2L was taken from 20 000–40 000 l of condensate collected daily in the air-handling units.[10] In a system developed from previous NASA work, wastewater was sent first to anaerobic holding tanks and then to a marsh bed, where bacteria broke down waste products to nutrients which were then supplied to the agricultural system.[20] All food was grown inside the structure without addition of artificial fertilizer or additional illumination. In contrast to spacecraft missions, where typically several hundred trace gases pose health risks, outgassing from structural material and paint was not a problem in B2L because of a careful selection of those materials and because of air filtration by plants and soils.[6] Atmospheric pressure differences, occurring due to the heating of the building, were buffered by two variable volume chambers made of steel and rubber membranes; the two so-called "lungs". In theory, the building only had to be fed with energy to provide air-conditioning and to enable communication between inside and outside. It was the most tightly sealed building of such a scale ever made, with an atmospheric leakage rate of less than 10% per year.[31] The climate control system was equipped with the best computer technology available at that time[32] and is still enormously powerful, demanding an energy bill of around US $800 000 per year. All essential functions were and are backed up, and hence the environmental conditions for plant life inside B2L have never been endangered. If the catastrophic event of a complete power failure occurred in this system, temperatures of more than 60 °C inside the glasshouse would be reached within minutes.

The only technical feature that was incorporated in the building, but was not used in the end was a soil bed reactor. In the test module (and potentially also in the agricultural biome of B2L), the entire air volume was pumped through the soil for air purification. In the test module, NO_x, ozone, sulfur dioxide and methane remained in non-toxic levels, and toluene and tetrahydrofuran levels were even depleted relative to the concentration upon closure of the structure as a consequence of this.[6] However, in the agricultural biome, air quality (and agronomical yield) might have been diminished in the main experiment since bacteria might have been released from the soil into the atmosphere.[9] Technically, it turned out that everything was figured out fine, when Mission 1 finally started.

What was not figured out fine was how to maintain the balance of this assembly of ecosystems. The idea of bringing in organically very rich soils that would produce a maximal agricultural output proved to be a most serious flaw which could not be corrected. The soil was a mixture of soils from a nearby cattle pond and from commercial components. The mixture was varied from biome to biome. The topsoil of the agricultural system consisted of 70% clay loam, 15% commercial compost, and 15% commercial peat.[15] The rainforest topsoil was mixed from 50% loam, 25% gravelly sand and 25% coarse organic material.[25] Other sources for obtaining more realistic soil were taken into consideration, but were not used in the end because of the enormous costs of soil transportation and because of the restrictions to import soils into Arizona.

Despite the high nutrient availability of the soils, the agricultural yield of Mission 1 produced only about 80% of the consumed nutrition;[15,16] the other 20% came from stored reserves that were grown inside B2L before starting Mission 1. Harvests were much smaller than anticipated due to low irradiation levels (about 50% of the incident irradiation[23]) inside the structure, which were caused by the glazed glass roof and by shading from the roof-mounting space frame structure. Also, high soil salinity and unforeseen pests such as broad mite, powdery mildew, cockroaches, aphids, crazy ants and many others led to significantly decreased harvests.[9] The worktime spent for agriculture and food preparation was higher than expected[15] (45%); the Biospherians worked an average of 66 h/week, spending less time than expected on research and data analysis[2] (10%). Due to the high workload and the sparse nutrition (about 2000 kcal), the Biospherians lost about 20% of their body weight during Mission 1.[13,23] Hunger and exhaustion were constantly present[33] and were exacerbated by the low atmospheric oxygen content, which at times was comparable to the natural oxygen content at an elevation of around 5000 m. The life of the "Biospherians" taking part in Mission 1 is described in Alling *et al.*[33]

It was anticipated initially that the atmospheric composition would reach a steady state quickly and that comfortable living conditions for the inhabitants would emerge. The Gaia-theory of a self-regulating living system[34] was the basis for composing the entire ecosystem inside the building, but self-regulation went in the wrong direction. The high proportion of organic material in all topsoils provided optimal conditions for soil microorganisms, which released large amounts of CO_2 and consumed equivalent amounts of O_2. Within sixteen months, a third of the initial oxygen content was deprived from the atmosphere. The only reason, why CO_2 did not rise to skyrocketing levels, but remained on a non-life-threatening concentration of around 4000 ppm was that the vast amount of uncured concrete within the structure was able to react with most of the emerged CO_2 to form calcium carbonate.[35] The O_2 level was kept above 14% subsequently by pumping tons of liquid oxygen into the lung of the system.

Self-regulation did not only affect humans but also the rest of the fauna in unforeseen ways. Initially, more than 3000 plant and animal species were present in B2L.[20,36] Overall, only very few of the introduced animal species survived the experiment. In particular, the insect community changed in an unpredictable way. None of the originally introduced 11 ant species survived Mission 1; from the mid 1990s on, the insect community was dominated by a tramp ant, *Paratrechina longicornis,* which entered the building during the construction phase and has thrived ever since, feeding on homopteran honeydew[37] and on dead cockroaches (*Periplaneta australasiae*), being the dominant nocturnal animals today. The enormous success of this ant species is based on its rapid, elaborate chemical communication pathway (personal communication Witte, manuscript in preparation). All pollinators went extinct due to predation by ants and cockroaches.[6] In the ocean biome, species surveys in 1992 and 1996 revealed a declining number of animal species (141 *vs.* 74), but an almost constant number of algae species[23] (31 *vs.* 28). The number of plant species in the rainforest was reduced from 280 before Mission 1 to 170 after Mission 1 and to a relatively stable community of 70 species today.[22,38]

The conditions that evolved in this experiment of self-containment were definitely not favorable for humans, nor were they for most animals. In this sense, the initial experiment failed. But the engineering design of the building was indeed a success. The degree of material closure and of self-sustainability of artificial ecosystems attained in this project had never been reached before and will probably not be reached again without substantially stronger funding for such a venture.

2. The present: Biosphere 2 as an experimental climate change science facility

A few months after Mission 1 started, the oceanographer and geochemist Wallace Broecker from Columbia University in New York was asked to help with the problem of uncontrolled evolution of CO_2 and loss of O_2.[39] His team finally solved the riddle of the unknown Biosphere 2 carbon sink by identifying the concrete structure of the building as the main counterpart of soil respiratory processes.[33,39]

In several workshops and meetings, it became clear to a wide part of the scientific community that the building was doomed to failure in its initial aim but provides an excellent tool for environmental studies if managed in a proper way and if the system-immanent flaws of the highly ingenious and expensive construction are taken into account. In 1996, B2C's owner transcribed the intellectual leadership of B2C to the Earth Institute at Columbia University, who proposed to lead the facility to a world-class center for environmental studies, especially in the field of global climate change. While this manuscript was being prepared, the campus was under the leadership of plant biologist C. Barry Osmond and 20 to 30 research staff members were constantly on site. The campus provides laboratory and office space and is equipped with modern analytical facilities for all kinds of laboratory or field experiments. Moreover, it provides excellent infrastructure for student education and public outreach.[40] An average of 170 000 visitors come to B2C each year and several graduate and undergraduate student education programs have taken place throughout the last few years in the student village. From January 2004 onwards, the campus will be closed for all kinds of activities until further notice which will be commented on later on.

2.1 Technical specifications of the apparatus

Presently, B2L is operated in flow-through contained mode. This means, fresh air from outside B2L is provided on demand by push-pull-fans. The airmass exchange and the exchange of water vapor and CO_2 with the outside atmosphere are monitored and quantified continuously.[41] Moreover, the facility's ecosystems, which can best be characterized as mesocosms,[42] are now separated from each other by transparent polyvinyl-chloride (PVC) curtains to achieve full control over the environmental parameters in each of the mesocosms (Fig. 2).

CO_2 injection into the mesocosms occurs *via* Sierra mass flow controllers (60 l min^{-1}) using Licor "Gashound" infrared gas analyzers. CO_2 suppression is realized *via* air exchange with the outside atmosphere. Push-pull-fans, rated around 100 m^3 min^{-1}, extract high CO_2 air from inside and replace it with equal volumes of low CO_2 air from outside. CO_2 concentration can be suppressed to ambient +15 ppm during night respiration. Soil drainage water, purified *via* reverse osmosis is taken for irrigation *via* rotary sprinkler heads, mounted high in the roof-support system (space frame). Atmospheric vapor pressure deficit is lowered by using high pressure fogging nozzles and raised by removing water vapor as condensate in the air handling system. The air handling system is served by the energy center near B2L. Two backup power generators (diesel/natural gas, each with a capacity of 1 500 kW – the peak demand of B2L) provide electricity in case of a failure of the local grid. Heating and cooling are provided by hot and cold water circulated from the energy center through the air-handling units of B2L. The summertime cooling requirement of B2L can reach 4×10^4 J h^{-1}; wintertime heating requirement can reach 1×10^4 J h^{-1}. A refrigerative chiller system (4 °C) and a tower water chilling system (some degrees below ambient temperature) can circulate a maximal flow of 2×10^4 l min^{-1}, respectively. The hot water system (85 °C) has a maximal flow of 1.5×10^3 l min^{-1}.

The climate of each mesocosm is controlled by several air-handler units in the basement, which provide an air turnover of several thousand m^3 min^{-1} per mesocosm (Table 1). Each of those mesocosms has an area of more than 500 m^2 and is 23–28 m high from the bottom liner to the rooftop, allowing for

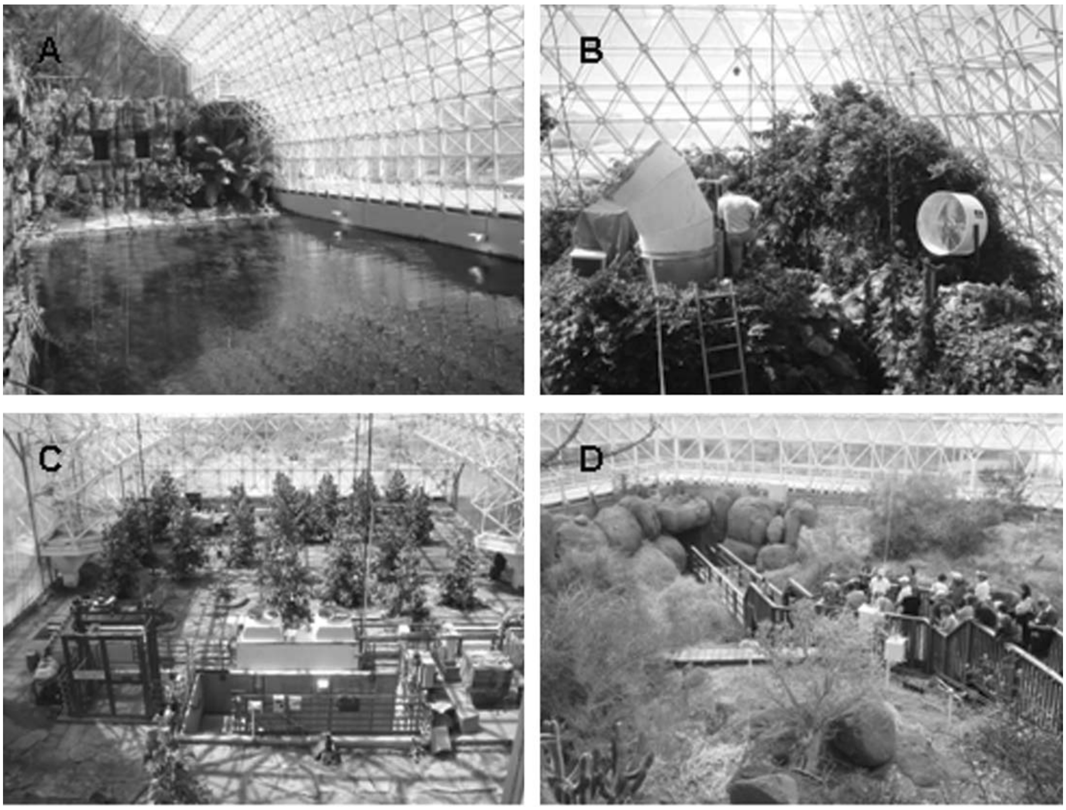

Fig. 2 Central mesocosms in Biosphere 2 Laboratory. (A) Ocean. (B) Rainforest. (C) Agriforestry, some weeks after resprouting of the cottonwood plantation in the center bay. (D) Desert, visited by a guided tour.

tree heights of 15–20 m, maximal soil depths of 6 m and a maximal ocean depth of 7 m. The total area to be used for scientific research currently equals approximately 7 600 m^2, the total volume (including the air mass in the basement, soils and water) equals roughly 140 000 m^3. The leakage rate of the research-driven biomes to the outside atmosphere equals about 1–2% per day. Incident light levels in the mesocosms reach maximal values of up to 1500 μmol m^{-2} s^{-1} PAR. A walkway through the savanna, the mangroves, the thornscrub and the desert is used for touristic purposes. Each day, five to ten groups of 10 to 20 persons are guided through those mesocosms, through the basement and through the south lung on a 60 min tour. Access to the rainforest and the agriforestry mesocosm is restricted to scientists only. Visitors have access to most parts of the former habitat area; only office and lab spaces are restricted there.

Throughout each mesocosm, CO_2 concentration, temperature, vapor pressure deficit and irradiation are measured at several locations and at different heights. This data is collected by Campbell Scientific CR 10X data loggers and is managed by Campbell Scientific Loggernet software. The data can be retrieved as numerical tables or trend graphs either on site or *via* remote access.

While separation by PVC-curtains put an end to investigations into the interactions between the different mesocosms,[43] it allowed for manipulative research in each of the mesocosms

Table 1 Dimensions of the mesocosms within Biosphere 2 Laboratory

	Agriforestry	**Rainforest**	**Ocean + connected mesocosms**	**Desert**	**Total**
	West Bay		Ocean		
	Center Bay		Mangrove, Marsh, Savanna, Thornscrub		
Mesocosm	East Bay				
Dimensions/m (north–south/east–west/height)	41/17/24 41/20/24 41/17/24	44/44/28	84/30/27	37/37/23	165/90/28
Area/m^2	553 653 553	1 950	700 1 800	1 400	7 600
Air volume/m^3	11 500 12 400 11 500	27 000	42 000	18 000	122 000
Soil or water volume/m^3	550 650 550	6 000	2 600 4 000	4 000	18 000
Air handling capacity/m^3 min^{-1}	4 080 4 080 4 080	8 150	9 500	4 080	34 000

separately. The mesocosms most actively used for research purposes are the tropical rainforest, the former agricultural area (agriforestry mesocosm), which is now used as a cottonwood plantation and the ocean with its Caribbean coral reef. The agriforestry mesocosm is separated by PVC-curtains into three almost identically-sized compartments (bays). Atmospheric exchange rates between those bays are 2–5% per h. Those bays are kept on CO_2 levels of 400, 800, and 1200 ppm, respectively. Recently, the desert was separated from the adjacent wilderness mesocosms that are still connected to each other (savanna, thornscrub, mangrove, marsh, ocean).

Today, B2L is well suited for studies of mass balances, because it is still operated with closed circles of nutrients and water and because air mass exchange with the atmosphere is monitored and quantified. The properties of B2L mesocosms are captured in different models, describing ecosystem growth parameters,[44] the relation between litterfall and respiration,[45] heat budgets[46] and water cycles.[47] The model of the global water cycle in B2L can be applied to calculating responses towards a tracer or pollutant infiltration. This model captures the way a tracer or contaminant is spread around the B2L water system and characterizes the dynamics of its time-dependant concentration. Even seasonal patterns can be taken into consideration.[48] By calculating reservoir turnover times, Tubiello *et al.*[49] showed that there are three main pools of water inside B2L. A fast pool comprising 60% of the available water used daily is recycled within a month through the air handlers' condensation system as a consequence of evapotranspiration; a medium pool of 30% moves through the soil profile and is recycled within a year; the remaining pool of 10% moves through the ocean and has a turnover time of several years.

The value of B2L for the ecological scientific community has risen over the past few years, since the soil has stratified and is therefore more realistic as in the beginning. In the cottonwood plantation soil, nutrient conditions resemble those of commercial forest plantation sites. Both the soil organic carbon (24 g kg^{-1}) and the C:N ratio (12) have dropped significantly since the start of Mission 1 by about 25%, having reached levels which are commonly found in agricultural soil systems.[50,51] In the rainforest soil, the organic carbon content is within the range of natural tropical rainforests.[38] Although some soil development took place during Mission 1, in 1993 the soil was still more alkaline and contained higher concentrations of calcium, magnesium and potassium than typical rainforest soils.[52] Moreover, the micrometeorology within the biomes is comparable to field conditions. Another practical advantage of B2L as a tool for environmental experiments is that the space-frame structure of the roof provides access to the outermost layers of the canopy *via* a specially designed access system,[53] rope-climbing techniques or space-frame climbing. Thus, upper layers of the canopy can be reached without stressing the plants by putting up ladders or tower structures.

A disadvantage compared to other settings for biological experiments is that proper replicates for ecosystem studies are not possible except for the agriforestry mesocosm, but have to be replaced by replicates in time. Furthermore it is at least difficult if not impossible to change the soil properties due to the enormous costs. Certain parts of the facility contain a new species of nematode, discovered in B2L,[54] which is a putative pest for crop plants and is not likely to be native to Arizona. Hence, the biomes are under quarantine. The space frame structure and the laminated glass still reduce the irradiation in the biomes to about 50% of the incident irradiation and block out UV radiation completely. CO_2 concentrations change diurnally (throughout 24 h) by as much as 500 ppm,[55] due to the large ratio of phytomass to airmass inside B2L; nevertheless diurnal mesocosm gas exchange characteristics are comparable to field situations.

All those features can be seen as advantages or disadvantages, depending upon the research facility with which B2L is compared. In comparison to field experiments, the Biosphere plant communities are artificial, but the possibility to control environmental parameters is much better. In the field, one is restricted to mere observations of global climate change. Manipulative experiments are practically not possible there, which is an enormous drawback for establishing causal relationships. As compared to growth-chamber scale studies, B2L provides a much more realistic model of what is going on in a natural system, but the flexibility to manipulate the B2L system is of course smaller. By many standards, research using the soils in B2L is a vast improvement over research on plants grown in small containers of vermiculite or commercial potting mix. Data from such small-scale studies is currently used to parameterize large-scale models for global change.

Because of the lack of manipulative studies in appropriate spatial and temporal scales, there is a large gap between climate predictions originating from models and experimental results of ecosystem research. It will be important in the future to bridge this gap between the 'Newtonian' and 'Darwinian' approach in environmental sciences; the one relying on theoretical models, the other relying on observations.[56] The way to mediate between them is to perform manipulative experiments on meaningful scales. Moreover, ecophysiological experiments that are currently performed on the leaf- or single-plant level can be scaled up to reactions of the entire canopy in B2L; a step that is very important for reaching realistic estimations of all kinds of environment-related processes.

2.2 Major research achievements

Today, there is hardly any doubt that man drives global climate change and that this has been dramatically affecting the earth's ecosystems within the last decades.[57] The goal of the current management of B2C is to offer B2L as a unique masterpiece of ecological engineering to a scientific community that is interested in performing manipulative experiments on a sufficiently large scale to address ecosystem responses towards global climate change. If this mission proves successful, B2L might be a prototype apparatus for an emerging discipline, which brings together the resources and experience of natural sciences and engineering, social sciences and economics in the field of experimental climate change science or earth systems science.[58] Efforts have to be made to provide more facilities for studies in appropriately large scales. May[59] observed that *"many of the most intellectually challenging and practically important problems of contemporary ecological science are on much longer time-scales and much larger spatial-scales"* than currently investigated. He noted surveys in 1999 showing only 25% of manipulative field studies exceeded 10 m in size, and 40% lasted less than a year, with only 7% exceeding 5 years.

Within the last few years, B2L has delivered novel, process-level, mechanistic insights to ecosystem functions in the soil–plant–atmosphere and in the benthos–ocean–atmosphere continuum, mainly by research efforts in the ocean, in the rainforest and in the agriforestry mesocosms. While species surveys and observational, descriptive studies are still performed at B2C, manipulative experiments have clearly come into the focus of attention (Fig. 3). CO_2 has been the most important parameter to be manipulated in those studies, either by increasing its concentration in a biome transiently and repeatedly for a certain period in time or by comparing results from the three different agriforestry mesocosms. For example, it was shown that the water-use efficiency of desert plants increases in elevated CO_2. While evapotranspiration remained constant, carbon uptake rose with increasing CO_2.[44] There have also been several studies dealing with the effect of changed temperature regimes and drought. Currently, a wide range of manipulative ecological studies is performed at B2C.

B2L is proving its capacity as a valuable tool for application

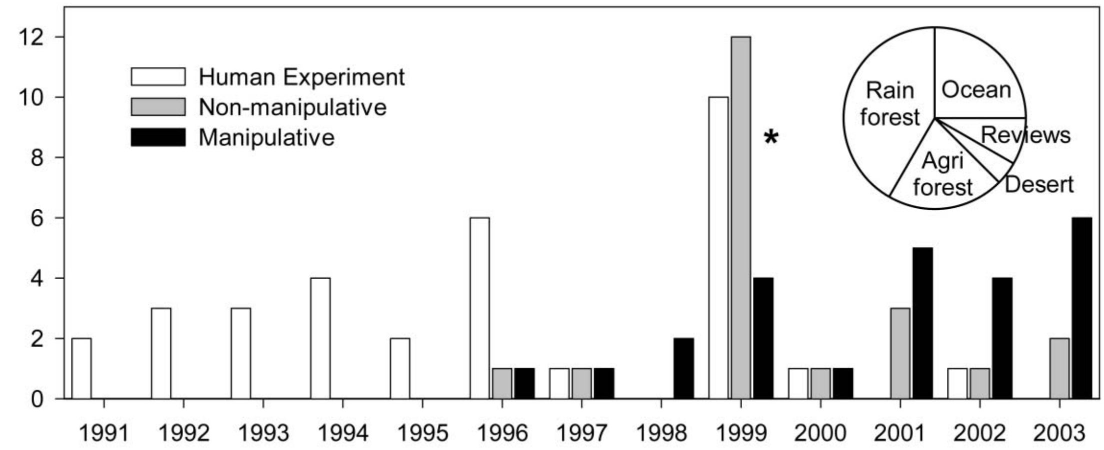

Fig. 3 Numbers of publications from Biosphere 2 Center research. Publications are split up in three groups: (non-manipulative) studies from the human experiment (test module, Mission 1 and Mission 2), non-manipulative studies under the leadership of Columbia University, and manipulative studies under the leadership of Columbia University. Data for this figure covers all original studies and reviews published from B2C research as full articles in peer-reviewed scientific journals ($n = 78$). Inset: distribution of studies from manipulative experiments among the mesocosms. *In 1999, a 22-paper special issue on B2C was published in the journal *Ecological Engineering*.

of emerging remote-sensing techniques to quantify the state of an ecosystem,[60,61] for studying the interaction between photosynthesis and growth processes,[62] for scaling studies from leaf to ecosystem level[63] and for studies in chemical ecology (personal communication Witte, manuscript in preparation; personal communication Berger, manuscript in preparation). Current research in the desert mesocosm is investigating germination under different water regimes (personal communication Schwinning, manuscript in preparation). Rain events are unpredictable in deserts and *El Niño* events even add to the variation. The test module has been remodeled to a cactus habitat, in which the carbon balance of those drought-adapted plants is investigated integrally for the mesocosm and differentially for above- and below-ground biomass.[64] The growth chambers have been used to assess water- and nutrient efficiencies of desert plants in future climates.[65] In addition to the research initiatives described above, also the general mission of the project, both during the time of Missions 1 and 2[2,5,9] and presently[66,67] as well as the philosophy with which science was conducted in both phases,[58,68] and the way education and public outreach is performed in B2C[40], is reflected critically in several scientific publications.

A more detailed representation of the various studies mentioned above would exceed the scope of this review. To give an impression of the way B2L might serve as a tool for experimental climate change science, the core findings of the most interesting studies performed in the three central 'research mesocosms' of B2L are discussed below in more detail.

Ocean. Biological oceanographers have been trying for years to bridge laboratory and field research and have been more successful in this than scientists in most other areas. An established culture of planning long-range, sometimes long-term, experiments at sea using well-equipped research vessels, has helped to build a strong collaboration of oceanic and atmospheric sciences in the interest of earth systems science. There are, however, many experiments that have simply not been possible to conduct at sea.

For example, it has long been observed that there is a correlation between the calcification rate of a coral reef and the saturation state ($[Ca^{2+}][CO_3^{2-}]/K_{sp}$) of the overlying water concerning calcium and carbonate.[69–73] The observations showed that the longer water resides over any particular reef, the more the carbonate ion activity and hence carbonate saturation state is reduced and the lower the calcification rate of the reef. Although a strong correlation between coral reef calcification and saturation state of the water was shown, coral reef ecologists did not accept this as evidence of a cause and effect relationship because it went against existing theories of coral calcification. Those theories predicted that corals were not very sensitive to changes in the external carbonate cycle because they achieve high rates of calcification by pumping Ca^{2+} and CO_3^{2-} ions from the environment into a highly supersaturated internal space.

Only when Ca^{2+} and CO_3^{2-} concentrations were manipulated independently under controlled conditions in the laboratory[74,75] and in the B2L mesocosm,[76,77] were coral reef ecologists convinced that coral growth was indeed very sensitive to changes in the carbonate saturation state of the water.

A doubling in atmospheric CO_2 will strongly (40%) reduce the production of $CaCO_3$ by the coral reef community.[76] Even after several years of exposure, the organisms exhibit no capacity for acclimation to high CO_2 conditions. The elevated CO_2 conditions in the water body were achieved by changing the pH of the water accordingly. Coral growth was depressed across the full range of light intensity and hence water depth in which the organisms were found.[77]

Although elevated CO_2 stimulates C-influx into the marine ecosystem, it has no effect on the net production of organic matter because the cycling of organic carbon is also stimulated.[78] Taken together, these results allow us to predict that rising atmospheric CO_2 will not boost macroalgal growth, but it will drive a change in coral community structure due to the negative impact on coral growth. We can also predict that coral reefs will not become sinks of atmospheric CO_2 in the long-term, but they will become a weaker source as calcification rates decline.

In a series of experiments, in which nutrient uptake kinetics were followed as a function of water velocity, the following hypothesis was confirmed: uptake kinetics of a benthic community are controlled by diffusion through a nutrient-depleted boundary layer, the thickness of which is controlled by water velocity.[79,80] Recent work attempts to derive the nutrient uptake kinetics directly from measurements of turbulence in the water column, which controls the thickness of the nutrient-depleted boundary layers around the benthic organisms. Also, principal relationships between organic carbon production and respiration[81] as well as processes affecting the diurnal carbon cycle[82] and the impact of rain on gas exchange between a body of saltwater and the atmosphere were investigated. To test the effect of rain on gas exchange, specially designed sprinkler systems were mounted above the ocean mesocosm to produce rain that was as close to natural rain as possible in terms of drop size distribution and rain rate. In addition to quantifying gas flux with a gaseous tracer, various state-of the art instruments were applied to quantify waves, currents, near surface turbulence, skin temperature, profiles of temperature and salinity, rain rates and raindrop size distribution. For future research, the effects of increased temperature and CO_2 are envisaged to be the focus of investigation.

Rainforest. A crucial question of interdependencies between terrestrial ecosystem functions and global climate change is whether CO_2 sink/source behavior of forests will be changed during global warming and with increased CO_2 levels. While the above-ground biomass is a sink for CO_2 due to photosynthesis during daytime and a (weaker) source during respiratory phases (at night and in winter), not even the sign of the overall effect of a forest ecosystem can easily be predicted due to the variable strength of soil respiration. Some model simulations,[83] atmospheric CO_2 analyses[84] and flux tower measurements,[85] indicate that a significant fraction of the terrestrial carbon sink (approximately 1 Gt C per year, 1 Gt = 10^{15} g) may be located in tropical rainforests. There are enormous concerns that this sink may switch to a source of CO_2 in response to global climate change hence producing a positive feedback for the greenhouse effect, and the lack of experimental research on this topic is expressed clearly in the recent report of the IPCC.[86]

It is essential that such ecosystem processes are properly represented in earth system climate models, and it is true that the predictive capacity of such models has been advanced by improved representation of ecosystem processes.[87,88] Predictions of the magnitudes of pool sizes, fluxes and residence times of carbon and key nutrient elements as rate limiting mechanisms in global carbon budgets have been addressed with models derived from extensive observations on natural systems,[89,90] but have not been linked with controlled experiments at appropriate spatial scales. It is inappropriate that most of those models have such a limited experimental basis, and often rely on a few laboratory scale studies to parameterize processes over vast areas such as the Amazon rainforest.

Large-scale experiments on the question of tropical rainforest carbon sink capacity in a changing environment have been performed in B2L. A remarkable correspondence between daily carbon fluxes measured in B2L (personal communication Lin, manuscript in preparation) and data from flux tower observations in the Amazon[91] was found. Both approaches show that drought – as *e.g.* in *El Niño* years – decreases ecosystem carbon influx and efflux[61] as well as growth, but does not switch the tropical forest from a sink to a source of CO_2. Models also suggest that the atmosphere is approaching CO_2 concentrations at which CO_2 influx is limited by the rate of photosynthetic processes. This projection from leaf scale experiments to ecosystem properties has been explored in B2L experiments. Those experiments suggest that the sink capacity of the tropical forest may saturate by the mid 21st Century.[92] The rate of photosynthetic fixation of CO_2 in leaves,[93–95] trees and whole biomes[38,55,96] increases with rising atmospheric CO_2 levels, but saturates at a certain level. The exact system response depends on the species composition. Increased photosynthetic activity and hence plant productivity in elevated CO_2 might be explained by interesting changes in leaf structure.[97] In leaves from plants in elevated CO_2, mitochondrial number is increased and chloroplast fine structure altered. When extrapolating results from the B2L rainforest mesocosm to the real world, it has to be kept in mind that although many micrometeorological parameters resemble those in rainforests of the Amazon basin, temperature stratification above the forest canopy is pronounced and differs from natural situations[98] due to little turbulent mixing caused by the comparably small volume of the mesocosm in relation to plant biomass.

Yet, the large ratio of leaf biomass to chamber volume of the B2L rainforest provides more advantages than disadvantages. For example, it leads to high signal to noise ratios and excellent sensitivity for diurnal profiles of net ecosystem CO_2 flux at present and future atmospheric CO_2 concentrations.[92] When coupled with analyses of the stable isotope compositions in the atmosphere of the chamber, the signatures associated with stable isotope discrimination during diffusion, evaporation, carboxylation and other processes occurring during the assimilation of CO_2 and its release from respiration into the ecosystem can be measured in real time. In the course of a day, the manifold aspects of this greenhouse gas flux in the Biosphere 2 rainforest can be evaluated with 10× greater sensitivity and 100× more rapidly than comparable data can be obtained from the inter-annual variation of these signatures in the planetary atmosphere. Just as we have made progress in understanding regulatory interactions in biochemistry through the application of control theory, there is every reason to believe the isotopic signatures of global enzymes[99] as revealed in Biosphere 2 experiments, will help uncover control principles that *"are analogous at the ecosystem, population, organism, and even enzyme reaction level"*.[100]

Agriforestry. Natural forest systems are increasingly replaced by managed forest systems. Hence, the effect of global climate change on their carbon sink capacity will gain importance throughout the upcoming centuries. The sink capacity of temperate forests has been investigated in field experiments using 'Free Atmosphere Carbon dioxide Enrichment' (FACE) techniques under mid-21st Century treatments. Nutrient limitations,[101] acclimation of assimilatory processes,[102] and soil responses to warming[103] only accelerate the time at which the sink capacity of forests will be reached. Given the broader knowledge about effects of CO_2 elevation on temperate forests *via* FACE and other medium-scale experiments, research in the cottonwood plantation at B2L was mainly focussed on the combined effects of temperature and CO_2 on the mesocosm and especially on evaluating the validity of current scaling models.[104]

For plant growth, basic research is currently performed to evaluate growth reactions in elevated CO_2 at different levels of plant organization. The results indicate that reactions on the leaf level cannot be easily extrapolated to reactions on higher levels.[63] While this might seem self-evident for growth reactions, it is the classical assumption for gas-exchange models, where traditionally, models of stand-level gas exchange have been based on single-leaf measurements. Evaluations of leaf *versus* stand level carbon fluxes at B2L have indeed shown that ecosystem warming stimulates leaf respiration more than measured from leaf warming alone.[105] Furthermore, it has been found that considerable within-canopy variation in rates of respiration and its temperature response can confound canopy efflux estimates.[106] The variation between leaves at different heights within the canopy is correlated with naturally occurring gradients in leaf carbohydrate and nitrogen content. Hence, quantifying the vertical distribution of leaf respiration is important for sensitive models of ecosystem gas exchange.

It has also been shown in B2L that nocturnal warming stimulates leaf respiration, lowers reserve carbohydrates and stimulates subsequent photosynthesis.[107] Since future climate scenarios suggest a differential rise of diurnal and nocturnal temperature levels with increased effects at night,[108] this effect has to be taken into account in future carbon cycle models. Another significant improvement for carbon cycle models was achieved through experiments on soil respiration in the coppiced cottonwood plantation before resprouting of the trees in spring. There, it was shown that point measurements of soil CO_2 efflux on a small volume of soil may not necessarily reflect the overall community response. Due to the high variability within a set of point measurements (10 randomly distributed measurement points in each bay of at least 550 m^2

area), the projected value from point measurements and the value of the community response differed by 36%.

All these experiments demonstrate clearly that leaf to canopy and landscape-level processes cannot be simply extrapolated for modeling purposes and they also build a strong case for refined remote sensing methods to monitor effects of environmental changes on agricultural and natural field plots instead of extrapolating results from small-scale measurements. The feedbacks identified could have many significant influences on plant and ecosystem carbon exchange under global change scenarios.

Another lively field of research activities in the B2L agriforestry mesocosm is the investigation of trace gases and stable isotope fluxes. Recent studies in the agriforestry mesocosm of B2L have included stand level C-flux measurements using temporary closure in order to explore responses to multivariate changes in environmental factors. Furthermore, combined effects of drought and temperature are under investigation and on-line stable isotope pulse experiments are revealing the sources of carbon used in respiration.

Trace gas monitoring, which is also available on-line at B2L, recently showed that elevated CO_2 reduces isoprene emissions.[109] Almost all commercial agriforestry species emit high levels of isoprene; hence proliferation of agriforest plantations has significant potential to increase regional ozone pollution and enhance the lifetime of methane, an important determinant of global climate. The isoprene emission study, performed in B2L benefited from the absence of isoprene-anabolizing UV light. It showed that the negative air-quality effects of proliferating agriforests may be offset by increases in CO_2.

2.3 Relation to other existing and potential facilities

Different facilities such as Terrestrial Ecosystem Research Facilities (TERF), FACE-sites, open-top-chamber facilities, eddy-covariance flux towers or classical greenhouse and laboratory facilities pursue similar research goals as B2C. It is often difficult to combine elevated CO_2 treatments with other treatments such as temperature, precipitation (amounts and timing), and other atmospheric components such as O_3 in non-enclosed facilities like FACE sites or open-top-chambers.[101,110] The mini-FACE experimental design,[111] which sacrifices plot area but incorporates multi-factorial climate change parameters (*e.g.*, warming, N-deposition, precipitation) with elevated CO_2, is especially suited to grasslands and small model systems, but dramatically increases edge effects. A general advantage of non-enclosed sites in comparison to B2L or other enclosed settings, such as the ecotron facility in the UK,[112] where 16 chambers of 4 m^2 are used to work on mini-ecosystems, is the more natural light environment and an often more realistic micrometeorology. Yet, the artificial light environment in B2L with complete absence of UV-radiation can also be beneficial for some studies, like in the measurement of UV-photolabile isoprene emissions.[109]

Another major difference between B2L and other manipulative facilities is that the basic costs of facility establishment differ enormously. Since it will be impossible to construct a "control" B2L, replication is restricted to replication in time. While taking replicates in time is an established tradition in laboratory research, ecological research usually relies on replication in space, which can be much more efficiently realized in FACE or flux tower experiments due to their lower construction costs. Yet, in terms of operational costs, B2L compares favourably with established FACE-sites. The costs of homogeneously gassing a field plot of a comparable size to a B2L mesocosm with CO_2 over longer periods of time can easily exceed the costs of providing appropriate climate control for such a mesocosm in B2L (personal communications Stan Smith, Nevada Desert FACE site and George Hendrey, Duke Forest). The costs for providing elevated CO_2 levels in the open flow but contained B2L are only a 2% fraction of the total operational costs – mainly energy costs.

In conclusion, a wise solution for efficiently exploiting the full potential of the existing variety of experimental climate change science sites would be to initiate a concerted effort to define experiments individually suited to the specifications of each site. Of course, such an effort would require an intensified global linking of the different sites with a central management or at least frequent meetings to coordinate future aims of the partners, ideally in coordination with ongoing long-term observational ecological studies and modeling efforts. Whether such an international effort can be successfully realized or not will depend on whether or not the existing scientific communities dealing with ecological questions are willing and able to communicate with each other and to define common aims.

Apart from being an element of the network of experimental climate change science sites, B2L can also play a vital role in the design of future facilities for contained ecosystems with a wide range of specific scientific or commercial aims. Those facilities should take advantage of the lessons learned at B2L over the last two decades. Some examples in which the B2L experience is currently stimulating new developments are the Boreosphere project in Sweden, the Biosphere 3 project in Japan, the Biotron facility in Canada and enclosed life support systems for space stations planned in the US and in Japan.

The Boreosphere design aims at enclosing and minimally disturbing existing plots of boreal forest. Those plots will be equipped with soil monitoring and temperature control systems as well as with aboveground climate control systems. The Biosphere 3 design will contain genetically modified crop plants, designed for optimal performance in future climates that can be safely evaluated there in a realistically simulated environment. In the Biotron facility, small plots of controlled ecosystems will be made available for research in biotechnology to elucidate mechanisms of interaction between plants, microbes and insects in extreme environments. Last but not least, the dream of sending man to Mars and providing him with an amenable and self-sustaining environment instead of feeding him from tubes and carrying in every mole of water and oxygen is still alive. Current considerations at NASA are based on crews of eight members that would live in a complex of approximately 1000 m^3–0.5% the size of B2L.[113] There, 40% of the space would be operated as agricultural area, providing the majority of the crew's caloric and nutritional requirements, 20% would be operated as water recovery system, including the services of higher plants and microbes. The balance of CO_2 and O_2 would mainly be regulated *via* technical means, as it is currently done at the International Space Station ISS, for which the use of natural ecosystems for food production or other purposes is not envisaged.

3. The future: Anything between ruin and research hot spot

B2C was designed and operated in the past as a visionary observational experiment, it has since then been operated as a scientific facility dealing with experimental climate change science and has just started to transform its character more and more towards a multi-user facility. The vision of the current management has been, to operate B2L in a similar way as a research vessel or experimental platform, such as a telescope or a particle accelerator, with research teams joining the long-term studies in the different mesocosms for specific short-term experiments or with research teams remotely monitoring experiments online. First experiences with this mode of operation have been made recently in experiments of all three major research mesocosms.

As an outcome of an international meeting of environmental scientists held recently at the B2C, several core experimental areas were pointed out that should guide the use of B2L for experimental climate change science in the coming years:

(1) Measurements of pools, fluxes and residence times of carbon and other elements (N, P) as rate limiting mechanisms in marine and terrestrial ecosystems that will permit application of control analysis, for example, to understanding responses of ecosystems to changing climate and their impacts on predictions of atmospheric CO_2 concentrations.

(2) Experiments that will complement and extend observational ecosystem level research done in FACE, flux tower and Long Term Ecological Research (LTER) programs.

(3) Experiments that will exploit the greater sensitivity and more rapid response time of changes in the natural abundance isotopic composition of ecosystem components and gases in B2L to understand processes and mechanisms controlling biogeochemical cycles in natural ecosystems.

(4) Experiments to calibrate remotely sensed optical signals against whole ecosystem carbon fluxes in B2L for application in natural ecosystems.

(5) Research that will advance and validate modeling methods through the control of environmental variables and capacity for replication in time in B2L.

(6) Experiments that will manipulate biocomplexity (biodiversity) to determine its role in robustness of ecosystem responses to changing climate.

(7) Research that will expand our experience in the operation of a collaborative environment to support an inclusive multiuser facility as an open ecological observatory for experimental climate change science.

(8) Research that will create an intellectual center for experimental climate change science and become a nucleus for development of new theories and methods for field research in the natural ecosystems of Biosphere 1.

As can be deduced from those goals, B2C has been planned to be open for interactions with a variety of scientific communities. The flow-through contained mode of B2L operation provides a very good basis for manipulative research in the field of environmental monitoring. Conceivable studies comprise investigations of tracer or contaminant distributions, mass balancing of different compounds or monitoring of environmental changes of all kinds during manipulative experiments. There is a lot that can be done with this unique tool. Yet, the compromises that have to be taken and the resistances that have to be overcome when applying a new tool in an environment of established tools are manifold and have been pointed out in this review. The sum of those compromises, a lack of interest from grant organizations within the US and a reorientation in the structure of the Earth Institute at Columbia University have led to the decision that Columbia University will not operate B2L any longer although there was a contract to operate the facility until 2010.[114] From January 2004 onwards, the building will be closed for research, education and public outreach, but shall be reopened for touristic purposes soon. The biomes will be kept in some form of hibernation state and wait for better times to come. The opportunities that are provided by this facility were and are unique. Whether they meet the political agenda of the time and the country in which the facility is located, and whether the people and organizations that have been responsible for tapping the potential of the building since its foundation was laid were chosen with good fortune remains questionable. Given the size of the facility and the long-term nature of environmental studies, three points seem to be absolute prerequisites for using the potential of this facility to conduct environmental research of any kind there in future: (1) the organization or the consortium managing the facility has to have a long-term interest in the nature of the studies conducted at the facility; (2) it has to have a clear concept of how to conduct research in this unique building and (3) it has to have a long term concept on how to finance this venture. We would like to conclude this review with the words of a senior researcher, who has been associated with this project for several years (J. Berry in ref. 67): *''This place will attract risk takers. It fosters innovative thinking and creative research. It's a place for people who think about ecosystems differently and who want to test new methods. What's happening at the Biosphere now is just a beginning.''*

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