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A coordinated set of ecosystem research platforms open to international research in ecotoxicology, AnaEE-France

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Abstract

The infrastructure for Analysis and Experimentation on Ecosystems (AnaEE-France) is an integrated network of the major French experimental, analytical, and modeling platforms dedicated to the biological study of continental ecosystems (aquatic and terrestrial). This infrastructure aims at understanding and predicting ecosystem dynamics under global change. AnaEE-France comprises complementary nodes offering access to the best experimental facilities and associated biological resources and data: Ecotrons,-seminatural experimental platforms to manipulate terrestrial and aquatic ecosystems, *in natura* sites equipped for-large-scale and long-term experiments. AnaEE-France also provides shared instruments and analytical platforms dedicated to environmental-(micro) biology. Finally, AnaEE-France provides users with data bases and modeling tools designed to represent ecosystem dynamics and to go further in coupling ecological, agronomical, and evolutionary approaches. In particular, AnaEE-France offers adequate services to tackle the new challenges of research in ecotoxicology, positioning its various types of platforms in an ecologically advanced ecotoxicology approach. AnaEE-France is a leading international infrastructure, and it is pioneering the construction of a European AnaEE infrastructure in the field of ecosystem research. This infrastructure is open to the international community of scientists in the field of continental ecotoxicology.

Keywords

Ecotoxicology, Ecotrons, Environmental simulations; Environmental survey, Process measurements, Genetic resources, Biochemical markers, Environmental stressors, Higher tier testing, Ecologically-based procedures, Mesocosms, Information system, Modeling

Introduction: from ecosystem understanding to ecotoxicology

Human activities have altered continental ecosystems worldwide and generated a major environmental crisis (Pereira et al. 2010). These alterations raise urgent societal questions on how to best produce goods while at the same time preserving ecological services. In this respect, understanding and predicting the dynamics of ecosystems and biodiversity, and the provision of ecosystem services under global changes are a key scientific issue with a high social value (Stockad 2005). Yet, ecosystems are inherently complex objects characterized by interacting abiotic and biotic processes, strong temporal and spatial heterogeneity, coupled ecological and fast evolutionary dynamics, and a huge diversity from genes to communities (reviewed in Loreau 2010). To truly develop integrative ecosystem biology and to assess the consequences of various forcing factors, we therefore need new approaches that bridge the traditional gap between life and environmental sciences.

Experimental approaches with model ecosystems provide the best means to achieve these goals (Stewart et al. 2013). Today, a major focus in ecological sciences is therefore on the production of quantitative, experimentally testable approaches using advances in our ability to characterize complex ecological systems from genes to ecosystems (Loreau 2010). Global changes are generally affecting organisms at the individual level. Individual responses can, however, have cascading effects at higher biological levels such as populations, communities, and ecosystems (Rustad 2008). To deal with this complexity, we need experimental tools spanning contrasted ecosystem types and spatiotemporal scales (Osmond et al. 2004). The role of all biological compartments in the ecosystem must be determined, including micro-organisms that require specific approaches to assess their importance and function, and it is also essential to monitor emergent properties of ecosystems (Jessup et al. 2004). In addition, one must find ways of reducing the level of complexity of ecological systems using models to assimilate date and to make predictions. By offering open-access platforms to experiment, analyze, and model complex ecological systems, the new ecosystem research infrastructure AnaEE-France (for-“Analysis and Experimentation on Ecosystems-France,” http://www.anaee-s.fr/) has been specifically built to solve these three grand challenges. AnaEE-France brings together an integrated network of major experimental, analytical, and modeling platforms dedicated to the study of continental ecosystems (aquatic and terrestrial) in France. It provides tools to conduct innovative fundamental research on gene- environment interaction, biodiversity, biotic interaction and ecosystem functioning, and eco- evolutionary processes.

One key issue of AnaEE-France is to understand and predict biodiversity and ecosystems dynamics under global changes, including chemical contamination. In this context, some of its platforms provide relevant tools for addressing new perspectives in the field of ecotoxicology. For many years, the usual procedures for ecological risk assessment (ERA) have been based on tests providing information on the effects of single substances on isolated groups of individuals of model species maintained under simplified and constant laboratory conditions (Vighi and Villa 2013). These approaches led to the development of internationally acknowledged tools supporting regulation ~~(*e.g.*,~~ (e.g., Technical Guidance Document; European Commission 2003). However, most of these tests, used for the assessment of the adverse effects of pollutants on ecosystems, suffer from a lack of ecological realism. The need for more-ecologically based approaches is now recognized by the scientific community in ecotoxicology and has been highlighted by a recent document of the European Commission (European Commission 2013, Fig. 1). In that-context, AnaEE- France offers adequate platforms to tackle the new challenges of ecotoxicology, positioning the various types of platforms in an ecologically advanced ecotoxicology approach. Below, we present these platforms and highlight how they can provide fruitful research perspectives in ecotoxicology for the future.

AnaEE-France platforms and their relevance for ecotoxicology research

AnaEE-France is a distributed infrastructure made out of five thematic nodes, each of them lining up a coherent set of coordinated services (Fig. 2). The core of AnaEE-France is made of experimental facilities, including (i) two-Ecotrons that provides means to control and monitor ecosystems *ex natura* (node 1 in Fig. 2), (ii) three seminatural experimental platforms to manipulate ecosystems in situations intermediate between Ecotrons and the field (node 2), and (iii) 21 sites dedicated to large-scale and long-term (spanning decades) experiments *in natura* in major biomes (forests, croplands,-grasslands, and lakes, node 3). These experimental facilities encompass a range of experimental conditions, ecological complexity, or human pressures according to a logic that is essential to make scientific progress-toward a comprehensive understanding of ecosystem biology. AnaEE-France provides also shared instruments and analytical platforms dedicated to environmental biology (node 4). It rests on shared information systems, databases, andmodeling tools aiming at coupling ecological, agronomical, and evolutionary approaches (node 5). Below, we describe the relevance of several service already used, or that ban be used for research in ecotoxicology.

Ecotrons

Ex natura systems, such as climate chambers or other controlled environment facilities, are important tools for the analysis of toxicity such as the analysis of the dynamics of substances (e.g., photochemical chambers) or of their effects on individual organisms (e.g., environmental test chambers for plants). Since ecotoxicology is increasingly moving toward the analysis of the impact of chemicals on complex communities and ecosystems (Van Straalen 2003; Vighi and Villa 2013; Calow and Forbes 2014), it may also take advantage of advanced controlled environment facilities such as Ecotrons. An Ecotron can be defined as an experimental facility where ecosystems are confined in enclosures allowing simultaneously a-multifactorial control of environmental conditions and some accurate, on-line measurements of ecosystem processes. A scheme of the Ecotrons principles is presented in Fig. 3. An Ecotron also has series of independent experimental units suitable for the statistical comparisons of observations between treatment groups, such as different climate conditions or soil types (Stewart et al. 2013). Tools such as Ecotrons are important because they offer the possibility for coupled experiments that help understand the dynamics of natural systems, complementing the more realistic *in natura* experimental facilities.

The first Ecotrons provided technological tools similar to classical plant growth chambers, but they were primarily designed to understand and monitor whole-ecosystem processes (Lawton et al.-1993; 1996). In AnaEE-France, Ecotrons offer improved the control of the environment and the measurements of ecosystem processes. They consist of five complementary experimentation platforms located in two sites (http://www.ir-ecotrons.cnrs.fr/), the European Ecotron of Montpellier (43° 40′ 54″ N, 3° 52′ 36″ E) and the Ecotron Ile-De-France nearby Paris (48° 17′ 15″ N, 2° 40′ 35″ E). The platforms differ by their size, controlled conditions and measurements capacities, and the type of ecosystem they can host (foe examples, see Fig. 4). The largest units are in the-“terrestrial Macrocosms” platform of Montpellier designed to host intact or reconstituted samples of continental terrestrial ecosystems from 2 to 15-t each, for several month- to a few year-long experiments (Milcu et al. 2014). This platform has 12-day lit units of 40 m^3 each and can accommodate soil cores from 2 to 5 m^2 with a depth from 0.5 to 2 m.

Weather (temperature, humidity) and atmospheric conditions (CO_2, $^{13}C\text{-}CO_2$) above the soil core can be controlled in each macrocosm independently. Natural light is reduced by only 10 % (or more with-shadings), and a large proportion of UV is kept. Sunlight can be blocked and artificial light sources can be used. A soil temperature gradient can be created, and the soil profiles of volumetric water content,-

temperature, and CO_2 concentration are measured. Ecosystem exchange measurements are made online (evapotranspiration and flux of CO_2, CH_4, N_2O) and measures of isotopologues ^{13}C-CO_2, ^{15}N-N_2O, ^{18}O-CO_2, ^{18}O-H_2O, ^{17}O-H_2O, and ^{2}H-H_2O allow the study of additional processes.

Two other platforms offer the possibility to manipulate smaller systems similar in size to mesocosms (Stewart et al. 2013). The Montpellier “terrestrial Mesocosms” platform includes 16-day lit plant units made out of a combination of modular lysimeters (up to 1 m^2 surface and 1 m deep soil) and of a canopy enclosure up to 1.8 m high. Weather conditions (positive or negative temperature, humidity, and optional controlled light) and the CO_2 content (preindustrial or-postindustrial) of the atmosphere can be controlled. The organic matter can be continuously ^{13}C-labeled. This platform benefits from the same automated measurement of ecosystem processes than the “terrestrial Macrocosms” platform. The “terrestrial and aquatic-Mesocosms” system at Ecotron Ile-De-France is made out of replicated, controlled environment chambers with artificial light conditions and simulated weather and atmospheric conditions (Verdier et al. 2014). This system integrates technology optimized for accuracy and reliability in a highly contained 13-m^3 chamber to host communities of aquatic and terrestrial species inside a temperature-controlled lysimeter (up to 80-cm deep soil or water). Climate (temperature, humidity, rainfall, irradiance) and atmosphere (O_2 and CO_2 concentrations, O_3 concentrations) conditions can be controlled, as well as the temperature in the lysimeter. The soil profiles of volumetric water content,-temperature, and CO_2 concentration are measured. Climatic and atmospheric variables can be programmed to follow natural, dynamical trajectories, and simulate gradual as well as step changes.

Two other smaller systems, called microcosms, allow for laboratory experiments on miniature ecosystems including microorganisms and small plants and animals from soils and waters. The Montpellier “terrestrial Microcosms” platform consists of 13 growth chambers (1 m-× 1 m-× 1.5 m) that can accommodate dozens of model microcosms with soils or with soils and small plants or insects (Coulis et al. 2015). Climatic conditions,-preindustrial or postindustrial CO_2 -level, and the $\delta^{13}C$ of the atmospheric CO_2 can be controlled. A subsample of the individual microcosms can be connected on demand to various-analyzers.

The “aquatic Microcosms” platform at Ecotron Ile-De-France consists of tens of chemostats and open-top microcosms that can accommodate a few-liters of fresh or marine water. They allow confining bacteria,-and phytoplankton and zooplankton to study the impact of environmental change on the biodiversity and functioning of aquatic microorganisms communities.

They are designed specifically to allow the study of the carbon cycle. All terrestrial and aquatic microcosms can be equipped with interchangeable lighting including plasma lamps with a spectrum similar to the sunlight or panels of LED with adjustable spectral composition.

Although these platforms were designed without a clear focus on toxins and pollutants, the Ecotrons are also important tools that could be used to address three important challenges raised by Vighi and Villa (2013) for the future of ecotoxicology. First, Ecotrons could allow testing the effects of toxicants under realistic physical, chemical, and biological conditions, including both aquatic and terrestrial ecosystems with a significant number of species representative of complex biological communities (e.g. Milcu et al. 2014). In particular, Ecotrons can reproduce the climatic conditions of almost all European regions with their daily and seasonal patterns, and intact ecosystem samples can be introduced into most of the platforms. Chemicals could be manipulated in the atmosphere, in soil or water, and their dynamics could be tracked by direct sampling of gas, soil, liquid, and biological samples. Second, Ecotrons make it possible to investigate interactions among various stress factors of different origins (Vighi and Villa-2013; Callow and Forbes 2014). This should be a major improvement to test the hypothesis that the effect of a toxicant will become more severe in the presence of another stressor (e.g., Heugens et al. 2001). For example, specific experiments in Ecotrons can be designed to simulate various environmental conditions and their interactions using factorial designs: One chemical treatment could thus be crossed with elevated temperature, elevated CO_2 concentration, or with a second chemical treatment. Another exciting possibility would be to conduct-dose-response analysis at the ecosystem level and construct-dose-response curves. What would be the shape of the response curve? Would we observe a threshold after which the ecosystem would collapse? Third, Ecotrons are designed with instruments suitable for the study of the impact of toxicants at the levels of populations,-communities, and ecosystems (Van Straalen 2003). Since the aim of conservation biology has shifted from a focus on species conservation to the sustainability of ecosystem services (e.g., Pereira et al. 2010), it is of prime importance to test the impact of toxicants on biodiversity and ecosystem functioning, including biogeochemical cycles or ecosystem productivity.

The ecotoxicological endpoints could also be the resistance and resilience of the community structure and of the ecosystems functions. Ecotrons were designed to provide measurements of ecosystems functions (through flux measurements of several gases), and they allow the manipulation of environmental conditions to generate extreme events and the recovery thereafter. They are thus extremely well suited for the purpose of whole-ecosystem assessment of ecological risk.

Seminatural experimental platforms dedicated to aquatic ecology and ecotoxicology

AnaEE-France offers several experimental platforms for experimentation in-seminatural conditions. These platforms allow experiments close to natural conditions, in which environmental factors can be realistically manipulated. They provide a powerful tool to link small-scale laboratory experiments including a single or a few species only or *ex natura* experiments to field or *in natura* experiments.- Seminatural experimental platforms are considered to offer the experimental ecosystem closest to the real world, without losing the advantage of reliable reference conditions and replication. By integrating over multiple direct and indirect species effects up or down the food web, the responses obtained from studies in these platforms can also be used for parameterization in ecosystem and biogeochemical models. Moreover, their moderate size offers the opportunity of replicates of experimental conditions or treatments during the experiments. There is a long-standing tradition of ecotoxicological studies on aquatic organisms and ecosystems, and controlled experimentation in seminatural platforms has played a major role in the identification of major pollutants, of sensitive bioindicators and biomarkers, and of potential disruptive direct and indirect effects on whole ecosystem structure and processes (reviewed in Caquet-2013; Caquet et al. 2000).

With a focus on freshwater and standing water bodies, experimental platforms available in AnaEE-France offer a combination of equipment to manipulate the exposure of aquatic species or communities to various substances and to analyze consequences from the (sub)organism to the ecosystem level. These experimental systems also allow to couple manipulation of chemicals and of other global change processes and drivers, such as increased temperature, biodiversity, trophic network structure,- eutrophication, or habitat quality, similar to next-generation mesocosm facilities elsewhere in the world (reviewed in Stewart et al. 2013). The platforms are currently operated by two complementary facilities including one specifically designed to conduct ecotoxicological studies, managed by the INRA Experimental Unit U3E (Unité Expérimentale Ecologie Ecotoxicologie Aquatique), hereafter referred to as PEARL (for Platform for Experiments on Aquatic ecosystems Rennes-Le Rheu, http://www6.rennes.inra.fr/u3e) platform, and another one designed to investigate the relationships between biodiversity and ecosystem functioning, hereafter referred to as PLANAQUA (for Plateforme nationale expérimentale en Ecologie Aquatique, http://www.cereep.ens.fr/).

The PEARL facility is involved in multidisciplinary projects that address all the components of continental hydrosystems. Current experimental facilities included in AnaEE-France are located on the

Agrocampus Ouest campus (48° 06′ 45″ N, 1° 42′ 20″ W) and on the INRA Le Rheu site (48° 12′ 27″ N, 1° 79′ 51″ W), nearby Rennes in Brittany, Western France. They include two experimental buildings (400 m^2 each), a 5000-m^2 outdoor platform comprising several types of pond mesocosms (70 ponds in total, volume 3 to 30 m^3, with a degree of replication from 12 to 24 depending on the type of pond), 500 m^2 of concrete wall basins and greenhouses for aquatic organism stocking and breeding, and a 5-ha pond station with 30 ponds of various size (from 100 to 1000-m^2 area and from 0.70 to 1.20 m deep). The PEARL facility is extremely well-suited to tackle research questions on the biology and auto-ecology of aquatic organisms submitted to stressors. The indoor facilities include a set of 15 rooms, and some rooms are dedicated to experiments including chemicals or invasive species (Gorzerino et al. 2009; Coutellec et al. 2011; Barsi et al. 2014). In addition, a lab is available for preliminary treatment of samples or other general purposes. The outdoor basins and mesocosms facilities host a hundred of experimental ponds (volume ranging from 0.5 to 30 m^3; Fig. 5a). Initial conditions (e.g., water level, sediments, macrophytes) may be controlled. Monitoring and the number and nature of in-pond devices (e.g., temperature probes, artificial substrates, emergence traps; Fig. 5b) may be customized according to the requirements of users (Caquet et al. 2001). Water level may be controlled and adjusted if-needed, and between-pond connectivity may be manipulated (Hanson et al. 2007). The main parameters informing the physicochemical water quality and meteorological conditions can be recorded. Eight ponds out of a series of 16 (volume 9 m^3) are equipped with heating devices that allow to have a constant difference (up to +4 °C) in water temperature between heated and control ponds.

Increased temperature may be crossed with toxicants or another factor to study the interaction between global warming and various stressors. Depending on the pond type and experimental design, experiment duration may range from a few weeks to 2–3 years, therefore giving the opportunity to study the whole life cycle of many species or to perform experiments encompassing several generations for model species thus providing a support for evolutionary ecotoxicology experiments (Coutellec and Barata-2011). Exposure patterns to single or mixture of toxicants may be acute (e.g., single exposure event; Hanson et al. 2007; Caquet et al. 2007) or chronic (e.g., repeated treatments; Auber et al. 2011; Bayona et al. 2014), therefore mimicking point-source or diffuse pollution, respectively. In addition,-30 large ponds (surface ranging from 100 to 1000 m^2) are available. They may be used for rearing model organisms or for functional studies (e.g., pond aquaculture).

The PLANAQUA facility was primarily designed to explore the effects of disturbances on the ecology and evolution of biodiversity, and cascading effects on ecosystem functioning. It is located at the field station of the Ecole Normale Supérieure nearby Paris-(48° 17′ 15″ N, 2° 40′ 35″ E) and includes a 1000 m^2 platform hosting 72 outdoor mesocosms, a 20,000-m^2 platform of outdoor aquatic macrocosms (48° 16′ 57″ N, 2° 40′ 20″ E) and a set of instruments, and indoor space for the breeding and maintenance of model organisms and basic laboratory work. The mesocosms platform consists of standard ponds varying from a volume of 1 to 10 m^3 and from 30 cm to 1.5 m deep with a high degree of replication (from 10 to 16 replicates per type of pond). These ponds can host standing water bodies including trophic networks up to small planktivorous fish species, and experiments in these systems will typically last from a few weeks to less than 2–3 years (Harrault et al. 2014). The platform also includes 12 nonstandard mesocosms, making it possible to manipulate the water physical structure as well as temperature (Fig. 6a, Blottière et al. 2014). All these mesocosms can be customized with model sediments, planktonic species, macrophytes, and fish species for the purpose of specific ecological studies. The aquatic macrocosms platform includes 16 artificial lakes with an individual volume of 700 m^3, connected to each other by dispersal corridors, and equipped with an automated instrumentation (Fig. 6b). They are complemented with a storage pond and a sewage pond of 4000 m^3 each, which can accommodate the inputs and outputs of water needed for the functioning of the artificial lakes. The macrocosms were not designed to allow for direct manipulation of toxic chemicals because of the high costs of maintenance and removal of pollutants at this spatial scale. However, they can be used to track the natural inputs and flows of toxins inside each lake and to link this dynamic with relevant environmental and/or bio-manipulation conducted in each lake. This approach is similar to the design of long-term observatories but retains some of the power of experimental designs such as the presence of independent replicates.

Thus, PEARL and PLANAQUA are dedicated to the implementation of experiments on seminatural aquatic ecosystems. The effects of physical and biological characteristics of the ecosystems, and those of various stressors(e.g., toxicants) or forcing factors (e.g., nutrients, temperature) may be assessed at several levels of organization (e.g., individuals, population, community, ecosystem). The spatial scales ranging from few to hundreds cubic meters, the diversity of pressures, and the range of instruments designed to probe functional properties of aquatic systems generate unique opportunities for ecotoxicological research at PEARL and PLANAQUA. In particular, it is possible to combine manipulation of organic and inorganic pollutants like in standard ecotoxicological studies with other types of stressors such as climate warming, turbulence-regimes, or biodiversity loss. This would allow to test hypothesis and provide data on the dynamics of aquatic ecosystems exposed both to global-(e.g., climate change, land use changes, biological invasions) and local (e.g., pesticides derived from local agricultural activities) pressures. Among key issues is the analysis of the relationships between species diversity and ecosystem functioning under control and stressed conditions, and between species diversity and vulnerability to combined stressors. This capacity will be essential to understand the strength of direct toxicity versus indirect toxicity mediated by changes in trophic networks and biodiversity. Furthermore, the combination of replicated, outdoor facilities with indoor room for the analysis of toxic effects on organisms makes it possible to investigate transgenerational effects of stress, including evolutionary dynamics, and to replicate studies in two separate climate zones.

In natura sites

AnaEE-France offers access to more than 20 *in natura* experimental sites that cover a range of continental ecosystems (forests, grasslands, crops, aquatic systems) with the aims of harmonizing measurement protocols and data management as well as promoting site and data access. These *in natura* sites are open to external fluxes (biotic, chemical, physical) that may have strong impacts on ecosystem dynamic and evolution. They host long-term manipulations spanning several decades where it is possible to take into account ecosystem inertia and feedback loops than cannot be detected on the short term in Ecotrons or seminatural platforms. If needed, these sites can also provide intact ecosystem samples, differentiated by long-term manipulations, for specific experiments (Milcu et al. 2014).

Among the experimental sites that cover a range of continental ecosystems (forests, grasslands, crops, aquatic systems), the SOERE PRO (observatory for monitoring and experimentation on agroecosystems receiving fertilizers of residual origins) is a network of long-term field experiments dedicated to the study of the benefits and risks associated to organic residues (OR). The SOERE PRO provides data (1) to better evaluate the effects of regular OR application on organic matter dynamic and potential C storage in soils, biogeochemical cycles of nutrients (C, N, P), fate of potentially present chemical and biological contaminants, and soil biological activities, and (2) simulate the long-term consequences of regular application and integrate them in environmental analysis that will allow to (3) test various alternative scenarios of application (Fig. 7). The SOERE PRO offers a large variety of contaminant concentrations in cropped soils in France including the following: (i) ongoing sites where regular applications of OR at doses representative of those usually applied by the farmers and where contaminant concentrations have barely increased but where cocktail of recent contaminants could have potential synergistic impacts; and (ii) "historical sites" that present high concentration levels in contaminants without any recent application, making it possible to study the resilience of contaminated cropped soils.

The SOERE PRO includes four main sites receiving regular applications of OR which allow the exploration of various agro-pedo-climatic contexts: QualiAgro started in 1998 (Fig. 7a) is located in the Paris Basin and compares urban composts and manure; Colmar (Fig. 7b), started in 2000, is located in northeast of France and compares composted and noncomposted residues); EFELE (Fig. 7c), started in 2012, is located in Brittany and compares different manures; and la Mare (Fig. 7d), started in 2013, is located on the Reunion Island and is dedicated to the study of sludge and manures on sugar cane fields. Identical instruments are installed on these ~~4~~four sites to monitor the hydrodynamic functioning of soil and soil water quality: TDR probes, tensiometers, temperature sensors, and lysimeters. Climatic data are monitored on all sites. Gas emissions (N_2O, CO_2) are followed by gas measurement chambers (since 2013 on EFELE, starting in 2015 for the other sites).

Two historical sites (La Bouzule and Couhins) are also associated to the SOERE PRO. In these sites, the applied OR included some highly contaminated wastes. The OR spreading has stopped since several years, and sites are resilient. These two last sites are not equipped as the four main ones.

The applied OR, soils,-crops, and waters (rains and leached waters) are sampled and analyzed. Identical analyses (parameters, analytical methods, laboratories) are done on samples for the four main instrumented sites of the SOERE PRO. Data management is centralized at the SOERE PRO level, with the development of databases for field experiment data, analytical data of organic wastes applied in France, and for traceability information concerning SOERE PRO samples. The information system is developed by INRA permanent engineers devoted to develop and manage the information systems of the INRA long-term observatories (INRA Orléans). Samples of OR, soil, and crops are long-term stored under harmonized conditions to allow future analyses and/or investigations.

Main SOERE PRO results showed positive results on biological indicators such as the increase of microbial and earthworm biomass with modification of biological diversity due to the increase of organic matter (Houot et al. 2009; Capowiez et al. 2009; Leyval et al. 2009; Pérès et al.-2011) or lack of negative impacts due to very low level of contaminants when rates of applications remained within the regulatory limits. An ecotoxicological indicator based on the accumulation of contaminants in a snail indicator did not point out any negative impacts of the recycling of urban composts in the Qualiagro after-seven applications (Pauget et al. 2013).

Analytical platforms

AnaEE-France nodes 1 to 3 provide experimental and data collection services, and share two sets of priorities. The first one deals with field samples and requires analytical platforms for analyses on biotic and abiotic samples that cannot be replicated in each site. The second deals with the requirement of efficient, specific and standardized methods for organism and ecosystem characterization. To achieve these goals, the node 4 developed efficient devices offering rapid and/or high throughput characterization of samples from experimental sites and environmental survey networks by accessing to cutting edge equipment. If a mobile platform is offered for sampling,-in situ preparation, and analysis of all samples, with a special interest for plant samples, other platforms are specifically designed for environmental microbiology and studies on fauna (invertebrates and small animals).

Mobile platform and shared instruments

One challenge is thought to avoid/limit disruption and transformation between-in situ sampling and analysis in the laboratory. Indeed, environmental samples (solid or liquid) contain labile molecules and dynamic microbial communities, which are both subject to change and transformation even over short time periods. Such transformations may be very rapid and/or intense due to the disturbance associated with the sampling process. Keeping in mind these constraints, it is clear that it is necessary to take advantage from equipment able to preserve samples,-analyze them, or monitor their evolution.
The objective of the Mobile Platform for Observation and Experimentation in Terrestrial Ecosystems (M-POETE) is to bring state-of-the-art equipment directly into the field (Fig. 8). The mobile platform is hosted by INRA Nancy in Champenoux (48° 41′ 03″ N, 06° 11′ 05″ E). It is then composed of a certain number of modules and complimentary toolboxes. Currently, four modules are operational: - One dedicated to soils, one dedicated to the vegetation, one dedicated to the environment, and one dedicated to sample preservation and analysis. In the light of the use of M-POETE for ecotoxicological purposes, the analytical module (including the toolbox) seems to be most appropriate. As the mobile lab is-per se mobile, it is possible to load the adapted equipment for each mission and then act locally. For such missions, the following equipment-(nonexhaustive) could be used: (1) highly precise GPS system to locate the site, the area of intervention, and the sampling points, (2) necessary material to collect solid or liquid samples, (3) mobile NIRS, suitable to characterize the initial samples (solid and liquid), (4) freeze dryer to preserve the samples, and (5) microbiological toolbox to prepare and preserve samples for further analyses or using the qPCR for-in situ identification of microbes and use

of an ultrasensitive VOC analyzer.

In the context of ecotoxicological studies, M-POETE is specifically expected to:

- Provide facilities for soil, sediment and water sampling (corer, sieving, pump, filters), sample preparation (extraction, homogenization, centrifugation, weighting, drying), plant tissue and DNA extraction (using storage in liquid N_2) and quantification, and rapid identification and characterization of soils and biological.
- Allow the characterization of soil, sediment, and water processes through new approaches using gas analyzers which can provide information on processes intensity (e.g., CO_2 fluxes), characteristics of processes (isotopes such as ^{13}C and ^{18}O), and the type and diversity of the biotic communities involved in these processes thanks to their VOC signature measured by proton transfer reaction-mass spectroscopy (PTR-MS) .method.

Environmental microbiology

AnaEE-France comprises two analytical platforms, GenoSol and Biochem-Env, that provide responses to a major knowledge gap associated with the lack of standardized information about "micro-organisms" (bacteria, fungi, and fauna) diversity and functioning in soils, sediments, and water. They are involved in the improvement or development of analytical methods in the fields of genetic or biochemical-characterization and contribute to the standardization of procedures or molecular and biochemical tools, with a view to normalization (AFNOR and ISO). In addition, they develop statistical tools and modeling approaches to study the spatial distribution and temporal evolution of microbial diversity, e.g., a pipeline for bioinformatics analyses adapted to the new generations of sequencing of taxonomic or functional genes (pyrosequencing; Terrat et al. 2012). The two platforms are already involved in research programs intended to study the effects of chemical contamination due to agricultural or industrial activities on the soil microbial community.

GenoSol (http://www.dijon.inra.fr/plateforme_genosol), hosted by INRA at Dijon (47° 19′ 03″ N, 5° 04′ 10″ E) in Burgundy, aims at providing an appropriate logistic structure for the acquisition, storage, and characterization of soil genetic resources obtained by extensive sampling and to make these resources readily available for the whole scientific community and policymakers. The ultimate goal is to produce a reliable reference system based on molecular characterization (taxonomic and functional features) of the soil microbial communities that provide scientific interpretations of the analyses from large scales of time and space sampling (Fig. 9a). The GenoSol platform has three principal facilities:

(1) a national Genetics Resources Centre (GRC) dedicated to the storage, conservation, and use of genetic resources (soil DNA), (2) a technical platform for the development and technological surveillance of methods for extracting nucleic acids from soils and characterizing microbial genetic resources (Plassart et al. 2012; Terrat et al. 2015), and (3) an information system to store data from analyses (Morin et al. 2013). The database is being used to develop a reliable reference system for the interpretation and translation of soil microbial diversity which will serve to define a "Normal Operating Range" for soil biodiversity and develop biological index to evaluate environmental impact of human activities (agricultural practices, industrial impact…).

The platform Biochem-Env (http://www.biochemenv.fr), hosted by INRA in Versailles (48° 48′ 14″ N, 2° 05′ 09″ E) nearby Paris, aims at providing skills and innovative tools for biochemical characterizations of microorganisms living in soils and sediments. Biochem-Env has two facilities: (1) a technical platform for the development and technological surveillance of methods for measuring enzymatic activities and microbiological descriptors in soils and sediment, and (2) an information system for managing the traceability of samples and data. The platform develops high- throughput tools for the biochemical characterization of solid samples (soils, sediments) to access their functional diversity (Fig. 9b) and phospholipidic fatty acid (PLFA) analysis of the structure of soil communities. Both platforms are involved in improving the standardization of procedures and molecular and biochemical tools, with a view to normalization (AFNOR and ISO).

Biochem-Env makes it possible to assess ecosystem functioning in responses to global changes including the effects of chemical contamination on soil enzyme activities (Riah et al. 2014). For example, soil enzymatic activities have been studied in samples collected in sites exposed to two different types of pollution. The effects of metal contamination on a combination of biological parameters were studied in Mediterranean soils (de Santiago-Martin et al. 2013). Enzyme activities (phosphatase, urease, β-galactosidase, arylsulfatase, and dehydrogenase activities) were strongly affected by metal contamination. The decrease of fungal DNA concentration in metal-spiked soils was negligible, whereas the decrease of bacterial DNA highly depended of the contamination level. In another project, the fate of microcystins and their effects on soil microorganisms and higher plants were studied. Microcystins, hepatotoxins produced by cyanobacteria of the genus *Microcystis*, are the most frequent cyanotoxins detected in irrigation waters, often at high concentrations (ca. 100 µg eq. MC- LR/L) leading to an important input to irrigated soils. Soil biological activity was globally unmodified by toxin inputs, but the nitrification activity was stimulated (Corbel et al. 2015).

Studied on invertebrates and small animals

AnaEE-France offers also platforms to study the responses of fauna exposed to chemical stressors in the environment. These platforms allow the determination of biochemical biomarkers in macrofauna and modifications of internal organs in small animals.

In addition to microbial descriptors, Biochem-Env (http://www.biochemenv.fr) allows the measurement of biochemical biomarkers (e.g., lipids, proteins, glucides, enzymes involved in the responses to oxidative stress) in terrestrial (e.g., earthworms) and benthic macrofauna (e.g., gammarids). Biomarkers are assumed to be direct measurements of pollutant bioavailability (Lanno et al. 2004).

Recently, Beaumelle et al. (2014) studied the influence of metal concentration, multicontamination, and soil characteristics on earthworm energy response to metal availability. At low concentration and despite the influence of multicontamination and soil characteristics, they found a link between earthworm energy reserves (protein and lipid) and metal availability. Another study aimed at assessing the effects of different doses of a commercial formulation of epoxiconazole (Opus®), a persistent and widely used fungicide, on the earthworm *Aporrectodea icterica*, a species commonly found in cultivated fields (Pelosi et al. 2015). This study highlighted moderate sublethal effects of the commercial formulation for earthworms, regarding mortality, uptake, weight gain, enzymatic activities (catalase and glutathione *S*-transferase), and energy resources (lipids and glycogen content).

By the end of 2015, the “Station d’Ecologie Expérimentale à Moulis” (SEEM) will house a small animal Magnetic Resonance Imaging (MRI) laboratory. This lab (42° 57′ 30″ N, 1° 05′ 12″ E) will offer a 4.7 Tesla super conducting cryogen-free magnet with superior gradients capable of receiving animals up to 7 cm in diameter. The lab will offer a number of rf coils adapted to different sizes and forms that will permit the acquisition of high-resolution images. Even aquatic organisms may be considered with the development of species-specific cells (e.g., containment of still or flowing water, etc.). There will be a number of applications for ecotoxicology, for example, monitoring the effects of toxins by following changes in shape and form of internal organs. This MRI is not limited to animals, since high-resolution images of plant or other materials may also be generated. This system is also equipped with MR spectroscopy allowing biochemical research by studying metabolites. These noninvasive methods are undertaken in-vivo; therefore, animals are not sacrificed and can be used for long-term studies with repeated measurements throughout the development of the animal (Fig. 10).

Resources for data management and modeling

Research in ecotoxicology has to face several challenges-(i) assessing the effects of low-concentration and long-term exposure to low concentrations of-pollutants that may impair the fitness and viability of organisms, populations, or ecosystems, (ii) define the baseline to discriminate between exposed and unexposed test organisms, (iii) consider the effects of multiple stressors that organisms are simultaneously or sequentially exposed (e.g., pollutants and nonpollutant stressors such as rising temperatures, altered CO_2 concentrations, changes in the combination of nutrients…), (iv) consider complex interactions in ecosystems and confounding factors,-(v) integrate data (from mechanistic molecular to systemic ones) to develop integrated dynamic models that will improve ecological risk assessment… Thus, covering a large area of experimental, analytical, or modeling platforms as well as databases, the AnaEE-France infrastructure has to face the challenge of managing dispersed and heterogeneous information from various disciplines including ecotoxicology.

To address these goals, AnaEE-France developed a general Information System based on (i) the description of resources using metadata, (ii) a unified access to the distributed components (databases of the experimental or analytical platforms and modeling platforms), and (iii) the interoperability among all these components.

Interoperability, within and outside AnaEE-France, will rely on the use of shared vocabularies and international standards ensuring notably the compliance to the European INSPIRE Directive (http://inspire.ec.europa.eu/).

The vocabularies already used in the AnaEE-France resources were merged with selected concepts imported from existing thesauri in order to generate the AnaEE-France thesaurus. Moreover, with the aim of fully describing the AnaEE-France resources using semantics, an ontology based on the OBOE ontology (Madin et al. 2007) is under development. OBOE covers the field of ecological observations offering a core ontology with generic classes and several thematic extensions. Part of the AnaEE-France thesaurus concepts is integrated as classes of a new extension to satisfy the specific needs of the infrastructure. The ontological annotation of the resources will offer semantic interoperability.

A metadata catalog (http://w3.avignon.inra.fr/geonetwork_anaee) provides the first-level description of AnaEE-France resources using keywords from the thesaurus. A semantic webportal will offer a unified access to databases and modeling platforms.

This organization allows a standardized access to the distributed information, offering the opportunity to deal with disciplinary data, for example in ecotoxicology, as well as to address larger ecological questions with multidisciplinary data sets.

The modeling services are jointly developed and operated by INRA and by CNRS. INRA started developing modeling platforms some years ago, and existing platforms include Record Platform (http://www.inra.fr/record), “VSoil” (http://www6inra.fr/vsoil/), and Capsis (http://www.inra.fr/capsis). Record and VSoil were initiated from the necessity of building modular models to simulate complex dynamic systems involving multiple interacting mechanisms and can be used for research in ecotoxicology. The platforms provide means not only for easily using existing models (sensitivity analysis and model comparison for example) but also for developing new modules and for coupling them in order to build new models adapted to specific problems. Thanks to the use of a common thesaurus and ontology, platform users will be able to easily access data bases and to use them for model evaluation, for forcing variables, or for parameters estimations. As an example, WORMDYN is a model of earthworm population dynamics accounting for the agronomic practices (Pelosi et al. 2008). It is a stochastic stage-based matrix model allowing the prediction of *Lumbricus terrestris* dynamics in ploughed and superficially tilled plots. It is being incorporated into the VSoil platform as a “biological module” pluggable to other modules describing other soil functions, e.g., fate of soil contaminants, which could be used in the future for modeling exposure-effects of pesticides or other contaminants.

Concluding remarks

AnaEE-France offers to the international community of environmental scientists and notably ecotoxicologists a coherent set of coordinated platforms (seminatural and *in natura* services, analytical platforms, data management, and modeling resources) and efficient methods for their optimized management and functioning. The platforms are closely associated to research laboratories from French research institutes or universities that host relevant practical and scientific skills. As a consequence, experienced technicians, engineers, and scientists are involved in the design, implementation, and monitoring of experiments, in sample analysis and in data processing and interpretation. They have deployed quality management systems based on internal referential of their hosting institutions, and some of them are engaged into the ISO 9001 standardization.

Thus, AnaEE-France offers three main kinds of services open to national and international research in ecotoxicology (i) access to experimental and analytical facilities, instruments, and modeling platforms, (ii) access to stored samples of biological-resources, and (iii) access to large data sets. Access policy will include a single access point with updated information on services and fees (web platform including the Information System for Infrastructure Administration of AnaEE-France), a single track to evaluate the scientific and technical relevance of proposed projects and common procedures for fees, and a common feedback mechanism allowing users to evaluate our services.

AnaEE-France offers several advantages to the research in ecotoxicology. Its platforms are closely associated to research laboratories from French research institutes or universities that host relevant practical and scientific skills. Experienced technicians,-engineers, and scientists may be involved in the design, implementation, and monitoring of experiments, in sample analysis and in data processing and interpretation. The platforms of AnaEE-France have deployed quality management systems based on internal referential of the hosting institutions (INRA and CNRS). Some of them are engaged into the ISO 9001 standardization. GenoSol and Biochem-Env possess French permission to import and perform experiments on Quarantine Material (soils, living organisms…). The platforms have accepted principles of ethical and professional conduct, especially if the research involves animals. Regular meetings of working groups on the most relevant methodological issues for the development of the infrastructure and its services are organized. They address various topics such as standardization of measurement protocols and experimental design, the development of new instruments, the use of standard models organisms and ecosystems, and solutions for a common system of archiving and stocking of biological resources. The infrastructure also operates higher education and scientific training, as well as technical training.

Then, AnaEE-France was designed in line of AnaEE-Europe Infrastructure concept, which is currently on the ESFRI roadmap under preparation phase. The integration of AnaEE-France into the European structure will enlarge the range of services with new ecosystem manipulation possibilities, a wider range of ecosystems and a diversification of analytical platforms.

Finally, by taking into account new challenges of research in ecotoxicology, the various platforms of AnaEE-France allow an advanced assessment of ecological risks. Supporting research groups in their analysis, assessment, and forecasting of the fate of chemicals and of their impact on ecosystem functioning and provided services, AnaEE-France is a unique research infrastructure for experimental manipulation of managed and unmanaged terrestrial and aquatic ecosystems.

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Compliance with ethical standards

The paper follows the rules of good scientific practice presented in the "Instructions for authors." Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the information presented. There are no potential conflicts of interests that are directly or indirectly related to the work presented.

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Fig. 1. A scheme for an ecologically based procedure for ecological risk assessment (modified from European Commission 2013)

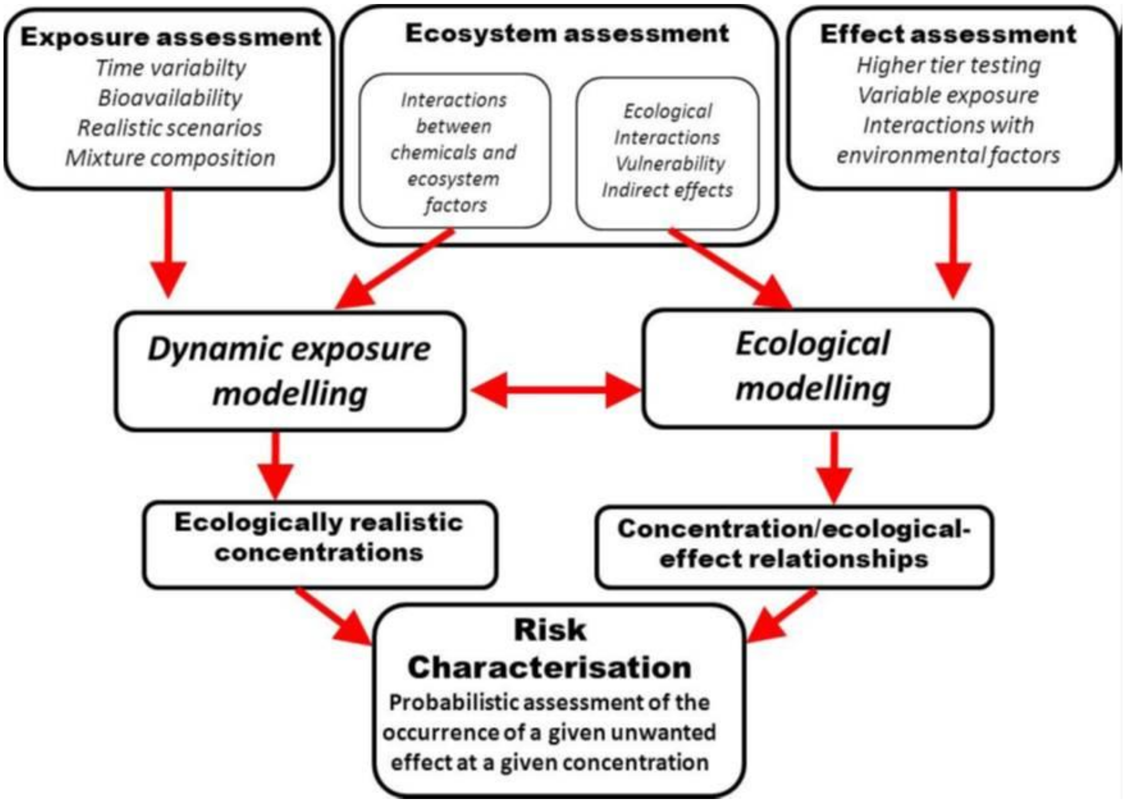

Fig. 2. Scheme of AnaEE-France illustrating the functional relationships between each node. Researchers conducting experiments on organisms to communities and ecosystems can access ecotrons, seminatural, or *in natura* sites to study processes involved in ecotoxicology at various spatial and temporal scales (see “Ecotrons,” “Semi-natural experimental platforms dedicated to aquatic ecology and ecotoxicology,” to “*In natura* sites”). Analytical platforms aim at offering the capacity of characterizing variety of parameters (see “analytical platforms”). Finally, modelers are interested in accessing data for model implementation and validation (see-“Resources for data management and modeling”)

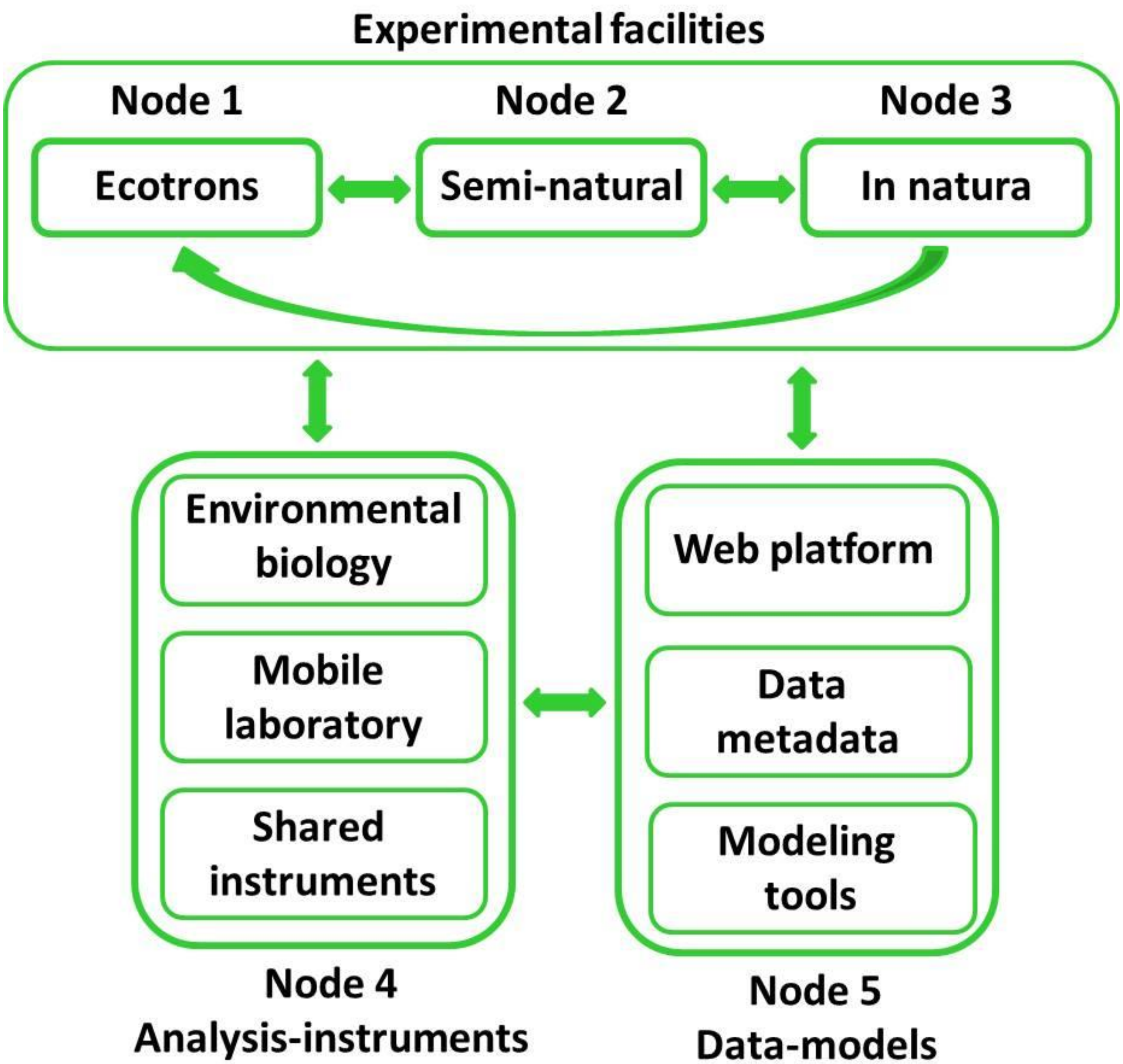

Fig. 3. Scheme of the Ecotrons principles combining improved environmental simulations and process measurements through ecosystems confinement

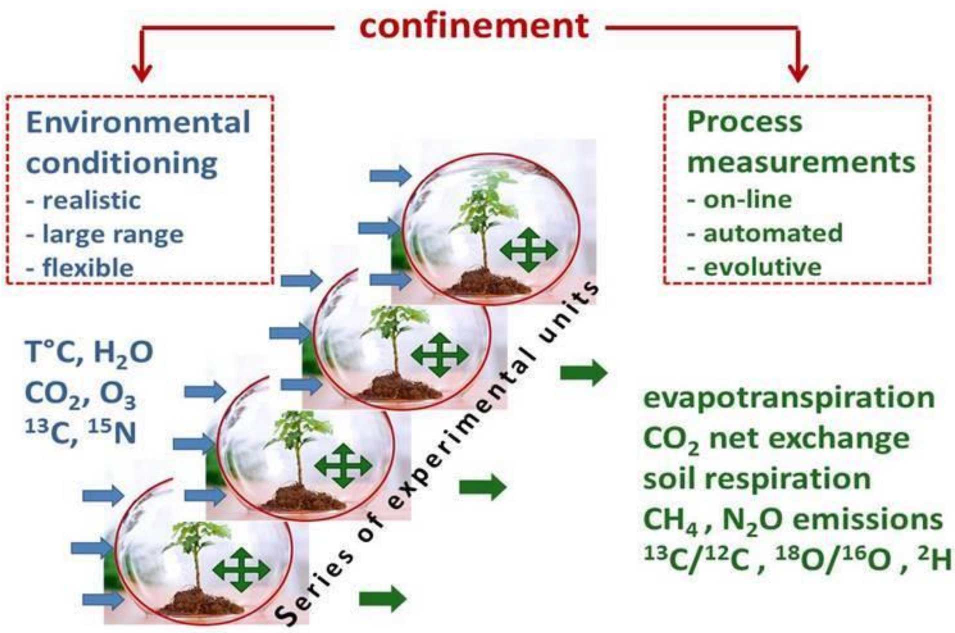

Fig. 4. Views of the Ecotrons. **a**. Online measurements of ecosystem processes in a lysimeter of the Montpellier Ecotron (evapotranspiration, soil respiration, and respiratory quotient) (© J. Roy, CNRS). **b**~~.~~ Aquatic microcosms designed to investigate the ecology and evolution of planktonic communities in Ecotron IleDeFrance. Each microcosm includes a bioreactor fitted with a chamber to allow gas measurements and isotopic analyses, and a culture medium for chemostat-like experiments (© S. Fiorini, ENS)

Fig. 5. Examples of PEARL experimental facilities. **a**. Mesocosm platform. In the foreground, the series of 16 circular mesocosms (volume: 9 m^3), at the back, 12 rectangular mesocosms (volume 30 m^3). Schematic representation of a mesocosm with some sampling systems. **b**. ~~.~~Schematic view of a pond mesocosm (modified from Caquet et al. 2001. *EIS* emerging insect sampler, *Mac* macrophytes, *MdIS* macrophyte-dwelling Invertebrate Sampler, *PS* Periphyton-Sampler, *SdIS* sediment- dwelling Invertebrate Sampler, *Sed* Sediment)

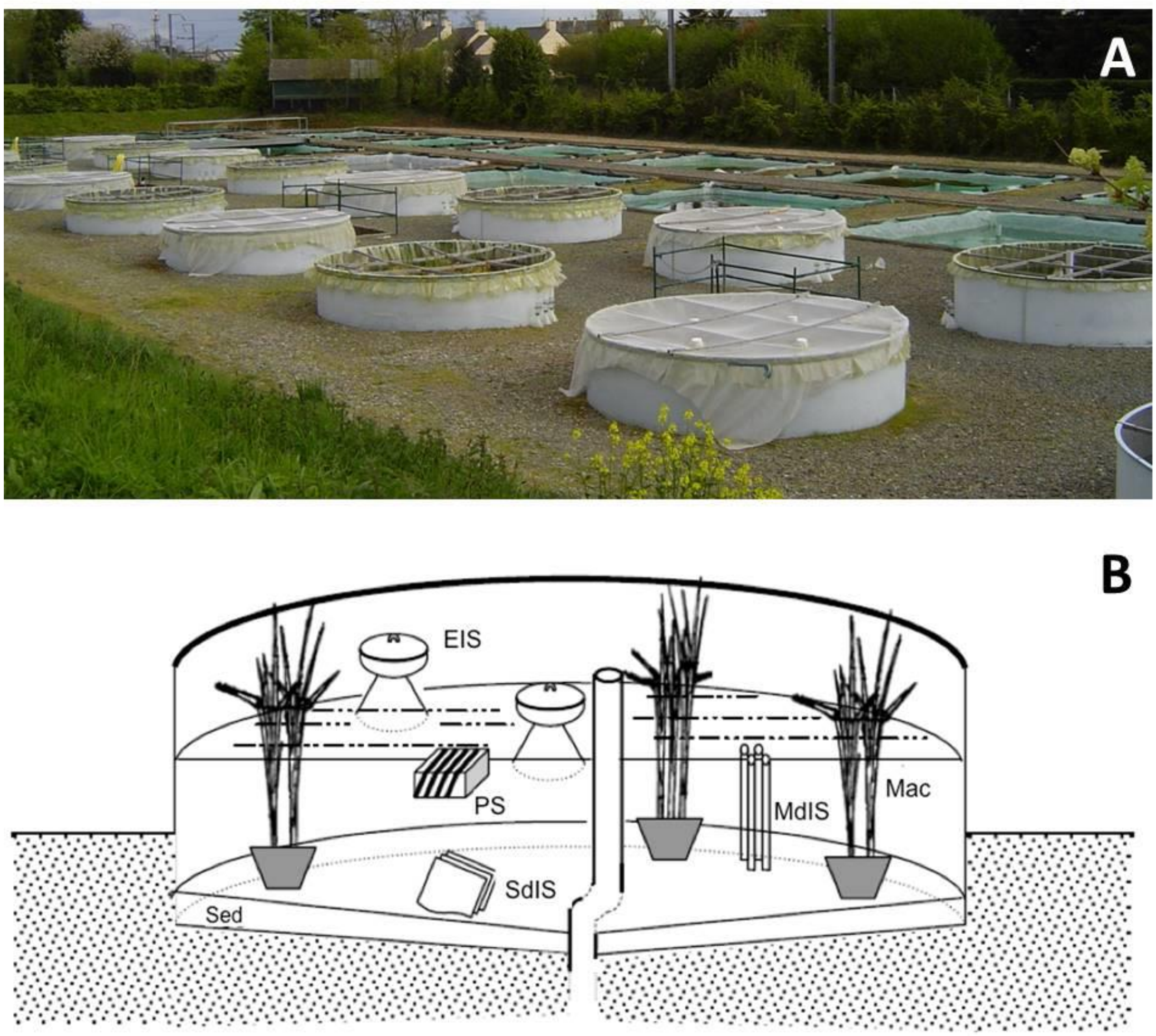

Fig. 6. Examples of PLANAQUA experimental facilities. **a.**–Mesocosms equipped with beaters to generate waves (*left part of the mesocosm*) and transparent covers to generate a greenhouse effect to increase surface water temperature (not shown). Each mesocosm is 10 m long, 1.7 m large, and 1.5 m deep. The angle and frequency of the wave beater can be automatically manipulated within a range allowing for subsurface wave and water stratification to full water mixing. The percentage of surface cover can vary manually from -0 to 100 %. This system makes it possible to investigate effects of water turbulence on the fate and effects of contaminants. **b**. Artificial lakes equipped with buoys installed on footbridges. Each lake constitutes a miniature replicate of a natural shallow pond integrating littoral, benthic, and pelagic zones, the spatial structure of the landscape can be manipulated to some extent, and real-time biophysical data are automatically recorded down to the temporal scale of a few minutes. To our knowledge, such highly replicated and instrumented systems have no analogue elsewhere in the world

Fig. 7. Examples of SOERE-PRO sites. **a**~~.~~Experiment Qualiagro. Image “Wordview 1” (resolution 2 m) on bare soil in March 2010, showing the differentiation between treatments after ~~7~~ seven applications of organic residues. **b**~~.~~View of the site in Colmar. **c**~~.~~View of the site EFELE in Brittany including an experiment studying different organic residues or the interaction between residue application and tillage practices. **d**~~.~~View of La Mare site in La Réunion Island

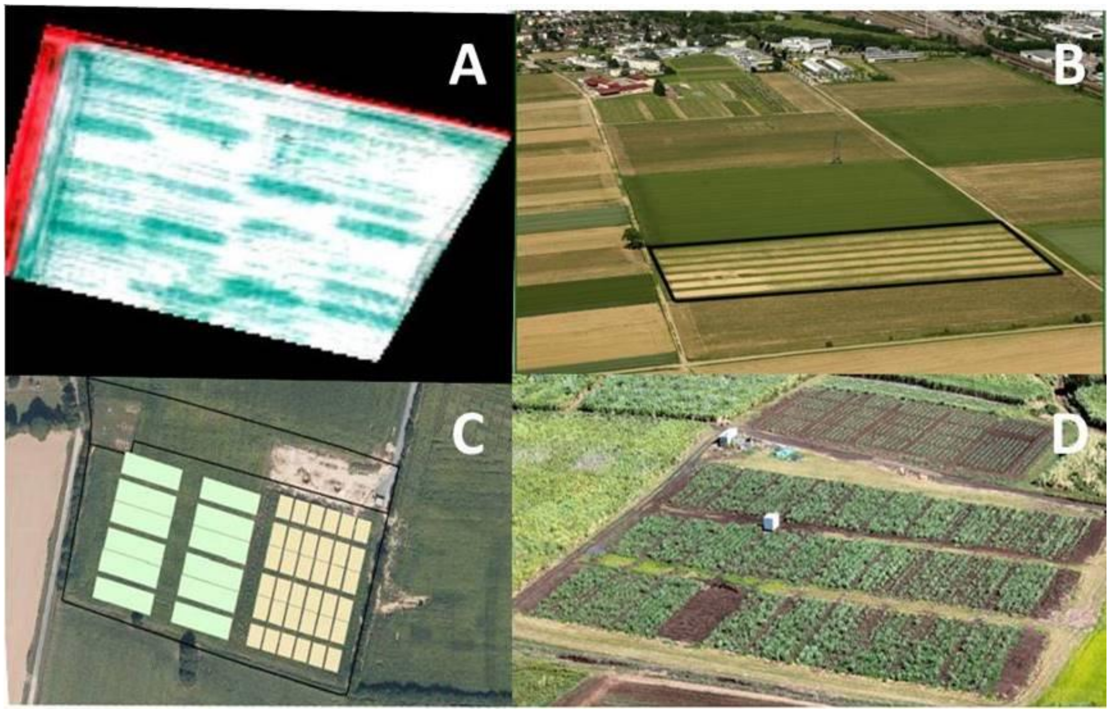

Fig. 8. The Mobile Platform for Observation and Experimentation in Terrestrial Ecosystems (E).
a. View of the truck. **b.** Monitoring of soil water content using decagon probes. **c.** qPCR device

Fig. 9. Platforms of environmental microbiology. **a**~~.~~ Genosol: the national Genetics Resources Centre unique in Europe for the storage and conservation of genetic resources from the environment and making them available to the scientific community. **b**~~.~~ Biochem-Env: the robot-supported equipment for the biochemical characterization of environmental samples (soils, sediments)

Fig. 10. An example of the Magnetic Resonance Imaging (MRI) system potentialities to diagnose effects on the internal body structures by a noninvasive way. Common Roach (*Rutilis rutilis*) with developed endo-parasite: the cestode, which can grow up to 25 cm long, can only been seen to deform the body cavity of the fish at a very developed stage. MRI can also be used to visualize the morphological effects of chemical contaminants on internal organs in small animals