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European Regional Development Fund

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Product E2.1

**GUIDE TO BEST PHYTOMANAGEMENT
PRACTICES FOR THE RECOVERY OF
BIODIVERSITY IN DEGRADED AND
CONTAMINATED SITES**

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1. PHYTOMANAGEMENT

1.1. Phytomanagement and phytotechnologies

Metal(loid)s (trace elements, TE), mineral oils (e.g., diesel fuel) and polycyclic aromatic hydrocarbons (PAHs) are among the most widely spread contaminants affecting European top-soils (Panagos et al., 2013; Van Liedekerke et al., 2014). These are also what regulators are looking for first. Xenobiotics such as organochlorines, paraquat, glyphosate or similar compounds and their derivatives are probably subject also to a widespread diffuse contamination. Activities such as mining, metallurgy, electronics, agriculture and the use of fossil fuels discharge a considerable amount of metal(loid) contaminants into soils, whilst accidental spills of petroleum-based products used for transportation (typically diesel-type fuels) are the principal cause of contamination with organic compounds (Barrutia et al., 2011). Soil contamination is often more complex since these contaminants frequently occur simultaneously (Agnello et al., 2016).

Over the last few decades various Gentle soil Remediation Options (GRO) have been developed to (phyto)manage contaminated soils (Kidd et al., 2015; Mench et al., 2009, 2010, 2018; Vangronsveld et al., 2009). GRO include *in situ* stabilisation (“inactivation”) and plant-based (“phytoremediation”) options. Conventional methods of remediation are based on civil engineering techniques (e.g., encapsulation, vitrification, soil washing, etc.) which have a high environmental impact (destroying soil structure and function) and elevated cost. GRO offer alternatives, which are considered to be less invasive, more cost-effective and more sustainable.

Phytoremediation was initially proposed (early 1990s) as plant-based methods to remediate contaminated environments, and alternatives to conventional civil engineering-based techniques. In the case of organic pollutants, plants and their associated microorganisms are used to degrade the contaminants to non-toxic metabolites, either within the plant tissues





(phytodegradation) or in the root-soil interface or plant rhizosphere (due to microbial activity or release of enzymes from plants: rhizoremediation). In TE-contaminated sites, GRO aim to decrease the labile (“bioavailable”) pool and/or total content of metal(loid)s in the soil through their uptake and accumulation in harvested plant parts (e.g., phytoextraction), or to progressively promote *in situ* inactivation of TE by combining the use of TE-excluding plants and soil amendments (e.g., phytostabilization). Both strategies have been subject to much discussion regarding their intrinsic limitations, such as the long time required to effectively extract metal(loid)s from medium to highly contaminated sites (although this can be overcome by considering “bioavailable contaminant stripping”). As a result, the concept of phytomanagement evolved which combines sustainable site management with gentle remediation options leading to the reduction in pollutant linkages alongside the restoration and/or generation of wider site services (Burges et al., 2017; Cundy et al., 2016). Phytomanagement approaches promote the use of gentle remediation options (based on the interaction between plants, microorganisms and soil amendments) within an integrated, mixed, site risk management solution or as part of a “holding strategy” for vacant sites. The use of profitable plants and the manipulation of the soil-plant-microbial system can control the bioavailable pool of soil contaminants, while maximizing economic and/or ecological revenues but minimizing environmental risks. Potential benefits include water runoff/drainage management, green space provision, soil erosion prevention, renewable energy and material generation, restoration/rehabilitation of plant, microbial and animal communities, greenhouse gas mitigation and carbon sequestration, recovery of land values, amenity and recreation, etc. (Cundy et al., 2016; Evangelou et al., 2015; Kidd et al., 2015; Smek et al., 2016; Touceda-González et al., 2017; Xue et al., 2018).

In recent years, phytomanagement has moved from a bench-scale level to full-scale deployment in the field. However, the long-term effects of various soil phytomanagement options on soil functionality, biodiversity, ecological functions and ecosystem services have been poorly assessed and reported. The objective of the PhytoSUDOE project was to





increase our understanding, and to provide evidence from long-term field sites, of the effects of phytomanagement on soil functionality and provision of ecosystem services.

1.2. Phytomanagement options

Gentle remediation options (GRO) have been developed as eco-friendly alternatives to traditional, civil-engineering methods of soil remediation (Kidd et al., 2015). These remediation options include *in situ* stabilization (inactivation) and plant-based (generally termed as phytoremediation) options, and are addressed to decreasing the labile (bioavailable) and/or the total content of contaminants (Cundy et al., 2016). These techniques are mainly based on the use of plants, soil microorganisms and amendments, also aided by agronomic management, which effectively reduce pollutant linkages while preserving the soil resource and remediating ecological functions (Vangronsveld et al., 2009). The use of contaminated land for the production of valuable biomass (such as the production of timber, bioenergy crops, biofortified products, ecomaterials, etc.) falls within the concept of phytomanagement (Robinson et al., 2009) and is considered essential for the commercial success of these phytotechnologies (Conesa et al., 2012). The guides which have been produced as part of the PhytoSUDOE project are based on experiences in metal(loid)-contaminated soils. Different options for the phytomanagement of contaminated soils are described below:

Phytostabilization uses tolerant plant species with a TE-excluder phenotype to establish a vegetation cover and progressively stabilize and/or reduce the availability of soil pollutants (Dary et al., 2010; Mench et al., 2006, Ruttens et al., 2006a, 2006b; Vangronsveld et al., 2009). The incorporation of amendments into the soil or use of microbial inoculation (aided phytostabilization) (Mench et al., 2010) can further decrease the bioavailability and phytotoxicity of pollutants located in the root zone, while improving plant establishment. Phytostabilization does not lead to the actual removal of contaminants but reduces pollutant



bioavailability and transfer to other environmental compartments. The mechanical action of the plant roots reduces soil erosion and transport of soil particles through natural agents, while evapotranspiration minimizes leaching during the growing season and therefore contaminant dissemination. In addition, the adsorption, precipitation, and accumulation of the contaminants in the rhizosphere (in collaboration with microorganisms associated with plant roots) entail their immobilization (Mench et al., 2010).

Phytoextraction is based on the use of TE-tolerant plants that take up contaminants (in general two or three metal(loid)s, rarely more) from the soil and accumulate them in excess in their harvestable aboveground biomass as compared to their common ranges (Vangronsveld et al., 2009). Phytoextraction can be aided by soil amendments, chemical agents and soil microorganisms (aided phytoextraction). When marketable TEs (such as Ni, Au, etc.) are recovered from the plant biomass (bio-ores), it is known as phytomining (Chaney et al., 2007). Another option is to pyrolyse / calcine such metal(loid)-rich biomass and to use the biochar or ashes as ecocatalysts in the biosourced fine chemistry (Clavé et al., 2016; Escande et al., 2014).

Phytovolatilization exploits the ability of plants to transform pollutants into volatile compounds either outside or inside some plant parts after uptake or to absorb and transport volatile compounds from the soil to the aboveground biomass where they can then be released to the atmosphere (Wenzel, 2009). When the contaminant is transformed and released directly from the soil surrounding plant roots (rhizosphere), it is usually termed as rhizovolatilization (Zhang and Frankenberger, 2000).

Phytodegradation or phytotransformation uses plants (and their associated microorganisms) to degrade organic contaminants to non-toxic metabolites having at their concentrations less or no toxic effect (Weyens et al., 2009b). When the degradation takes place in the rhizosphere of plants (due to microbial activity or release of enzymes from



plants), terms such as phytostimulation or rhizodegradation are more correct (Becerra-Castro et al., 2013c).

Rhizofiltration is based on the use of aquatic plants to absorb in and/or adsorb on their roots the contaminants present in water, sediments or aqueous wastes in their roots. The use of aquatic macrophytes as biofilters in natural and constructed wetlands and wastewater treatment facilities has gained interest due to their well-known bioaccumulation properties (Marchand et al., 2010; Salem et al., 2014).

1.3. Advantages and constraints

The remediation of contaminated soils by phytotechnologies is considered an environmentally-friendly, aesthetically pleasing and economically viable alternative to harsher civil engineering-based methods. Moreover, phytomanagement can be applied *in situ* and on a large scale. Establishing an extensive plant cover prevents the dispersion of contaminated soil particles by wind and/or water erosion and can decrease contaminant availability and mobility through root accumulation, rhizosphere-induced adsorption and precipitation and/or degradation (Vangronsveld et al., 2009). However, these techniques do of course present a series of limitations and still require optimization before they can become fully implemented on a wide-scale. In addition to the inherent problems associated with any agronomical practice (such as the dependence on climate and season, outbreaks of pests or disease, etc.), a major problem associated with these techniques is the length of time required for the clean-up process (of particular concern in phytoextraction). Several authors have suggested that to be realistically viable the clean-up time should not exceed 10 years (Robinson et al., 2009; Vangronsveld et al., 2009). The time length required can also be significantly reduced if the target values are based on the available pool of contaminants and the pollutant linkages instead of total soil contaminant concentration. As mentioned above, the shift from phytoremediation strategies to phytomanagement options, in which remediation strategies



are combined with sustainable site management options, result in a net gain (or at least no gross reduction) in soil functions and ecosystem services, as well as achieving effective risk management (Cundy et al., 2016). The provision of ecosystem services may compensate some of the limitations of the remediation process. In this context, (aided) phytostabilization should be considered as a management strategy for contaminated sites which offers economic, environmental, and societal benefits (Cundy et al., 2016).

Climatic conditions pose a crucial and obvious limitation to the success of phytomanagement. Temperature controls transpiration, water chemistry, growth and metabolism of plants, and therefore directly affects both contaminant uptake and their fate in plant parts and other ecosystem compartments (Bhargava et al., 2012). Soil moisture affects both plant growth and contaminant transport in soil, and GRO management also needs water management, especially in arid and semi-arid areas that undergo relatively long periods of drought and heatwaves. Prolonged drought induces stress which enhances plants' sensitivity to pathogens or herbivory and, more importantly, reduces plant growth with negative implications on the phytoremediation success. Additional site-specific problems concern mining areas and sandy soils where soils are often characterized by a low water retention capacity (Kidd et al., 2015).

As mentioned above, a major limitation of phytoextraction is the very long time required to effectively extract metal(loid)s from soils, particularly in medium and highly contaminated sites (Zhao et al., 2003). However, if the aim of the phytoextraction strategy is only to strip the bioavailable metal(loid) fraction from soil ("bioavailable contaminant stripping") and not to reach total metal(loid) concentration targets established by legal frameworks, then the time required for successfully reaching this target is much shorter (Mench et al., 2018; Vangronsveld et al., 2009). Also, for phytoextraction, the low biomass and slow growth of most hyperaccumulators are largely responsible for the long time required. This limitation can be overcome to an extent by using plant species that provide an added value in order to



obtain economic benefit during the phytoextraction process itself. Energy crops, such as *Miscanthus* spp., *Ricinus communis* L., *Brassica napus* L., have been proposed due to their metal tolerance and accumulation capacity along with their usefulness for biofuel production (Burges et al., 2018). Other commercial applications of plants used in phytoremediation, such as biochar production, raw materials for industries (oil, paper, bio-chemicals, essential oils, etc.) and medicinal purposes are being studied (Pandey et al., 2016). The use of fast growing trees offers the possibility to combine metal (Cd, Zn, Ni) extraction with production of biomass for bioenergy and other end-products (e.g., timber, resin, adhesives, etc.) (Schroder et al., 2008). Recovery of high-value metals or strategic elements, from metal-rich plant biomass is another means of increasing the economic viability of phytoextraction (in this case termed as phytomining), while simultaneously eliminating the need for disposal of the contaminated biomass. Chaney et al. (2007) demonstrated that phytomining of Ni can be highly profitable in Ni-contaminated soils.

Additional aspects that should be considered include the degree of soil contamination, the bioavailability and accessibility of the contaminants, and the capacity of the plants and their associated microorganisms to adsorb, accumulate and/or degrade the contaminants (Vangronsveld et al., 2009). Assisted phytoextraction using chelates has been proposed as a means of increasing metal bioavailability, but an important limitation of chelate-induced phytoextraction is the possibility of promoting metal leaching to other environmental compartments (e.g., groundwater) (Burges et al., 2018).

The establishment and growth of plants on contaminated sites are other major obstacles (Mendez and Maier, 2008; Tordoff et al., 2000). In addition to the phytotoxic concentrations of pollutants, contaminated soils usually present edaphic conditions which can severely limit plant growth (nutrient deficiency, poor soil structure, low organic matter, etc.). The careful selection of tolerant and resilient plant species is vital for the long-term success of phytomanagement strategies (Batty, 2005; Clemente et al., 2012; Parraga-Aguado et al.,



2014). The efficiency of phytotechnologies can also be enhanced by incorporating agronomic practices. For example, plant cropping patterns (rotation, intercropping) can improve plant growth and performance and, depending on the phytotechnology, can be designed so as to enhance or mitigate metal(loid) availability, uptake and accumulation (Kidd et al., 2015). Intercropping, traditionally used in agriculture to increase crop yield, can pair phytoextracting plant species with other crops, in order to promote remediation while providing economic benefits (Burgess et al., 2018). The use of deep-rooting plants, mycorrhizal plants or bioinoculants can enhance plant growth and GRO efficiency (Kidd et al., 2009, 2015). The use of organic and inorganic amendments may optimize plant growth and performance by improving soil physicochemical properties, fertility and microbial activity and diversity (Bolan et al., 2011, Pardo et al., 2014b, 2014c). In addition, amendments directly or indirectly influence the availability and mobility contaminants through the modification of soil physicochemical and biological conditions (pH, redox conditions, concentration of chelating and complexing agents, cation exchange capacity, and biological activity) (Kidd et al., 2015; Pardo et al., 2016a; Pérez-De-Mora et al., 2006). Depending on site characteristics a selection of the most appropriate phytomanagement options will be necessary; in some cases the implementation of several approaches may be needed. The combination of different options can be more effective in site remediation than using a single approach.

1.4. Current status

Phytomanagement requires the use of appropriate agronomic and crop management practices, and can be assisted through the application of soil amendments. However, long-term field experiments are crucial for monitoring the efficiency and sustainability of phytomanagement options over time. A growing number of studies under field conditions can be found in the literature and these should contribute towards reaching full-scale deployment of these techniques. Such field studies have shown that phytostabilization can effectively reduce trace metal(loid) mobility by altering speciation, as well as to improve soil



physicochemical properties and fertility, increase microbial diversity and restore functionality in the long-term (Clemente et al., 2012; Kumpiene et al., 2009; Mench et al., 2018; Pardo et al., 2014c, 2014d, 2016a, 2017a; Quintela-Sabaris et al., 2017; Xue et al., 2015, 2018; Zornoza et al., 2012). At any given site, it will be necessary to implement a long-term monitoring programme so as to ensure that any reduction achieved in metal toxicity and improvement in soil quality are maintained (Epelde et al., 2014a).

Phytoremediation processes are governed by the interactions between three key players: soil, plants and microorganisms, and some biotic interactions. The last few years have seen a growing interest in the influence of microorganisms on plant growth and contaminant bioavailability and degradation. A growing body of results indicate a crucial role of plant-associated microorganisms in improving phytoremediation success (Afzal et al., 2014; Benizri and Kidd, 2017; Deng and Cao, 2017; Feng et al., 2017; Kidd et al., 2017; Lenoir et al., 2016; Sessitsch et al., 2013; Thijs et al., 2016). Rhizosphere and endophytic organisms that have received much attention because of their beneficial effects on plant growth health and resistance to stress are the plant growth-promoting bacteria (PGPB), mycorrhizal and endophytic fungi (Coninx et al., 2017; Mendes et al., 2013). Microorganisms can increase the availability of essential plant nutrients, such as nitrogen (N_2 -fixing organisms), phosphorus (by solubilization or mineralization through the production of organic acids and/or phosphatases) or iron (by releasing Fe(III)-specific chelating agents or siderophores). Plant growth-promoting bacteria can also directly influence plant growth and physiology through the production of phytohormones (e.g., IAA or by reducing stress ethylene levels in plants through the production of the enzyme 1-aminocyclopropane-1-carboxylate deaminase). Some bacteria can inhibit or reduce plant diseases indirectly by competing for nutrients and space (niche exclusion), producing antimicrobial compounds or through the induction of plant defence mechanisms (Compant et al., 2005; Lemanceau et al., 2007).



Several field-based trials implementing phytostabilization in metal-contaminated soils have shown the benefits of organic-based amendments for recovery of soil biological fertility. Microbial biomass and soil enzymatic activities were higher in acidic mine soils amended with pig manure/sewage sludge/marble waste than in the untreated mine tailings (Zanuzzi et al., 2009; Zornoza et al., 2012). Touceda-González et al. (2017) amended highly acidic Cu mine tailings with composted municipal sewage wastes and established a SRC system and a grassy cover. Microbial activity was stimulated and led to the establishment of vital biogeochemical cycles. Pardo et al. (2014d) successfully used olive-mill waste compost as a soil amendment to promote the growth of a native legume (*Bituminaria bituminosa* (L.) C. H. Stirt.) in a mine-affected soil from a semi-arid area (Southeast Spain) contaminated with trace elements (As, Cd, Cu, Mn, Pb and Zn).

However, the use of amendments has to be carried out with caution as amendments can have undesirable effects: for instance, an inappropriate use of organic amendments can result in underground water contamination by nitrates, antibiotics, hormones, and loss of soil biodiversity, posing a risk to environmental and human health (Burgess et al., 2016, 2018; Goss et al., 2013). Organic and inorganic amendments can induce other negative effects like destruction of soil structure, addition of potentially toxic compounds, immobilization of essential nutrients, etc. (Alkorta et al., 2010). Moreover, although amendments have demonstrated to aid revegetation, plant roots may not extend readily from a fertile layer into underlying non-amended contaminated soil (Pulford and Watson, 2003), limiting the potential of this phytotechnology to the top layer of soil.

1.5. Legal and regulatory framework

Key concerns regarding the increasing loss of soil quality through degradation or contamination of soils led the European Commission to develop a Soil Framework Directive (EC, 2006) which presented a Thematic Strategy toward soil protection considering eight



main threats to European soils: (1) erosion, (2) loss of organic matter, (3) contamination, (4) compaction and other physical soil degradation, (5) salinization, (6) decline of biodiversity, (7) soil sealing by infrastructure, and (8) floods and landslides (EC, 2006). Unfortunately, this Thematic Strategy was not accepted by all EU Member Countries. The Global Soil Partnership (GSP) was established in 2012 by the Food and Agriculture Organization of the United Nations (FAO) in order to develop interaction and enhanced collaboration amongst all relevant stakeholders (from land users to policy makers) towards the development of soil legislation and sustainable soil management measures. This proposal was very important and promoted a discussion on how to translate soil science into environmental policies (Bouma et al., 2017). The Intergovernmental Technical Panel on Soils (ITPS), which was established at the first Plenary Assembly of the GSP in 2013, published the first-ever comprehensive report on the State of the World's Soil Resources (SWSR) (FAO and ITPS, 2015). Major threats to soil functions at a global scale were identified as soil erosion, loss of soil organic carbon, nutrient imbalance, and salinization and sodification. Requirements for soil protection are also often included in other EU policies, such as the Nitrates Directive and the Water Framework Directive, and in the national legislations of various European countries, specifically addressing water, waste, and mining regulations. Although these policies consider soil contamination and contribute indirectly to soil protection, they only feature soil as a secondary objective.

The legislation available in many industrialized countries, regulating local soil contamination, and guidelines for assessing potentially contaminated soils, is based on total contaminant concentrations. However, negative effects of metal(loid)s on soil functioning is known to be related to mobile/bioavailable elemental pools rather than total metal concentrations (Kumpiene et al., 2009). Therefore, the site-specific approach based on conceptual model, pollutant linkages and risk assessment is more and more adopted in European countries (e.g., France, UK, Germany, etc.). On the other hand, it is often the case that bioavailable concentrations show no correlation with total concentrations (Burgess et al., 2015). There is a





general consensus that metal(loid) bioavailability is more important for environmental protection and risk assessment than total metal(loid) concentrations because it represents the labile fraction subject to leaching and uptake by soil organisms (Madejón et al., 2006). In recent years, more sophisticated risk-based approaches to deal with the local effects of soil pollution have been developed, which include the concept of pollutant linkages (contaminant-receptor-pathway). Decision makers and regulatory organizations have accepted that bioavailability of soil contaminants is a key variable to be taken into consideration in risk assessment, regulation policies and soil remediation (Naidu et al., 2015). These risk orientated policies focus on the abandonment of policies aimed at restoring soils to their original 'clean' state. Some national trigger values classifying soils as contaminated or requiring remediation now have bioavailability explicitly (e.g., in the UK, Belgium, Switzerland) or implicitly (trigger values set according to the main influencing soil physicochemical properties, e.g. soil pH, granulometry, organic matter content) embedded within them. Several phytomanagement options are aimed at removing the bioavailable contaminant fraction ("bioavailable stripping"), a target which significantly reduces the length of time required for rehabilitation.

There is now an emerging consensus in the broad frameworks and approaches for sustainable remediation being developed around the world (Bardos, 2014) which is culminating in the drafting of international standards by ISO and ASTM International. The fundamental basis of sustainable remediation is to promote the use of more sustainable practices during environmental clean-up activities, with the objective of balancing economic viability, conservation of natural resources and biodiversity, and the enhancement of the quality of life in surrounding communities. In broad terms, concepts of sustainable remediation are based on achieving a net benefit overall across a range of environmental, economic, and social concerns that are judged to be representative of sustainability. This is a key goal in land remediation and land regeneration, given the large global contaminated land legacy and the overall resource and financial cost required to bring this land back into





beneficial use. The implementation of the International Organization for Standardization (ISO) Standard on Sustainable Remediation is now at an advanced stage (REFS). Remediation begins with an option appraisal that short lists strategies that could deliver the required reduction in risk. A remediation strategy comprises one or more remediation technologies that will deliver the safe and timely elimination and/or control of unacceptable risks. The ISO standard will help assessors identify the most sustainable among the shortlisted, valid alternative remediation strategies.



2. BIODIVERSITY

2.1. Basic concepts on biodiversity

2.1.1. Definition of biodiversity

One of the most commonly used definitions of *biodiversity* or *biological diversity* (both terms are often used interchangeably) is that endorsed by The International Convention on Biological Diversity (1992): “The variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species, and of ecosystems”.

The term *biodiversity* encompasses the whole range of species and biological communities, together with the genetic variation within those species and the features and

Populations: organisms of the same species that live in the same area and interact with one another.

Biological communities: all of the populations of different species in a specific place or time.

Assemblage: a taxonomically related group of species that occur together in space and time.

processes of the ecosystems where they live. Simplifying, the term *biodiversity* refers to the total sum of all biological variability from the level of genes to that of ecosystems, passing through *species*, *populations* and *biological communities*.

The number of interacting species in biological communities and the complexity of their interactions exemplify what is meant by the term *biodiversity*. Here, it must be taken into consideration that the relationships between species change over evolutionary time as species adapt to one another through coevolution.

The field of *Ecology* (the scientific study of the interactions between organisms and their environment) can be divided into: (1) *autecology*, dealing with the study of the interactions of an individual organism or a single species with the living and non-living factors of its



environment, i.e. the ecology of individual organisms and populations; and (2) *synecology* or community ecology, dealing with study of the organization and functioning of communities, i.e. the ecology of different species living in the same habitat.

Definitions of *species*:

- 1.- A group of living organisms consisting of similar individuals capable of exchanging genes or interbreeding.
- 2.- The largest group of organisms in which two individuals can produce fertile offspring, typically by sexual reproduction.
- 3.- A group of interbreeding natural populations that do not successfully mate or reproduce with other such groups.

There are certainly many definitions of *species*, the basic unit of taxonomic classification and biological diversity, but unfortunately all of them are unsatisfactory. Although the debate of the limitations and problems associated to the definitions of *species* is not within

the scope of this document, just to mention that the most frequently used definitions of *species* (see Box) are particularly inadequate when dealing with microbial life. After all, prokaryotes (bacteria and archaea) do not have sexual reproduction mechanisms and, in addition, they can transfer genes by horizontal/lateral gene transfer (see below). But, paradoxically, microorganisms (i) are responsible for most of the biodiversity in this planet, and, besides, (ii) remain the largest unexplored reservoir of biodiversity on Earth (Konstantinidis et al., 2006). Prokaryotic species are typically defined according to several criteria, including the well-known arbitrary requirement that a species' genome must have no more than 70% DNA-DNA relatedness or 97% 16S rRNA identity to its nearest relative of another species.

In any case, at present, there seems to be general consensus that circumscriptions of species for taxonomic purposes must be based on an extensive set of parameters, in order to guarantee the understanding of their uniqueness (the so-called 'polyphasic approach') (Gevers et al., 2006; Rosselló-Morá and Amman, 2015). In fact, according to Rosselló-Morá and Amman (2015), for a correct classification of a species, three major premises must be fulfilled: monophyly, genomic coherence and phenotypic coherence. In consequence, for



these authors, the term *species* refers to “a category that circumscribes monophyletic, and genomically and phenotypically coherent populations of individuals that can be clearly discriminated from other such entities by means of standardized parameters”.

Correspondingly, nowadays, the ‘polyphasic approach’ for bacterial and archaeal taxonomy indicates that a novel species must be recognized according to phenotypic, genotypic and chemotaxonomic traits of the organisms (Chun and Rainey, 2014; Tindall et al., 2010). The recent development of next-generation sequencing-NGS (see below) has provided, among many other benefits, a cost-effective method for obtaining whole-genome sequences of microbial strains. Undoubtedly, the development of these innovative genomic techniques, coupled with computational advances, has, and will, greatly advance the field of prokaryotic taxonomy and systematics (Chun and Rainey, 2014). In particular, the emergence of high-throughput genomic sequencing has emphasized the importance of genomic similarity in bacterial taxonomy (Zhu et al., 2015).

Although it is true that, at the present time, prokaryotic taxonomy relies strongly on *phylogenetics*, we must not forget that there are also non-phylogenetic alternatives for classification such as, for instance, *phenetics*, which classifies organisms based on similarity regardless of shared ancestry. Thus, while the task of phylogeny-based taxonomy (*cladistics*) consists of reconstructing organismal evolutionary history, phenetics attempts to cluster organisms into consistent classes on the basis of observable traits (Zhu et al., 2015).

Finally, biodiversity can be studied at three levels of complexity (Primack et al., 2008): genetic, species and ecosystems (the term *ecosystem* refers to the complex of living organisms, their physical environment, and all their interrelationships in a particular unit of space).



2.1.1.1. Genetic diversity

The term *genetic diversity* refers to the genetic variation within species (i.e., among individuals within a population and among different populations of a given species). Here, it must be taken into consideration that biodiversity is a consequence of the combined action of ecological and evolutionary processes (e.g., competition, speciation, natural selection, etc.). Indeed, genetic variation plays a critical role in evolutionary processes; after all, without genetic variation, there would be no evolutionary change (in other words, evolution is only possible in the presence of genetic variation). The origin of this genetic diversity rests upon the processes of *mutation* (generating new genetic variants) and *recombination* (generating new combinations of those variants).

Genetic diversity encompasses the components of the genetic coding that structure organisms (i.e., nucleotides, genes and chromosomes), as well as the variation in the genetic make-up between individuals within a population and between populations (Gaston and Spicer, 2004). The total number of *genes* and *alleles* in a given population is called its *gene pool*. An organism's *genotype* is the set of genes that it carries, which

Nucleotides form the basic structural unit of both DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and are composed of three subunit molecules: a nitrogenous base (purine or pyrimidine), a five-carbon sugar (ribose or deoxyribose) and a phosphate group.

Genes are functional units, at the DNA level, that act as the blueprints for proteins.

Alleles are different forms of a gene which arise through mutations of the DNA sequence, i.e. an allele is one of the versions of a gene that may exist at a locus.

Locus: the place in the DNA where a gene is located.

depends on the specific combination of alleles that it possesses. In turn, the term *phenotype* refers to all the observable characteristics (morphological, physiological, anatomical, biochemical, behavioural, etc.) of an organism, which are influenced by both its genotype and the environment. Finally, *chromosomes* are structures within the cell that bear the genetic material as a threadlike linear strand of DNA bonded to various proteins in the nucleus of eukaryotic cells, or as a circular strand of DNA in the cytoplasm of prokaryotes and in the mitochondrion and chloroplast of eukaryotes.



Traditionally, biodiversity research was focused on *taxonomic diversity*, notably species richness and turnover. But since species are not evolutionarily independent neither functionally equivalent, nowadays, much research is directed towards the understanding of (i) *phylogenetic diversity*, which reflects the accumulated evolutionary history of a community, and (ii) *functional diversity*, which reflects the diversity of morphological, physiological and ecological traits (Arnan et al., 2016). Indeed, in the last decades, for the measurement of biodiversity, the focus has shifted from the simple counting of species and their abundances (see below: species richness and species diversity) to the quantification of the evolutionary information represented within groups of taxa (i.e., *phylogenetic diversity*) and/or the diversity of ecological traits (i.e., *functional diversity*).

For the quantification of *functional diversity*, attention is paid to what organisms do in communities and ecosystems rather than to their evolutionary history. Nevertheless, the concept of *functional diversity* is unquestionably complex (Petchey and Gaston, 2006). Hence, not surprisingly, many definitions have been proposed for this type of biodiversity, e.g. “The value and range of those species and organismal traits that influence ecosystem functioning” (Tilman, 2001); “The functional multiplicity within a community” (Tesfaye et al., 2003); “The number, type and distribution of functions performed by organisms within an ecosystem” (Díaz and Cabido, 2001); “The distribution of species in a functional space whose axes represent functional features” (Rosenfeld, 2002); and so on. Importantly, the study of *functional diversity* (defined here as the value, range and relative abundance of traits present in the organisms of an ecological community) can reveal the functional response of species assemblages to environmental filters or their ability to occupy functional niche space in such a way as to optimize ecosystem functioning; in turn, the study of *phylogenetic diversity* provides information on a system’s capacity to generate evolutionary solutions in the face of environmental changes or to persist despite those changes (Arnan et al., 2016).



In relation to the concept of functional diversity, the term *functional group* refers to a group of organisms that respond to the environment or affect ecosystem processes in a similar way. Thus, the set of species co-existing in a given community constitute a functional group if they have similar functional characteristics related to one ecosystem service (see below) (Pla et al., 2012). Since a functional group is a collection of organisms with similar suites of co-occurring functional attributes, they have similar responses to external factors and/or effects on ecosystem processes (de Bello et al., 2010).

The term *functional trait* refers to the morphological, biochemical, physiological, structural, phenological or behavioural characteristics of organisms that influence performance or fitness (Nock et al., 2016). In the last years, many researchers working on the study of biodiversity have focused their interest on the diversity of organismal functional traits, i.e. on characteristics that define how organisms interact with their surrounding physical, chemical and biological environments (Nock et al., 2016). Here, it is important to differentiate (i) *effect traits*, which determine the influence of a given species on ecosystem properties and, hence, ecosystem services; and (ii) *response traits*, those that influence the abilities of species to colonise or thrive in a habitat and to persist in the face of environmental changes.

Importantly, microorganisms mediate most of the biogeochemical transformations that are critical for the functioning of ecosystems (Cantrell et al., 2013). In this respect, different *microbial functional groups* (these groups mediate ecosystem processes) can be defined, such as lignolytic and cellulolytic decomposers, nitrogen-fixers, nitrifiers, denitrifiers, methanogens, methane oxidizers, sulfidogenic bacteria, dissimilatory Fe(III)-reducers, etc. (Cantrell et al., 2013).

On the other hand, soil organisms are often divided into three functional groups: (1) *ecosystem engineers*: these organisms can modify or create habitats for smaller organisms by building soil aggregates and pores (e.g., earthworms, ants, termites, small mammals); (2)



chemical engineers: these organisms are responsible for the decomposition of organic matter (mainly, bacteria, fungi and protozoa); and (3) *biological regulators*: these organisms (e.g., nematodes, pot worms, springtails, mites) regulate the dynamics in space and time of other organisms, for instance, by means of being predators of plants, invertebrates or microorganisms.

The terms *functional group* and *guild* are frequently used interchangeably. Nonetheless, in strict terms, they do not refer to the same thing: the term *functional group* is concerned with how a resource or any other ecological component is processed by different species to provide a specific ecosystem service or function (i.e., the ecosystem processes these species eventually perform through resource exploitation); on the other hand, the term *guild* refers primarily to the mechanisms of resource sharing by species in a competitive context (i.e., the sharing by species of a similar resource) (Blondel, 2003). The term *guild* was initially defined by Root (1967) as “a group of species that exploit the same class of environmental resources in a similar way. This term groups together species without regard to taxonomic position that overlap significantly in their niche requirements”. In other words, the term *guild* refers to a group of species that exploit the same class of resources in a similar way. The terms *functional group* and *guild* have mainly been used in plant and animal science, respectively.

Functional diversity is related to but distinct from functional redundancy: functional diversity arises when different species that perform a similar function exploit different aspects of a habitat (Leslie & McCabe, 2013).



Next-generation sequencing

As abovementioned, in the last years, next-generation sequencing-NGS (the colloquial way to describe highly parallel or high-output sequencing methods that produce data at or beyond the genome scale) has evolved at an amazing pace, supported by the development of the field of bioinformatics as a major scientific discipline (Levy and Myers, 2016). In particular, the advent of NGS has revolutionized the field of Microbial Ecology, allowing, for instance, a high-throughput analysis of complex microbial communities (such as those present in the soil ecosystem, marine water, freshwater, etc.) and boosting the study of the microbiota of animals (above all, humans) and plants (Boughner and Singh, 2016). Next-generation sequencing is currently an inexpensive technique that facilitates the generation of massive sequencing results in parallel using, for instance, the 16S rRNA gene, an excellent phylogenetic marker. This gene is conserved across all prokaryotic species and it is more resistant to mutations than other conserved genes; nonetheless, some regions of the 16S rRNA gene, called hot spots, are different in all species (Clarridge III, 2004). More importantly, it is very improbable for the 16S rRNA gene to be horizontally transferred (see below: horizontal gene transfer-HGT), but such an event is not impossible (Kitahara and Miyazaki, 2013; Tian et al., 2015). In any case, unlike HGT of functional genes, the HGT of the 16S rRNA gene has been reported to occur at a low rate and only between closely related taxa (at intragenus or intraspecies levels) (Tian et al., 2015).

Indeed, NGS techniques have allowed us to analyse the *microbiome* and *metagenome* of environmental samples, as well as from samples taken from plants and animals (relevantly, humans), paving the way for a better understanding of the astounding biodiversity of microbial life on Earth. In fact, it has been estimated that Earth is home to upward of 1 trillion (10^{12}) microbial species (Locey and Lennon, 2016). Actually, most of the phylogenetic diversity on Earth is microbial (Pace, 1997). Besides, given the abundance, ubiquity and biogeochemical importance of microorganisms, the integration of microorganisms into studies of biodiversity considerably benefits the field of conservation biology. However, the vast majority of microbial taxa cannot be identified using standard culture-based methodologies. Fortunately, as described above, recent developments in molecular techniques, and in particular NGS, have greatly promoted surveys of the highly diverse microbial world (Fierer et al., 2010).



Notably, soils harbour an extraordinary bacterial diversity: thus, a single gram of soil can have up to 10^{10} bacterial cells and an estimated bacterial species richness of 4×10^3 - 5×10^4 species (Roesch et al., 2007; Torsvik et al., 1990). Soils are indeed among the most diverse microbial environments analysed to date (Youssef and Elshahed, 2009). Interestingly, despite this extremely high number of bacterial cells and species in soil, it has been estimated (Raynaud and Nunan, 2014) that the number of neighbours a single bacterium has within an interaction distance is relatively limited, as is the number of bacterial species. In turn, the concentration of viruses in soil has been estimated to be $\sim 10^9$ virus particles g^{-1} dry weight (Swanson et al., 2009; Williamson et al., 2005).

Currently, there are different approaches for the NGS analysis of microbial communities, such as, for example (Boughner and Singh, 2016): (i) *targeted amplicon sequencing*, a primer based method by which a gene or region of interest is sequenced; (ii) *metagenomics*, or shotgun sequencing, a method by which all the DNA extracted from an environmental sample is sequenced; (iii) *transcriptomics*, or RNA sequencing (RNA-seq), focused on the analysis of RNA present at the time of extraction; (iv) *proteomics*, which examines the proteins that have actually been produced from the mRNA (here, it must be remembered that not all amplified mRNA leads to synthesized protein); and (v) *transposon sequencing* (Tn-seq), a method used to examine gene functions and/or genes of essentiality within an organism under varying growth conditions.

[Note: there is some confusion in the vocabulary used in the field of microbial community analysis. Marchesi and Ravel (2015) proposed the following definitions: **(1) microbiota**: the assemblage of microorganisms present in a defined environment; **(2) metagenome**: the collection of genomes and genes from the members of a microbiota; and **(3) microbiome**: the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses), their genomes (i.e., genes), and the surrounding environmental conditions. This later definition is based on that of *biome*, the biotic and abiotic factors of given environments].



Horizontal/lateral gene transfer

The immense genetic diversity of prokaryotes depends on their having a variety of efficient genome modification mechanisms, which allow adaptation to changing environmental conditions and facilitate the colonization of a plethora of ecological niches (Norman et al., 2009). In particular, a most important mechanism for providing genetic variation is *horizontal gene transfer* (HGT), in which new genes are acquired via *mobile genetic elements* (such as, for instance, plasmids, integrons and transposons) or by direct uptake and incorporation of naked DNA by homologous or illegitimate recombination (Frost et al., 2005). Horizontal gene transfer occurs both between closely related and highly divergent species (however, it is easier in closely related organisms) (Andam and Gogarten, 2011; Boto, 2010), and can involve DNA molecules that are circular or linear, single-stranded or double stranded, self-replicating or not (Chu et al., 2018). Mobile genetic elements (MGEs) are segments of DNA that encode enzymes and other proteins that mediate the movement of DNA within genomes (intracellularly) or between prokaryotic cells (intercellularly). The three main mechanisms of intercellular DNA movement are (Frost et al., 2005):

(1) Transformation: free DNA is taken up from the environment by recipient bacterial cells and is then incorporated into their genomes. The transfer of DNA occurs between closely related bacteria and is mediated by chromosomally encoded proteins.

(2) Conjugation: temporary direct contact

between two bacterial cells that results in an exchange of DNA. This exchange is unidirectional, i.e. one cell is the donor of DNA and the other is the recipient. Thanks to this phenomenon, genes are transferred laterally among bacteria, as opposed to vertical gene

A **plasmid** is a collection of functional genetic modules that are organized into a self-replicating entity or replicon, which usually does not contain genes required for essential cellular functions.

Integrated conjugative elements (ICEs) are chromosomally located gene clusters that encode phage-linked integrases and conjugation proteins; *conjugative transposons* can be transferred between cells, whereas *genomic islands* cannot.

transfer in which genes are passed on to offspring. The mechanism of conjugation requires independently replicating genetic elements, such as *conjugative plasmids* and *chromosomally integrated conjugative elements*, which encode proteins that allow their own transfer and,



occasionally, the transfer of other cellular DNA from the donor plasmid-carrying cell to a recipient cell that lacks the plasmid or ICE.

(3) Transduction: a form of DNA transfer that is mediated by independently replicating bacterial viruses called *bacteriophages* or *phages*, which can accidentally package segments of host DNA in their capsid and then inject this DNA into a new host, in which it can recombine with the host's chromosome. Thus, bacteriophages function as vectors to introduce DNA from donor bacteria into recipient bacteria by infection.

In the case of intracellular movement of DNA, **transposons** (promiscuously recombining *loci*) randomly recombine or 'jump' between replicons. Transposons can 'hop' into phages or plasmids, and then be transferred with them into other bacterial cells. Other relevant MGEs are **integrons**. Integrons are genetic elements that encode an integrase enzyme, which can assemble tandem arrays of genes or gene fragments (*gene cassettes*) and provide them with a promoter for expression. Integrons, which usually reside on bacterial chromosomes (Gillings, 2014), have three main features: an integron-integrase gene (*intI*), a recombination site (*attI*) and a promoter (Gillings et al., 2015). Thus, integrons sample gene cassettes that encode functions of potential adaptive significance (e.g., integrons have often been associated with antibiotic multi-resistance). Although integrons are chromosomal elements, they can be mobilised onto plasmids (and then into other bacterial cells) by transposons.

Horizontal gene transfer can occur from Bacteria to Archaea, from Archaea to Bacteria, from Archaea to Eukarya, from Bacteria to Eukarya, from Eukarya to Bacteria and even within Eukarya (Boto et al., 2010). Nonetheless, HGT has been much more widely documented in Bacteria and Archaea. Koonin et al. (2001) estimated that between 1.6 and 32.6% of the genes of each prokaryotic genome are acquired by HGT. Dagan et al. (2008) reported a value of $81 \pm 15\%$ if the cumulative impact of HGT towards lineages is considered.

Thanks to HGT, prokaryotic genomes are highly dynamic systems in which considerable amounts of genetic material are being added or lost through promiscuous genetic exchanges (Norman et al.,



2009). As a matter of fact, it has been reported that HGT has accelerated the introduction of new genes into prokaryotes by a factor of at least 10,000 (Jain et al., 2003). Most importantly, HGT helps augment the genetic diversity of a microbial population or community and can confer novel traits that help microbes adapt to a broad range of environments (Chu et al., 2018; Koonin, 2016; Vos et al., 2015). Importantly, antibiotic resistance genes (ARGs) are associated with highly mobile genetic elements, with plasmids being the most common method of acquiring antibiotic resistance determinants (Ochman et al., 2000). However, transposable elements (the resistant determinant is flanked by two insertion sequences) and integrons (the mobility of integrons demands capture by insertion sequences, transposons or conjugative plasmids) can also disseminate ARGs.

In particular, the rhizosphere (the area of soil directly affected by a plant's root system, associated root secretions and microorganisms) is a major hot spot for HGT when compared to bulk soil (Van Elsas et al., 2000), due to, among other reasons, the enhanced nutrient input and water fluxes in the rhizosphere, which may stimulate bacterial metabolic activities and the elicitors of conjugative plasmid transfers between rhizosphere inhabitants (Van Elsas et al., 2002). Similarly, the phyllosphere (the total aboveground portions of plants as habitat for microorganisms) of plants has been shown to be equally conducive to conjugative plasmid transfer (Björklöf, 1995).

2.1.1.2. Species diversity

Species diversity refers to the whole range of species in a particular unit of study. It usually incorporates two main parameters: number of species (*species richness*) and number of individuals within each of those species (*species abundance*). In other words, species diversity, as a measure of community complexity, is a function of both: (i) number of different species in the community (species richness); and (ii) their relative abundances (or *species evenness*). The term *relative abundance* refers to the total number of individuals of one taxon compared with the total number of individuals of all other taxa in an area, volume, or community. Greater numbers of species and more even abundances of species result in higher species diversity.



Species diversity is possibly the most commonly used representation of biodiversity (in fact, many people use the term biodiversity as a synonym for species richness or species diversity). Actually, the *structure of a biological community* can be described by its species richness (number of species present) and species diversity (as mentioned above, a measure of both species richness and species evenness).

As expected, the structure of a given community (i.e., essentially, its composition) can be affected by different interacting factors such as abiotic parameters (climate patterns, geography, environmental heterogeneity, etc.), species interactions, level and frequency of disturbances, etc.

Remarkably, some species play unusually important roles on community structure:

Foundation species play a unique and vital role in defining a biological community, often by modifying the environment in such a way that it can support the other organisms that form the community. These species physically modify the environment and create and maintain habitats that benefit other organisms.

Ecosystem engineers modify their habitats through their own biology or by physically changing environmental (biotic and abiotic) factors: (i) *autogenic engineers* modify their environment by modifying their own biology; and (ii) *allogenic engineers* physically change their environment. Interestingly, *invasive species*, lacking local predators or abiotic factors to constrain them, can be ecosystem engineers and modify the environment in ways that inhibit the growth of the native species.



Keystone species have a disproportionately large effect on community structure relative to its biomass or abundance, and are more likely to belong to higher trophic levels and act in more diverse ways than foundation species.

The term **umbrella species** (often conflated with the term *keystone species*) also describes a single species on which many other species depend but, unlike keystone species, its value is tied to its geographic species range. Umbrella species have large habitat needs and, in consequence, their identification can be of great value for conservation purposes (actually, the minimum species range of an umbrella species has often been used as the basis for establishing the size of a given protected area).

Flagship species (often charismatic megafauna, with popular appeal due to their appearance or cultural value) are used for conservation purposes, acting as “mascots” for entire ecosystems.

Indicator species are very sensitive to environmental changes in the ecosystem and can, thus, provide early warning that a habitat is being altered or degraded. In fact, their status (presence, absence or abundance) can reveal the qualitative status of the environment or reflect a unique set of environmental qualities or characteristics found in a specific place. Indicator species can reveal a change in the biological condition of a particular ecosystem and are, therefore, being used as a proxy to diagnose the health of an ecosystem.

Sentinel species are similar to indicator species in that they can be used to predict health outcomes, but are, nonetheless, more commonly used as indicators of health threats to humans. To this purpose, habitually, animals are placed deliberately in an area of special interest to assess the impact of, for instance, contamination or remediation efforts, on human health.



Species diversity can be studied at three different levels (Primack et al., 2008):

(1) Alpha diversity: number of species in a given community (it is often used to compare it with the number of species of another community).

(2) Beta diversity: rate of change of species composition as one moves across a region (e.g., to measure changes in species composition with respect to changes in environmental conditions along a gradient).

(3) Gamma diversity: number of species found across a large region with a number of ecosystems.

Thus, alpha diversity refers to “species richness”, beta diversity is focused on “susceptibility to change”, and gamma diversity is similar to alpha diversity but measured over a large scale.

When studying the diversity of species belonging to a given biological community, it must be taken into consideration that biological communities change over time, a phenomenon known as *succession* or *ecological succession*. The term *succession* refers to a directional process of progressive change in the composition of a biological community over time, owing to changes in the environmental conditions and the replacement of species. Actually, succession has been defined as “the somewhat orderly and predictable manner by which communities change over time following the colonization of a new environment” (Fierer et al., 2010).

There are two types of ecological succession:



(1) **Primary succession**, when new land is formed or bare rock is exposed, providing a habitat that can be colonized for the first time, initially by pioneer species.

(2) **Secondary succession**, in which a previously occupied area is disturbed and then re-colonized following such disturbance. The biological community arising in the disturbed area then goes through a series of compositional changes over time (i.e., the ecological succession).

Traditionally, ecologists have thought that the end result of an ecological succession is a stable *climax community*, largely determined by an area's climate. Nonetheless, the concept of climax community as the end point of succession has been called into question, since ecosystems can experience frequent disturbances that prevent a community from reaching or maintaining an equilibrium state.

Most of the studies on succession have been carried out with plant communities. On the contrary, the successional patterns exhibited by microbial communities have received relatively little attention (Fierer et al., 2010). Since the abovementioned categories of primary and secondary succession have limitations for describing microbial succession (microorganisms are far more diverse, phylogenetically and physiologically speaking, than plant communities), Fierer et al. (2010) proposed three operationally-defined categories of microbial succession related to primary succession dynamics:

(1) *Autotrophic*: initial colonizers are predominately autotrophs using light or the oxidation of inorganic compounds to generate energy. In this case, little to no organic carbon is initially available.

(2) *Endogenous heterotrophic*: initial colonizers are predominately heterotrophs, respiring or fermenting organic compounds to generate energy. In this category, succession is primarily



fuelled by organic carbon derived from the substrate itself. In fact, the majority of organic carbon supplies in this endogenous succession are derived from a single initial input contained within the substrate itself.

(3) *Exogenous heterotrophic*: initial colonizers are predominately heterotrophic respirers or fermenters. Exogenous succession is fuelled by continuous external inputs of organic carbon.

In any case, microbial community assemblages can be regulated by (i) the local environment (species sorting); (ii) massive immigration (mass effect); and (iii) neutral process, which assumes that all species are similar in their competitive ability and in dispersal (López-Lozano et al., 2013). These mechanisms can co-occur, leading to microbial communities being structured by more than one of these processes (Langenheder and Székely, 2011). In any event, there is a long-standing debate about whether stochastic or deterministic factors are more important in controlling community assembly during succession. Furthermore, in the soil environment, successional trajectories for bacteria and fungi may be quite different (Schmidt et al., 2014). Bacteria are less likely to be dispersal limited than are larger microbes (fungi), which could result in a more deterministic community assembly pattern for bacteria during primary succession in soil. Besides, bacteria exhibit a much broader range of physiologies than do fungi and, in consequence, are more likely to be successful colonists of early-successional soils (Schmidt et al., 2014).

2.1.1.3. Ecosystem diversity

An *ecosystem* is a dynamic and complex system, acting as a functional unit, which includes all living organisms – archaea, bacteria, fungi, protists, plants, animals – in a given area, as well as the interactions among themselves and with their non-living environment. Thus, an ecosystem is formed by a biological community (*biocenosis* or *biocoenosis*) along with its physical environment (*biotope*).



Ecosystem diversity is focused on biological communities and ecological processes, together with the chemical and physical environment. The living and non-living components of an ecosystem are linked by a network of biophysical relationships involving the exchange of matter and energy; in other words, the biotic and abiotic components of an ecosystem are linked together through nutrient cycles and energy flows. Ecosystems, as systems defined by the network of interactions among organisms and between organisms and their environment, can be of any size but usually encompass specific, limited spaces.

The biotic interactions in ecological networks can be *trophic* or *symbiotic*. Concerning trophic interactions, the trophic level of an organism represents its position in the food chain (a *food chain* is a succession of organisms that eat other organisms and may, in turn, be eaten themselves). In this respect, organisms are often divided in: (i) *producers (autotrophs)*: primary producers that produce their own food by means of photosynthesis and, exceptionally (e.g., in deep-sea hydrothermal ecosystems), chemosynthesis; (ii) *consumers (heterotrophs)*: herbivores, carnivores and omnivores; and (iii) *decomposers (detritivores)*, which break down dead organic matter, thus releasing essential nutrients back into the environment. *Food webs* are formed by interconnections between different food chains (linear networks of links), which, in turn, usually consist of three or four trophic levels. At each trophic level, energy is lost in the form of heat, as organisms expend energy for metabolic processes.

When studying ecosystems and ecosystem diversity, common concepts are those of:

(1) *Habitat*, or the place where an organism, a population or a community lives, including all living and non-living components of the environment (in other words, a habitat is characterized by both physical and biological features).



(2) *Ecological niche*, which refers to the way a species or population relates to, or fits in with, its environment; in other words, the relational position of a species or population in an ecosystem. The niche of a species includes: (i) all of its interactions (competition, predation, parasitism, mutualism) with the other members of the community; (ii) how a population responds to the abundance of resources; and (iii) the abiotic environment, since the physical environment influences how species and populations affect, and are affected by, resources and the presence and activity of the other members of the biological community. In other words, the *niche* is made up of all the physical, chemical, and biological factors that a species needs to survive and reproduce, while the *habitat* includes all of the biotic and abiotic factors where an organism lives.

It must be remembered that the ecological niche evolves as physical and biological factors change, of course provided that those changes are slow enough for species to adapt. In any event, *niche width* (the breadth or diversity of resources used by an organism or taxon, i.e. the availability of resources over spatial and temporal scales) and *habitat diversity* (the structural complexity of the environment) are both critical important components of ecosystem diversity.

Regarding biological interactions, it is a well-known fact that the various species living in an ecosystem can relate to each other in different ways. Thus, the term ***symbiosis*** refers to an interaction between two organisms of different species that live in direct contact with one another (i.e., that share a close physical space). There are three different types of symbiosis: mutualism, commensalism and parasitism.



Mutualism is a symbiotic relationship where two organisms of different species benefit from the interaction (i.e., the interaction is mutually beneficial, for instance, in terms of protection, locomotion, nutrition, etc.). In some cases, the interaction is so close that the two organisms need each other to survive. In this respect, the mutualistic relationships can be obligate (the survival of either one or both organisms is dependent upon the interaction) or facultative (the symbiosis is not necessary to their survival).

Commensalism is another symbiotic interaction where one organism (the commensal) benefits and the other (the host) does not benefit nor is it harmed, i.e. an interaction in which one species obtains food or other benefits (shelter, locomotion, etc.) from the other without either benefiting or harming the latter. There are several types of commensalism, such as (1) inquilinism, when one organism uses the other for permanent shelter; (2) metabiosis, when one organism creates a habitat for the other organism to use; and (3) phoresy, when one organism uses another for travel.

Parasitism is a symbiotic relationship where one organism (parasite) benefits and the other (host) is harmed. Typically, the host harbours the parasite, which obtains shelter and nourishment. Usually, parasites do not usually aim to kill their host, but sometimes they do either unintentionally or intentionally. Thus, necrotrophic parasites kill their host intentionally. On the other hand, biotrophic parasites thrive off a living host.

In relation to non-symbiotic interactions, **competition** occurs when two organisms rely on the same resource, such as food, space, water, etc. The term **predation** refers to a non-symbiotic interaction in which a predator (the organism that is hunting) feeds on its prey (the organism that is attacked).

2.1.2. Biodiversity indices

Before starting, it must be stated that because the variety of life can be expressed in a multiplicity of ways, there is no single overall measure of biodiversity, rather there are multiple measures of different facets. After all, two species can be discriminated according to facets of their biochemistry, biogeography, evolutionary history, ecological role, genetics, morphology, physiology and so on (Gaston and Spicer, 2004).

A myriad of indices, many of them derived from information theory, can be used to measure biodiversity (i.e., the variety and heterogeneity of organisms or traits at all levels of the



hierarchy of life). However the quantification of biodiversity is somewhat confusing as there is no single index that adequately summarizes such concept. Richness (S), or the number of species or attributes present, is the simplest and most commonly used metric to quantify diversity. In turn, species diversity indices usually take two aspects into consideration: species richness and evenness (or *equitability*, i.e. the distribution of abundance among the species; low values of evenness indicate that one or a few species dominate, while high values indicate that relatively equal numbers of individuals belong to each species.).

Indeed, many diversity indices, such as Shannon's diversity (H') and Simpson's diversity (D_1), combine measurements of species richness and abundance (Magurran, 2004). Shannon's diversity (H') represents the uncertainty about the identity of an unknown individual. In turn, Simpson's diversity (D_1) represents the probability that two randomly chosen individuals belong to different species. Other commonly used indices of diversity are: Simpson's dominance (D_2), Berger-Parker dominance (BP), Pielou's evenness (J') and Simpson's evenness (E) (Morris et al., 2014).

Shannon's and Simpson's diversity indices are frequently used to quantify prokaryotic diversity based on operational taxonomic units (OTUs). Besides, in microbial diversity studies, rarefaction curves (they are used to

Operational taxonomic units: clusters of microorganisms, grouped by DNA sequence similarity of a specific taxonomic marker gene; normally, OTUs, as proxies for microbial species, are based on similar 16S rRNA sequences and they are defined according to a 97% similarity threshold).

compare observed richness among communities that have been unequally sampled) and ACE (Abundance-based Coverage Estimator) and Chao1 indices are often used (Kim et al., 2017).

Regrettably, traditional measures focusing on species richness and their relative abundance make no distinctions among the species involved (i.e., they are "species neutral") (Chao et al., 2010; Magurran and Dornelas, 2010). In this respect, there have been many proposals



on how to incorporate phylogenetic differentiation to non-neutral species diversity measurements (Cavender-Bares et al. 2009; Faith, 2002; Vellend et al., 2010). After all, it has frequently been recognized that, all else being equal, an assemblage of phylogenetically divergent species is more diverse than an assemblage consisting of closely related species (Chao et al., 2010). Interestingly, Chao et al. (2010) proposed a unified class of phylogenetic diversity measures that are based on Hill numbers and that obey the replication principle

On the other hand, functional diversity can be summarized using indices based on trait values and species importance in the community, like abundance, cover and biomass (Pla et al., 2012). Functional diversity metrics can be single-trait or multi-trait. Some well-known single-trait metrics and indices are the Community Weighted Mean, the Functional Divergence Index, and the Functional Regularity Index. In turn, common multi-trait indices are the Functional Attribute Diversity; Convex Hull Hyper-Volume; Quadratic Entropy; Extended Functional Diversity; and Functional Richness, Evenness, Divergence and Dispersion (Pla et al., 2012).

Finally, it must be taken into consideration that, although common indices of diversity may appear interchangeable in simple analyses, when considering complex interactions, the choice of index can strongly alter data interpretation (Morris et al., 2014). In any case, when using diversity indices, we must always remember that describing species diversity as a single value compromises much of the detailed structure of a community. Indeed, the use of indices inevitably results in “information compression” and often leads to an oversimplification of available information (Garbisu et al., 2011).



2.2. Values of biodiversity

In our modern society, many people tend to forget that human well-being closely depends upon biodiversity in many different ways and, more worryingly, that its current loss (see below) is adversely impacting the quality of our lives. Then, in the current scenario of biodiversity loss, it is becoming more and more important to properly conceptualize the link between biodiversity (genes, species, habitats, ecosystems, traits, etc.) and human well-being (i.e., health, wealth, security, etc.) (Naeem et al., 2016).

The values of biodiversity are frequently divided into two main categories: (1) *utilitarian* (or anthropocentric) value; and (ii) *intrinsic* (or biocentric) value.

The *utilitarian value* of biodiversity, determined by its use or function, is typically measured in terms of its use for humans (although it can also represent the value of an organism to other living things or its ecological value). In turn, utilitarian values of biodiversity are often subdivided into:

(1) *Direct use* values for those *goods* (e.g., food, medicines, fiber, fuel, building materials) that are consumed directly (these values incorporate the commercial and subsistence benefits of organisms to humankind).

(2) *Indirect use* values for those *services* (e.g., ecological, cultural, spiritual and aesthetic values) that support the items that are consumed, including ecosystem functions (Sterling et al., 2018). Indirect values of biodiversity (e.g. air and water purification, soil formation, nutrient cycling, waste disposal, education, recreation, etc.) do not involve harvesting and destroying the natural resource.



Finally, the utilitarian value of biodiversity also includes *non-use* values such as (i) *potential value*: future value either as a good or service; (ii) *existence value*: value of knowing something exists; and (iii) *bequest-serendipic value*: value of knowing that something will be there for future generations (future but as yet unknown value of biodiversity to future generations of humankind) (Sterling et al., 2018).

On the other hand, the *intrinsic value* of biodiversity describes the inherent worth of an organism, independent of its value to anyone or anything else, based on the notion that all living things are valuable simply because they have the right to exist. This concept aims for people to be aware of (and then internalize) issues such as the irreversible nature of extinction, the uniqueness of species, and their right to exist.

Certainly, biodiversity can do both satisfy basic needs like food and medicine and enrich our lives spiritually or culturally. From a functional point of view, biodiversity supports ecosystem functions (e.g., primary production, organic matter decomposition, nutrient cycling) which give rise to ecosystem services that improve human well-being. However, the effects of species on ecosystem function appear to depend on species functional traits, and the effects of species loss on ecosystem function can vary depending on the species, the community it is lost from, and the ecosystem function of interest (Thompson and Starzomski, 2006). Gaston and Spicer (2004) reported that there are three ways in which ecosystem processes might respond to reductions in species richness:

(1) *Redundancy*: beyond some minimum number of species necessary to carry out basic ecosystem processes, most species are equivalent and their loss of little significance.

(2) *Rivet-popping*: the loss of a few species may have no apparent effect, but beyond some threshold losses, the ecosystem processes will fail.



(3) *Idiosyncrasy*: as diversity changes so does ecosystem function, but the magnitude and direction of change is unpredictable.

Here, it is important to differentiate between ecosystem function and ecosystem structure. Thus, *ecosystem structure* has been defined as the taxonomic composition, biological diversity, or presence of specific habitats or species (Bremner et al., 2006). In contrast, other authors (Dernie et al., 2006; Elliot et al., 2006; Hiscock et al., 2006) consider that the concept of ecosystem structure includes the identification of both (i) biotic and (ii) physical-abiotic attributes that determine the sorts of organisms that are likely to exist at a specific location. According to Hiscock et al. (2006), while several biotic and abiotic attributes can be used to define the structure of an ecosystem, it may be required to identify 'key structural species' or 'ecosystem engineers' that play an essential role in maintaining the structural integrity of the ecosystem. On the other hand, Naeem et al. (2004) defined ecosystem function as "the activities, processes or properties of ecosystems that are influenced by its biota". In turn, Diaz and Cabido (2001) defined function as: "the flow of energy and materials through biotic and abiotic components of the ecosystem". In any case, there is much confusion and ambiguity with the term "function" in ecology and environmental sciences: in fact, there are at least four different meanings of "function": (1) functions as processes, (2) the function(ing) of a system; (3) functions as roles; and (4) functions as services (Jax, 2005). Furthermore, the terms "ecosystem functions" and "ecosystem processes", as well as the terms "ecosystem functions" and "ecosystem services" are often used interchangeably. Nevertheless, in general, ecosystem services are those ecosystem functions directly beneficial to humans.

At this point, it must be taken into consideration that the values of biodiversity are spatio-temporally dynamic, due to the fact that the value placed on biodiversity is, to a great extent, a reflection of underlying human values which, not surprisingly, vary considerably among



societies and persons. Regrettably, many of the uses of biodiversity are not incorporated in economic accounts, resulting in biodiversity being under-value in many contexts.

2.2.1. Biodiversity and ecosystem services

Part of our society is nowadays internalizing a position of nature in the centre of society, where ecosystems provide services for society representing the utility factor of nature: our natural capital (Vos et al., 2014). The term *natural capital* represents “the world’s stocks of natural assets which include geology, soil, air, water and all living things” (World Forum on Natural Capital). Thus, the term *natural capital* comprises two main components: (i) *abiotic natural capital* such as subsoil assets (fossil fuels, minerals, metals, etc.) and abiotic flows (e.g., wind and solar energy); and (ii) *biotic natural capital* (or ecosystem capital) which consists of the ecosystems that deliver a wide variety of ecosystem services that are essential for human well-being. It is important to always remember that many aspects of this natural capital (clean air, fertile land, clean water, biodiversity, etc.) are limited and vulnerable to disturbances.

At the present time, it is a well-recognized fact that humans derive a wide range of services (ecosystem services) from this natural capital. *Ecosystem services* have been defined as “the benefits people obtain from ecosystems” (Millennium Ecosystem Assessment, 2005). In other words, ecosystem services are the benefits that humankind receives from the multitude of resources and processes supplied by natural and managed ecosystems.

The Millennium Ecosystem Assessment (2005) identified four broad categories of ecosystem services:

(1) *Provisioning services*, or products obtained from ecosystems, such as food, fibre, fuel, water, pharmaceuticals, etc.



(2) *Regulating services*, the benefits obtained from the regulation of ecosystem processes, such as air-quality maintenance, climate and water regulation, erosion control, water purification, natural hazard (storms, floods, landslides) protection, bioremediation of wastes, etc.

(3) *Cultural services*, the non-material benefits that people obtain through spiritual enrichment, cognitive development, recreation, etc., such as spiritual and religious value, inspiration for art, folklore and architecture, social relations, aesthetic values, cultural heritage values, recreation, ecotourism, etc.

(4) *Supporting services*, necessary for the production of all other ecosystem services, such as soil formation and retention, nutrient cycling, primary production, water cycling, production of atmospheric oxygen, provision of habitat, etc.

In the last years, much debate and research has been focused on both the concept and the application of the ecosystem services paradigm. In particular, a variety of aspects of human-nature relations have been identified as frontiers of ecosystem services research (Peterson et al., 2018). Similarly, the notion of nature's contributions to people (Diaz et al., 2018) has recently been added to the agenda of studies on the benefits that humans obtained from the natural capital

Human well-being, a central component of the ecosystem services framework, is dependent on the flow of these ecosystem services. As a matter of fact, the Millennium Ecosystem Assessment put the relation between ecosystems and human well-being at the core of its framework (Jax and Heink, 2016). Although the debate on the advantages and limitations of the ecosystem services framework is not within the scope of this document, just to mention that ecosystems can also provide disservices which negatively affect human well-being.



Relevantly, the provision of ecosystem services is underpinned by a series of biophysical *processes* and ecological *functions* which themselves are driven by biological diversity (Balvanera et al., 2006). These ecological functions must be understood in order to properly manage and enhance ecosystem services provision (Banerjee et al., 2013). In this respect, de Groot (2002) illustrated the link between ecosystem function and human benefit by defining *function* as the capacity of natural processes and components to provide goods and services that generate human utility. However, our understanding of the quantitative relationship between biodiversity, ecosystem functioning, and ecosystem services is undoubtedly limited (de Groot et al., 2016; Turner et al., 2008). Although all ecosystem services appear to require some level of biodiversity to function, the exact nature of the relationship is indeed not well understood for most services (Reyers et al., 2012). Some authors have highlighted the potential for trade-offs between biodiversity and ecosystem services (Chan et al., 2007, Redford and Adams 2009). In any event, changes in biodiversity can certainly affect ecosystem processes and, in turn, the alteration of these processes can influence the provision of ecosystem services.

Within the context of climate change, the term *co-benefits* has gained increased importance. Simplifying, co-benefits are the added benefits, often unanticipated and unplanned, we get when we take measures to control climate change, such as, for instance, cleaner air, technological innovation, new business opportunities, energy efficiency, etc. On the other hand, potential conflicts or *trade-offs* can arise between policies aimed at climate change control and other objectives. In consequence, decision makers must take into account all the pros and cons of the different options, and implement measures that maximise co-benefits while minimising conflicts. Above all, the consideration of co-benefits can help to persuade decision makers and the general public of the desirability of a specific strategy. Finally, sometimes, co-benefits are easier to quantify than the direct benefits of a specific measure.



The often-discussed relationship between biodiversity and ecosystem services improves our understanding of how biodiversity contributes to human well-being and, from a sustainable management perspective, can help determine carrying capacity and sustainable use levels as essential information for such sustainable management (de Groot et al., 2016). In particular, much research effort has been directed at understanding (i) how biodiversity loss impacts ecosystem functioning and (ii) the influence of this on the goods and services that ecosystems provide (Cardinale et al., 2012). In general, biodiversity loss has been reported to reduce ecosystem functions and impair their stability over time (Cardinale et al., 2012). Thus, a reduction in ecosystem diversity (e.g., structural or species diversity) can have an effect on the system's resilience (Banerjee et al., 2013).

As abovementioned, biodiversity has been frequently reported to have a key role in ecosystem service delivery (Mace et al., 2012). Nevertheless, it is possible that, in the short-term, species composition and biomass may be more important than biodiversity itself (Mace et al., 2012). In particular, for some ecosystem services, specific functional traits or specific key species are important (Harrison et al., 2014). Also, much evidence points to a strong role for dominant species (e.g., keystone species or ecosystem engineers) in controlling ecosystem function, rather than biodiversity *per se* (Thompson and Starzomski, 2006). In any case, the relationship between biodiversity and ecosystem services will be negative, neutral or positive depending upon the specific ecosystem service under evaluation. However, the relationship between biodiversity and process rates (henceforth "function") is more likely to be positive (Wall and Nielsen, 2012).

Unfortunately, our understanding of the functional consequences of biodiversity loss belowground is very limited, compared to aboveground (Bardget and van Putten, 2014). This problem must be faced urgently, as belowground biodiversity represents one of the largest reservoirs of biodiversity in our planet. Besides, the extremely diverse belowground communities can have a major role in shaping aboveground biodiversity and, in general, the



functioning of terrestrial ecosystems. Nonetheless, it has often been stated that (i) soil communities have a high functional redundancy for nutrient mineralization, and (ii) changes in belowground community composition, rather than species diversity, are of most importance for ecosystem functioning (Bardget and van Putten, 2014).

Brussaard et al. (2007) reported that the soil biodiversity-ecosystem services relationship is more dependent on the presence of certain key species and/or species with specific traits than species richness *per se*. In any case, there is evidence that species richness is important in certain soils. Thus, positive biodiversity-ecosystem function relationships are more pronounced in species poor ecosystems than species rich ecosystems (Nielsen et al., 2011). Interestingly, some soil ecosystem services, such as carbon sequestration, are optimal in systems with inherently low species richness (carbon is best sequestered in soils where organic matter is allowed to accumulate due to impaired decomposition (Wall and Nielsen, 2012). But the question whether soil functioning depends on (i) the number of species present, (ii) key species, (iii) species traits or (iv) the composition of the soil biological communities remains unanswered (Wall and Nielsen, 2012). In any event, the paradigm of ecosystem services provides a great opportunity to promote soil conservation through the linkage of soils to human well-being. In fact, Velasquez et al. (2007) proposed a multifunctional indicator of soil quality (GISQ) that evaluates the services provided by the soil ecosystem, assuming that “the more ecosystem services are produced, the better soil quality”.

Finally, biodiversity and the ecosystem services it provides are essential for achieving the United Nations Sustainable Development Goals of the 2030 Agenda for Sustainable Development:



United Nations Sustainable Development Goals:

- (1) End poverty in all its forms everywhere.
- (2) End hunger, achieve food security and improved nutrition and promote sustainable agriculture.
- (3) Ensure healthy lives and promote well-being for all at all ages.
- (4) Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all.
- (5) Achieve gender equality and empower all women and girls.
- (6) Ensure availability and sustainable management of water and sanitation for all.
- (7) Ensure access to affordable, reliable, sustainable and modern energy for all.
- (8) Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all.
- (9) Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation.
- (10) Reduce inequality within and among countries.
- (11) Make cities and human settlements inclusive, safe, resilient and sustainable.
- (12) Ensure sustainable consumption and production patterns.
- (13) Take urgent action to combat climate change and its impacts.
- (14) Conserve and sustainably use the oceans, seas and marine resources for sustainable development.
- (15) Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.
- (16) Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels.
- (17) Strengthen the means of implementation and revitalize the global partnership for sustainable development. After all, biodiversity is at the centre of many economic activities (e.g., fisheries, agriculture, livestock, forestry, tourism, etc.) and of the fulfilment of the daily subsistence needs for many of the most vulnerable people.

After all, biodiversity is at the centre of many economic activities (e.g., fisheries, agriculture, livestock, forestry, tourism, etc.) and of the fulfilment of the daily subsistence needs for many of the most vulnerable people.

Detailed information on the importance of biodiversity for achieving the abovementioned Sustainable Development Goals (SDGs) can be obtained in “Biodiversity and the 2030





Agenda for Sustainable Development - Policy Brief & Technical Note” (Convention on Biodiversity; Food and Agriculture Organization of the United Nations; United Nations Environment Programme; United Nations Development Programme; 12 December 2016).

2.2.2. Biodiversity and ecosystem attributes

Following the same line of thought, Garbisu et al. (2011) proposed to link the concept of soil quality to that of ecosystem function (i.e., ecosystem health, *sensu* Rapport, 1998) through the grouping of soil microbial properties within a set of ecosystem attributes of ecological relevance. In particular, the health of the soil ecosystem can be defined in terms of a range of attributes of ecological relevance, such as (Garbisu et al., 2011):

(1) *Vigor*, quantified in terms of productivity or throughput of material or energy in the soil ecosystem.

(2) *Organization*, which depends on the diversity of components and their degree of mutual dependence.

(3) *Stability*, which refers to the soil's ability to maintain its structure and behaviour in the presence of disturbances.

(4) *Suppressiveness*, or the capacity of a given soil to maintain disease severity or incidence at a low level, despite the presence of a pathogen, a susceptible host plant, and climatic conditions favourable for disease development.

(5) *Redundancy*, so that soil functioning is not affected by the loss of a species, since other species are able to perform the same function. According to the ‘redundant species’ hypothesis, only a minimum number of species is needed for ecosystems to function and the



loss of a functionally redundant species would have very little impact, or none at all, on the ecosystem services provided (Hunt and Wall, 2002; Naeem et al., 1995).

As a matter of fact, Garbisu et al. (2011) defined soil quality as: “the capacity of a given soil to sustainably perform its ecological processes, functions and ecosystem services, and maintain a suite of essential ecosystem attributes of ecological relevance (vigor, organization, stability, suppressiveness, redundancy) at a level similar to that of a reference soil, without causing an adverse impact on the proper functioning of surrounding ecosystems or human health”. Thus, from all of the above, it can be concluded that soil properties can be grouped into ecosystem services or ecological attributes, an idea of great value for long-term monitoring programs since these higher-level categories can provide the required stability through time against changes in techniques, methods, interests, etc. (Epelde et al., 2014b).

Out of this five ecological attributes of ecological relevance, *stability* (or robustness) is of the utmost significance, given the crucial importance of maintaining ecosystem function in the current scenario of increasing environmental pressures. Within the field of ecological stability, two categories are distinguished (Botton et al., 2006; Griffiths and Philippot, 2013):

(1) *Engineering resilience*, which concentrates on stability near an equilibrium steady state, where the ability of a system to withstand a disturbance (*resistance*) and speed of return to the equilibrium (*resilience*) are used to measure the property.

(2) *Ecological resilience*, which considers the amount of disturbance required to move the system from one stable state to another alternate stable state.

Resilience is often considered an integrated indicator of an effectively functioning ecosystem (Dernie et al., 2006; Elliot et al., 2006), as an ecosystem is considered stable or robust if it (i)



returns to its original state after a perturbation, (ii) exhibits low temporal variability, or (iii) does not change dramatically in the face of a perturbation.

A priori, a greater biodiversity offers a greater potential for interactions, and a more complex network of interactions (e.g., biological communities with higher diversity form more complex trophic paths) is generally more adaptive to change and resilient to disturbance (Garbisu et al., 2011). Thus, an increase in species richness and complexity helps to buffer the community from environmental stresses, rendering it more stable. In fact, there are several mechanisms that underlie the relationships between biodiversity and ecosystem function:

(1) *Redundancy*: a priori, it can be expected that the more species there are that can perform a specific function, the more likely it is that if some of those species disappear the function will remain unaffected (the remaining species will keep on performing such function).

(2) *Repertoire*: the more the biodiversity the wider the suite of metabolic abilities and, hence, the wider the range of functions.

(3) *Interactions*: the more the biodiversity the greater the potential for interactions (a more complex network of interactions frequently leads to a more adaptive ecosystem to change and a more resilient ecosystem against disturbances).

In other words, biodiversity forms a structural and functional network (ecological infrastructure) that provides ecological stability (resistance and resilience) through a higher functional redundancy, a higher repertoire of metabolic abilities, and a higher number of interactions.

In any case, one must be cautious with the concept of functional redundancy; after all, redundancy is a highly context-dependent property: while two species can perform the same



function, they may not perform it under the same range of conditions or at the same time. Besides, it is obvious that organisms can contribute to more than just one function. Then, two species that are redundant for one specific function may play a key functional role elsewhere in the food web.

Maintaining biodiversity is essential to both (i) the supply of ecosystem services and (ii) ecosystem health and resilience. Nonetheless, the relationship between species diversity and ecosystem stability is extremely complex. A key question in ecology is whether ecosystem functioning depends on the number of species present, on key species, species traits or on the composition of the communities. A priori, there are three potential types of positive relationships between species richness and ecosystem functioning (Wall and Nielsen, 2012):

- (1) The *linear* relationship occurs if the addition of any new species enhances functioning.

- (2) The *redundancy* relationship occurs if multiple species have the same influence on functioning (then, adding a new species only have a positive influence on functioning if it possess a trait not already found in the community; the chance of this being the case decrease progressively as species richness increase).

- (3) The *idiosyncratic* relationship indicates a system where species differ in their ability to enhance functioning, or where biotic interactions enhance or inhibit functioning. In this case the inclusion of single species have disproportionally large negative or positive impacts on functioning and the overall community composition is therefore more important for functioning than species richness per se.

In any case, biodiversity appears to enhance the resilience of desirable ecosystem states, which is required to secure the provision of ecosystem services (Elmqvist et al., 2003). Luck



et al. (2003) emphasized the importance of diversity in species and populations within functional groups for maintaining ecosystem services (i.e., ecological redundancy or functional redundancy). Here, it must be considered that *response diversity*, or the diversity of responses to environmental change among species contributing to the same ecosystem function, is critical to resilience (Elmqvist et al., 2003). In fact, response diversity is particularly critical for ecosystem renewal and reorganization following change.

Finally, a related, but rather abstract, concept is that of *ascendency*, an attribute of an ecosystem which is defined as a function of the ecosystem's trophic network and which intends to capture in a single index the ability of an ecosystem to prevail against disturbance. Ulanowicz (1980) defined *ascendency* as "an index that quantifies both the level of system activity (measured by the sum of the magnitudes of all the trophic exchanges occurring in the system) and the degree of the organization with which it processes material in autocatalytic fashion". Thus, the organisation of the flow structure is captured by the average mutual information inherent in how the flows are put together (Patricio et al., 2006). Relevantly, the concept of ascendency was created to evaluate the developmental status of an ecosystem (Ulanowicz, 2000).



3. PHYTOMANAGEMENT AND BIODIVERSITY

Paraphrasing the three well-known M's of successful trading (Mind, Money management, Method), we can visualize the links between biodiversity and phytomanagement according to three M's of successful phytomanagement: (1) Mind: for effective phytomanagement, we must use our mind and creativity to design the best strategy for each specific site and casuistry (here, following the medical aforism "there are no diseases but sick people", we can state that there is no contamination but contaminated areas; all of them are different and require a case-by-case assessment). In this respect, biodiversity provides ideas, models and strategies (tested through millions of years of evolution) that we can learn from; (2) Management: for successful phytomanagement, we must apply scientifically-based adaptive management, especially under the current scenario of climate change). Biodiversity provides a myriad of species, metabolic capabilities, functional traits, etc. which we can use in response to changing conditions; (3) Money: a fruitful phytomanagement will provide economic value thorough products and ecosystem services which can help fuel our bioeconomy.

In this section, a series of Rules aimed at the proper application of phytomanagement practices for the recovery of biodiversity in degraded and contaminated sites will be presented. Some of the rules deal with the implementation of phytomanagement strategies/practices. Other rules deal with monitoring of the effectiveness of phytomanagement practices in terms of the recovery of biodiversity in degraded and contaminated sites.



RULE 1: Before applying any phytomanagement strategy, it is essential to study the degraded/contaminated site in search for valuable biodiversity that should be preserved

Before discussing the implementation of specific phytomanagement practices for the recovery of biodiversity at degraded and contaminated sites, it is essential to strongly emphasize that some contaminated sites, most relevantly mining sites, harbour a unique biodiversity that must be preserved.

Above all, there is an urgent need to conserve metallophytes (i.e., unique plant species that have evolved to survive on metal-rich soils), which are nowadays increasingly under threat of extinction from mining activity (Whiting et al., 2004). Metallophytes are the result of strong selective pressures over evolutionary time as a result of the presence of high levels of trace elements in the soil. The duration of the exposure to these high concentrations of trace elements governs the degree of specialization of the metal tolerance trait. Thus, some populations of plant species can evolve metal resistance within a few years, for example around metal smelters, if the selection pressure is high enough (Barrutia et al., 2011): these populations of common plants are termed *pseudometallophytes* and present a greater ability to withstand metal toxicity as compared with other populations of the same species from non-contaminated soils (Whiting et al., 2004). But, as the duration of metal exposure increases, the mechanisms of tolerance become progressively more specialized, resulting in true metallophytes or eumetallophytes that have evolved mechanisms to resist and thrive on metalliferous soils after a slow and strong selective pressure. In fact, true metallophytes have often diverged genetically and morphologically to form new taxa endemic to their native metalliferous soils (Barrutia et al., 2011). Regrettably, their restricted geographic range is, partly, responsible for the observed high rates of population decline or extinction.



In particular, plants growing in metal-contaminated soils can be classified as (1) *excluders*: these plants limit metal translocation and, then, maintain low levels of metal contaminants in their aerial tissues over an extensive range of soil metal concentrations; (2) *indicators*: these plants accumulate metals in their harvestable biomass at concentrations similar to soil metal concentrations; and (3) *accumulators/hyperaccumulators*: these plants increase internal sequestration, translocation and accumulation of metals in their harvestable biomass to levels that far exceed those present in the soil. Plants living in metal-contaminated soils are classified as resistant. Metal resistance can be achieved by avoidance and/or tolerance. The term *avoidance* refers here to the ability of some plants to prevent excessive uptake of metals into their tissues (i.e., avoiders prevent metal ions from entering their cytoplasm). The term tolerance refers to the ability of some plants to cope with metals excessively accumulated in their tissues (i.e., tolerant plants can detoxify metal ions that have crossed the plasma membrane or internal organelle biomembranes). In some cases, plants cope with the effects of high internal metal concentrations by the upregulation of the antioxidant defense system, which is activated to respond to the adverse effects caused by reactive oxygen species.

Importantly, apart from their intrinsic value as remarkable rare species, metallophytes are ideal candidates for the (i) revegetation of mining sites, (ii) rehabilitation of land contaminated with trace elements, and (iii) implementation of environmental phytotechnologies such as phytoremediation, phytomining and phytomanagement. Here, it is important to clarify the sometimes confusing terminology related to the use of metallophytes in metal-contaminated sites (i.e., to the revegetation of metal contaminated sites with metallophytes). Indeed, closely linked terms, such as restoration, rehabilitation, reclamation and remediation, are often confused, since all of them are aimed at improving the biological and physical conditions at the degraded site.



The term *ecological restoration* refers to the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed. Ecological restoration attempts to return the site to a state as close as possible to that existing before the degradation started (i.e., the aim is to rebuild the ecosystem that existed at the site before it was disturbed, in other words, ecological recovery). In many cases, there is no suitable information regarding the conditions of the ecosystem before being degraded/damaged/destroyed. In this case, very often, an appropriate reference ecosystem is chosen for comparison purposes during the ecological restoration process. Then, the term ecological restoration can only be applied to the use of metallophytes in revegetation of degraded sites when the site originally had those specific metallophyte species (Whiting et al., 2004). In any event, in most cases, a complete ecological restoration of the site is simply impossible. But although a disturbed ecosystem may never return to pre-existing conditions, it can certainly return to conditions that are functionally equivalent to those present before it was disturbed.

Similarly, through the proper implementation of suitable rehabilitation, reclamation and remediation practices, the establishment of a functional ecosystem can certainly be achieved. The term *rehabilitation* refers to the establishment of a stable and self-sustaining ecosystem, but not the one that existed before disturbing the site. In this case, metallophytes are exploited for the revegetation (for the establishment of a vegetative cover) of a metal-contaminated site, without aiming to reach pre-existing conditions. In other words, the aim is to recreate ecosystem processes, functions and services, but not to restore the pre-existing species composition and diversity.

In the mining industry, the term *reclamation* is used when the objective, usually after mine closure, is the physical stabilisation of the terrain, the improvement of landscape appearance, the restoration of the topsoil and the return of the land to a useful purpose.



Revegetation is often a component of reclamation, but it is limited to the establishment of a few species for site stabilization (thus, to reduce the risk of erosion and runoff).

Finally, the term *remediation* refers to the process of removing the toxic metal contaminants from the site or, alternatively, reducing the (eco)toxicity caused by those metal contaminants. Thus, in this case, the aim is to clean up the contaminated site to safe levels by removing or isolating/immobilizing the contaminants. When using metallophytes for these purposes, the term used is *phytoremediation* (mainly, through phytoextraction or phytostabilisation).

While a strict ecological restoration is impossible in most cases, nowadays, there is a growing interest in (1) the use of native species for the revegetation of degraded/contaminated sites, as opposed to the use of non-native introduced species, and (2) the promotion of a high (native) biodiversity, as opposed to the implementation of a monoculture of a metal-tolerant plant species. In this respect, a strong commitment to conservation of metallophyte biodiversity is self-evident (Whiting et al., 2002). After all, apart from their intrinsic value as unique, often endemic, metallophyte species, their conservation is essential to identify candidates for the revegetation of mine sites and the development of phytotechnologies for the recovery of metal contaminated soils, i.e. for restoration, rehabilitation and remediation purposes. In this respect, it is important to protect not only hyperaccumulators as endangered nature's oddities but also excluders, indicators and accumulators.

Then, before starting any phytomanagement initiative, it is imperative to study the native vegetation of the site in search of potential candidates (mainly, metallophytes) for conservation purposes. If such candidates are identified, then, an area of the site (preferably, the area where the most interesting metallophytes have been identified) must be left unmanaged, for conservation purposes. Regrettably, metalliferous ecosystems are currently threatened, at a global scale, by the growing of mining activities with concomitant high risks



on metallophyte diversity (Séleck et al., 2013). One of the best strategies to ensure the survival of metallophytes is to promote their use in ecological restoration and site rehabilitation at the point of mine closure.

Apart for protecting the native environment of the metallophytes (conservation *in situ*, in representative biotope “islands”), efforts must also be directed at conserving them *ex situ*, for example, in seed gardens, arboreta, botanic gardens, or germplasm banks (Whiting et al., 2004). On the other hand, when designing a strategy to preserve unique metallophytes, attention should also be paid to plant assemblages (metalliferous distinctive plant communities).

Finally, apart the presence of unique metallophytes in metal-contaminated sites, these degraded environments can also harbour a valuable microbial diversity that can be used for phytoremediation and phytomanagement initiatives. In particular, interesting metal-tolerant plant growth-promoting rhizobacteria and endophytes, as well as metal-tolerant mycorrhiza, can be isolated from metal-contaminated sites and, subsequently, be used to improve plant survival, growth and performance under the harsh conditions usually present in many metal-contaminated sites, particularly mining sites.

From all of the above, it can be concluded that we must find phytomanagement strategies that simultaneously protect and promote biodiversity. In other words, we must find a compromise between protection and recovery of biodiversity, but this is easier said than done because the word “compromise” usually implies a conflict of values, interests, principles, etc. and, in consequence, that the parties must agree to mutual concessions.



RULE 2: Select plants with the right traits to survive and grow healthy under the current scenario of climate change

Nowadays, the negative consequences of climate change can be identified in the alteration of natural and agricultural landscapes, owing to, for example, higher temperatures, more extreme droughts and storms, and an increased likelihood of heat waves and heavy precipitation episodes (Alkorta et al., 2017).

In consequence, plant survival and growth are nowadays being altered under these changing climatic conditions. Furthermore, increased CO₂ concentrations in the atmosphere are changing the physiology of plants, affecting, among other aspects, their growth rate.

In particular, regarding the choice of plant species for phytomanagement in southern Europe, and taking into consideration the critical importance of an adequate water regime for the success of any revegetation initiative, special attention should be paid to the selection of drought-resistant plant species and ecotypes, since the frequency and duration of extreme droughts is currently increasing in many regions of south Europe.

The possibility of irrigating phytomanagement crops is highly controversial, since water is a scarce resource in many parts of the world, including southern Europe. In fact, a proper management of water resources is one of the current greatest challenges for our society on a global scale. Above all, we must ensure availability of good quality water for human consumption and agricultural production. Besides, in the decades to come, water scarcity will probably be worse than it is today: an increase in world population will require more water for human consumption and, above all, an increased in agricultural production. Here, it must be remembered that agriculture accounts for around 70% of water used in the world today



(likewise, it contributes to water contamination from excess nutrients, pesticides, etc.). Then, the consumption of good quality water for irrigation of phytomanaged sites is, in general, not considered a valid option, especially in southern Europe.

A possibility often discussed regarding the need of water in phytomanaged sites is the use of wastewater for irrigation. Interestingly, urban wastewater is known to contain nitrogen and phosphorus, leading to an additional beneficial effect through plant fertilization. Since, for sanitary reasons, irrigation is recommended for use only in non-food, non-fodder crops, it appears an ideal option for phytomanagement. The main limitation of this approach is that it requires the presence of a wastewater treatment plant closed to the site to be phytomanaged.

Then, especially in southern Europe, it is strongly recommended to select plant species resistant to water stress and heat waves, in order to increase the long-term success of the phytomanagement initiative. For instance, as water supply and its distribution during the crop cycle is one key limiting factor for crop production in SW France, sunflower ability to resist to more frequent heatwaves and long droughts due to the climate change is an advantage (Kidd et al., 2015). Indeed, although thousands of plant species have been reported as metal accumulators, there is nowadays an urgent need to identify plants which can be used for phytoremediation and phytomanagement under the current scenario of climate change.

Apart from the direct consequences of climate change on plant survival and growth, it is important to take into consideration indirect effects of climate change in soil biota through the abovementioned changes in plant growth and physiology. Although higher levels of atmospheric CO₂ are not expected to directly affect soil microbial communities (CO₂ concentrations are much higher in soils than in the atmosphere), such higher atmospheric CO₂ concentrations have an indirect effect on soil microbial communities through higher plant growth and changes in litter deposition and root exudation (Burns et al. 2013, Phillips et



al., 2011). Soil microbial communities are affected by plant responses to higher atmospheric CO₂ concentrations, including water use efficiency, increased rhizodeposition and faster nutrient uptake (Alkorta et al., 2017). Climate change can alter the magnitude of the coupling between plants and soil microbial communities by, for instance, increasing root exudation and stimulating microbial activity (Bardgett et al., 2013). On the other hand, the effects of climate change on plant growth and physiology, rhizodeposition patterns, the diversity and activity of rhizosphere microorganisms, etc. can lead to altered metal bioavailability in soils (Rajkumar et al., 2013) and, hence, affect plant performance during phytomanagement. Indeed, changes in atmospheric CO₂ concentrations, temperature and drought will alter plant-metal interactions in contaminated soils (Rajkumar et al., 2013). Much research is still needed to better understand how climate change will affect the diversity and activity of soil microorganisms and their contribution to plant growth and phytomanagement performance in contaminated soils.

In particular, the impact of climate change (through elevated atmospheric CO₂ concentrations, drought, higher temperature, etc.) on beneficial plant-microorganism interactions (e.g., plant growth-promoting rhizobacteria, endophytes, mycorrhiza) are increasingly being studied (Compant et al., 2010). Interestingly, plant growth-promoting microorganisms (both bacteria and fungi) can positively affect plants subjected to drought stress, and then should be considered as a suitable tool for phytomanagement under climate change.

On the other hand, climate-induced changes in soil humidity and temperature can directly affect soil processes, such as the decomposition of organic matter and the cycling of nutrients (Burns et al., 2013), as well as the structure and function of soil microbial communities (Alkorta et al., 2017).



Interestingly, phytomanaged soils have a potential for sequestering carbon and thus mitigate climate change. Certain management strategies, such as low- or no-tillage practices and the use of crop residues, have positive effects on the capacity of soils to absorb and hold carbon. The incorporation of trees in phytomanagement initiatives (for instance, as part of intercropping systems) has also positive effects in this respect.

A well-known suitable option for adaptation to climate change in phytomanagement initiatives is to incorporate to the planting scheme as many plant species as possible (i.e., high plant richness), and preferably from different vegetation types: grasses, shrubs and trees. Moreover, the conservation of plant biodiversity (e.g., metallophyte diversity) is also a key option for adaptation to climate change, as part of an insurance policy: other species, varieties and ecotypes may be needed in the future as the environmental conditions are altered due to climate change (i.e., plants better adapted to the new conditions).



RULE 3: To promote biodiversity under phytomanagement, grow as many plant species and varieties/ecotypes from different vegetation types (grasses, shrubs and trees) as possible

A higher plant richness (in general, a more diversified vegetation) leads to a higher number of ecological niches and, hence, biodiversity. In particular, aboveground and belowground organisms are closely linked. In this respect, plants, through their photosynthetic activity, are known to provide (1) the organic carbon required for soil decomposers and (2) the resources for root-associated organisms such as root herbivores, pathogens and symbiotic mutualists (Wardle et al., 2004). In turn, soil decomposers break down dead plant material and regulate plant growth by determining the supply of soil nutrients. In fact, aboveground and belowground biological communities can be powerful mutual drivers, with both positive and negative feedbacks (Wardle et al., 2004). Plant species differ in the quantity and quality of root exudates that they release, thus affecting the activity and composition of soil microbial communities. Also, the quantity and quality of litter produced by trees has a strong effect on soil communities. Higher plant richness will lead to a higher variety of different exudates and types of litter, resulting in a higher diversity belowground. Many authors have proposed that a high aboveground diversity is linked to high belowground diversity because of diverse litter types and root exudates (Haichar et al., 2008; Wardle et al., 2004). Nonetheless, Li et al. (2015) reported no linkage between plant and bacterial α -diversity in the early successional forest, and even a negative correlation in the late successional forest. Similarly, a negative correlation between plant and ammonia-oxidizing bacterial diversity was observed by Kowalchuk et al. (2000) in grasslands at different successional stages. In any case, linkages between aboveground and belowground diversity appear to be functionally important at the ecosystem level in terms of the maintenance and stability of ecosystem processes and the



persistence of keystone species or other important species (Chapin et al., 1997; Hooper et al., 2000; Wall and Moore 1999).

Plant community composition is known to exert a strong effect on the composition of root-associated organisms. On the other hand, the presence of phytopathogens and root herbivores in the rhizosphere can produce a negative feedback on plant growth. By contrast, mycorrhizal fungi and plant-growth promoting rhizobacteria have a positive feedback on plant productivity.

But the evidence for correlation between aboveground and belowground biodiversity is mixed, and not all of the mechanisms by which organisms aboveground affect community composition and diversity belowground and vice versa necessarily lead to correlations of species richness in the two domains (Hooper et al., 2000).

Finally, an important difficulty to establish aboveground-to-belowground comparisons is the lack of information on soil organisms, especially for some taxonomic groups (e.g., microfauna and microorganisms).



RULE 4: Reach a compromise between the intrinsic value of biodiversity and the utilitarian value of biodiversity

Nowadays, it is a well-known fact that biodiversity provides a wide range of direct and indirect values. Indirect values include a wide list of values such as, for instance, ethical value, aesthetic value, cultural value, spiritual value, scientific value, ecological value, educational value, etc.

Arguably, the most important value of biodiversity to humans comes from the ecosystem services it provides. Indeed, biodiversity has a key functional value related to the services it provides and the preservation of ecosystem structure and integrity. Thus, biodiversity has a key value to humans because we are so dependent on it for our environmental, cultural and economic well-being. In fact, biodiversity forms the backbone of healthy ecosystems on which we depend on for our basic needs, security, and physical and mental health.

Interestingly, biodiversity has even a bequest (serependic) value regarding to future but as yet unknown values of biodiversity to ecosystems and humankind.

Although there are many lists of biodiversity values in the literature, here we will focus only on two aspects of this topic:

(1) For some people, biodiversity has an *intrinsic value* by itself and we have the moral responsibility to preserve it for future generations. In this sense, we have an inherent obligation to live as harmoniously as possible with the other organisms inhabiting our planet. Very often, modern arguments focus on the utilitarian value of biodiversity (see below).



Nonetheless, well-known nature writers such as Emerson, Thoreau, Leopold, Muir, etc. emphasized the intrinsic value of biodiversity.

(2) Other people are more interested in the utilitarian value of biodiversity, focused on the commercial and subsistence benefits of organisms to humankind. In this second aspect, the idea is to protect biodiversity so that we can utilize it later for our own benefit (e.g., food supplies, medicines, energy, raw materials and so on), including ecosystem services.

Most relevantly, this second aspect (the utilitarian value of biodiversity) is inextricably link to the concept of phytomanagement, i.e. the use of plants to reduce and control risks arising from soil pollution while contemporaneously making a profitable and sustainable use of this resource by producing marketable biomass. In other words, phytomanagement aims to create value from contaminated land while minimising environmental risk.

Both aspects are of equal importance (if such a comparison makes sense, which most likely does not) and, in consequence, when designing a phytomanagement initiative we must find a compromise between the intrinsic and utilitarian values of the biodiversity naturally or artificially present in the phytomanaged site (see Rule 1 above).



RULE 5: When quantifying biodiversity, do not take into consideration only richness

As indicated above, the concept of biodiversity is anything but simple. Among other things, it takes into consideration the following aspects: richness (number of species), evenness (relative abundances, rare and dominant species), composition (taxonomic groups), phylogenetic relatedness/distinctiveness (phylogeny) and spatial and temporal distribution (ecology).

Regarding species composition, we must not forget that species are not all equal in their contribution to biodiversity. Actually, in a seminal paper, Vane-Wright et al. (1991) emphasized that biological species are not all equal and, in this respect, more value should be assigned to those species that are more evolutionarily distinct, i.e. those that lack close relatives (Cadotte and Davies, 2010). Thus, if we prioritize evolutionary distinct species, the preservation of the evolutionary diversity of a clade will be maximized. In addition, by maximizing the conservation of evolutionary diversity, we maximize genotypic, phenotypic and functional diversity, and, hence, provide ecosystems with the most options to adapt to a changing world (Cadotte and Davies, 2010; Vane-Wright et al., 1991).

On the other hand, as abovementioned, there are foundation species, ecosystem engineers, invasive species, keystone species, umbrella species, flagship species, charismatic species, indicator species, chemical engineers, biological regulators and so on, leading us to the inevitable challenge to objectively prioritize among them (“the agony of choice”).

Besides, some species perform phylogenetically narrow processes (e.g., nitrification, atmospheric nitrogen fixation) while other perform phylogenetically broad processes (e.g.,



denitrification). In other words, the former show a lower degree of functional redundancy, compared to the latter.

In any event, it must be taken into account that the assessment of the impact of phytomanagement practices on biodiversity is anything but easy. After all, there are still many unanswered questions that research is yet to answer such as, for example, What number of species is a good number?, What species composition is best?, What degree of phylogenetic distance is more adequate?, How differently should we value the different types of species?, Are functionally redundant species less valuable than non-functionally redundant species?, etc.

Finally, it is important to quantify biodiversity at different spatial and temporal scales.



RULE 6: When quantifying biodiversity, take into consideration genetic, species and ecosystem diversity

Biodiversity should preferably be studied at three levels of complexity: genetic, species and ecosystems. But biodiversity is not simply the sum of all ecosystems, species and genetic material; instead, it represents the variability within and among them. Interestingly, the different levels of biodiversity are often interrelated. For instance, genetic diversity within populations and species diversity within communities are hypothesized to co-vary in space or time due to (i) locality characteristics that influence the two levels of diversity via parallel processes, or (ii) direct effects of one level of diversity on the other (Vellend and Gerber, 2005). Besides, genetic diversity can have significant effects on ecological processes such as primary productivity, population recovery from disturbance, interspecific competition, community structure, and fluxes of energy and nutrients (Hughes et al., 2008). In fact, genetic diversity can have crucial ecological consequences at the population, community and ecosystem levels (actually, in some cases, the effects might be comparable in magnitude to the effects of species diversity) (Hughes et al., 2008).

Partly due to its abovementioned complexity, real biodiversity can be extremely difficult and expensive to measure. In any event, one should make the best efforts possible to evaluate the impact of phytomanagement practices at the three levels of complexity.

In particular, for soil microorganisms, the assessment of genetic diversity is indispensable for microbial ecologists since, as indicated above, all the current definitions of “species” are inadequate for prokaryotes, among other reasons due to the common transfer genes between members of different taxa by horizontal gene transfer. Besides, most microorganisms cannot be cultivated and so we have no other choice but to study them by



means of the application of molecular biology techniques. Nowadays, most soil microbial ecologists are focused on (intoxicated by) the use of next generation sequencing (metabarcoding, metagenomics) for the quantification and assessment of soil microbial diversity. But we must not forget that next generation sequencing has still many technical limitations and then we must be cautious when drawing conclusions about the effect of a priori positive or negative disturbances on microbial diversity. Finally, most studies on the effect of phytoremediation or phytomanagement practices on microbial diversity are focused on soil microbial communities, especially rhizosphere microorganisms. In this respect, more attention should be paid to plant microbiota and plant microbiomes (e.g., phyllosphere) under phytomanagement.

Similarly, concerning genetic diversity, there are still many unanswered questions such as, for example, The more genes the better?, Are all genes equally important?, Are there “good” genes (e.g., genes involved in contaminant biodegradation pathways) and “bad” genes (e.g., antibiotic resistance genes) from an anthropocentric point of view?, How can we combine data from metagenomic, metatranscriptomic and metaepigenomic studies?, etc.

On the other hand, when dealing with ecosystem diversity (i.e., the richness and complexity of biological communities, including trophic levels and ecological processes, together with the chemical and physical environment), additional questions emerge: How many trophic levels do we need?, Are all of them equally important?, How many species per trophic level are needed?, etc.

As a final point, when interested on the links between biodiversity and ecosystem properties and functioning, one should also take into consideration the concept of *emergent properties* or those new qualities that appear on higher integration levels and represent more than the sum of the low-level components (Reuter et al., 2005). In consequence, emergent properties cannot be explained solely on the basis of the properties and relationships of the elements;



instead, to understand them, we need to introduce component interactions (Reuter et al., 2005). Emergent properties can be *aggregational* (properties that emerge as a result of an aggregation procedure by an observer on the higher level which does not make sense or is not applicable on lower levels) or *connective* (properties based on an interaction network of lower level entities, which brings about the specific system characteristic) (Reuter et al., 2005). This concept facilitates the investigation on the causal links across more than one hierarchical level (e.g., genes, species and ecosystems). In consequence, when possible, key biological interactions should be identified and studied during phytomanagement initiatives, since they support the functioning of the ecosystem and are the basis of emergent connective properties.



RULE 7: When promoting biodiversity under phytomanagement, include organisms from the different levels of the trophic chain

When promoting biodiversity under phytomanagement, it is important to always include organisms from the different levels of the trophic chain. Instead, when evaluating their effect on biodiversity, most phytomanagement initiatives only pay attention to aboveground botanical diversity (richness, composition, vegetation structure) and, occasionally include some belowground soil biota, in many cases just microorganisms owing to their well-known key role in critical soil processes and, hence, functions and ecosystem services. Nonetheless, for a biodiversity assessment to have more ecological relevance, it is desirable to include taxonomic groups from the different levels of the trophic chain. As a matter of fact, we should study as many taxonomic groups from the food web as possible (food webs are formed by interconnections between different food chains, each of them usually consisting of three or four trophic levels).

Simplifying, the aboveground food web includes producers (plants), primary consumers (herbivores) and secondary consumers (predators). Regarding consumers, there is an unresolved debate regarding the benefits and disadvantages associated to the presence of animals in phytomanaged sites.

When we are dealing with contaminated sites, in many cases, animals are deliberately excluded, in an attempt to avoid possible ecotoxic effects on exposed animals that take up the contaminants (the uptake and retention of a metal or any other chemical by an organism is termed bioaccumulation) and, above all, to minimize the risk of biomagnification (the process whereby contaminants are transferred from food to an organism resulting in higher concentrations compared with the source) (Mann et al., 2011). *A priori*, there are two main



groups of substances that can biomagnify along the food chain (Mann et al., 2011): metals and persistent organic pollutants (lipophilic organic substances that are not easily degraded or metabolized). Biomagnification occurs because successive trophic levels consume relatively large quantities of biomass to obtain the resources required for metabolic functioning. Then, if that biomass is contaminated, the contaminant will be taken up in large quantities by the consumer (in other words, when the mass of the contaminant is largely conserved along the food chain, while the biomass decreases).

Bioaccumulation and biomagnification depends on both contaminant bioavailability and the organism's capacity for uptake and subsequent excretion.

Pertaining to the soil ecosystem, an amazing biodiversity of organisms make up its food web: from the smallest organisms (one-celled bacteria, fungi, algae and protozoa) to more complex animals (nematodes, micro-arthropods) and then to visible organisms such as earthworms, insects, small vertebrates (mice, moles) and plants. Like all food webs, the soil food web is fuelled by the primary producers (plants, lichens, moss, photosynthetic bacteria, algae) that use the sun's energy to fix atmospheric carbon dioxide. A few prokaryotes, called chemoautotrophs, get energy from inorganic sources such as hydrogen sulfide, elemental sulfur, ferrous iron, etc., rather than from carbon compounds or the sun. The remaining soil organisms get energy and carbon by consuming organic compounds.



RULE 8: When monitoring the impact of phytomanagement practices on biodiversity, use an adaptive monitoring strategy

When dealing with long-term monitoring programs, such as the one here proposed for the impact of phytomanagement practices on biodiversity, we must expect that, as time passes, novel analytical techniques, methods and innovative equipment might appear in the market, different approaches might come up, changes in the ecosystem developmental stage might occur, unexpected environmental threats might emerge, budget fluctuations might threaten the initiative, and so forth (Epelde et al., 2014b).

For that reason, we propose that the paradigm of adaptive monitoring, which enables monitoring programs to evolve iteratively as new information emerges and research questions change, should be incorporated to the long-term monitoring of the impact of phytomanagement practices on biodiversity. To this purpose, among other aspects, we need (i) well-formulated, tractable questions posed at the outset of the phytomanagement initiative, (ii) a rigorous statistical design, and (iii) a conceptual model of the site under phytomanagement. In adaptive monitoring, question setting, experimental design, data collection and analysis, etc. are iterative steps (Epelde et al., 2014b). As part of the adaptive monitoring program, periodically - the time period will depend on the specific phytomanagement initiative -, an expert judgement (researcher, policy-maker, resource manager) analysis must be organized to revise and, if necessary, update the different aspects that make up the biodiversity monitoring program. Relevantly, expert judgement analyses frequently encourage the forging of partnership between researchers, policy-makers and resource managers (Epelde et al., 2014b).



RULE 9: Apart from the intended products, select from the beginning the ecosystem services envisioned to be provided by the phytomanagement initiative

As stated above, phytomanagement is based on the combination of profitable site uses with gentle remediation options, leading to reduction of contaminant linkages and the restoration and/or generation of economic gain and other wider site services. Indeed, along with risk mitigation, the accomplishment of economic, social and environmental benefits is a key aspect of phytomanagement. In particular, the provisioning of ecosystem services (carbon sequestration, soil fertility improvement, control of soil erosion, improvement of air quality, climate and water regulation, production of atmospheric oxygen, provision of habitat, etc.) is, in many cases, an important component of phytomanagement initiatives.

Most importantly, the provision of those ecosystem services is underpinned by a series of biophysical processes and ecological functions which themselves are driven by biodiversity. Certainly, changes in biodiversity can affect ecosystem processes and, hence, the provision of ecosystem services. In any case, in some situations, biomass, species composition, functional traits, etc. are more important than biodiversity itself for the provisioning of ecosystem services.

Nonetheless, by contrast, trade-offs between biodiversity and ecosystem services might arise in some situations. Also, trade-offs and conflicts between the different ecosystem services themselves might also emerge, and, then, it is desirable to select from the onset what specific ecosystem services to promote, and implement measures that minimise conflicts.



RULE 10: To quantify the impact of phytomanagement practices on biodiversity, apply different indices of biodiversity

Biodiversity is difficult to quantify, at least partly, due to the multitude of indices proposed to measure it (e.g., species richness, Shannon-Wiener entropy, Simpson's index, Berger-Parker index, etc.). To further complicate matters, there is no general consensus about which indices are more appropriate and informative. This is not surprising because of the complexity of all the aspects of the term biodiversity (see above), which inevitably leads to the fact that no single indicator for biodiversity can be devised (Duelli and Obrist, 2003). As a matter of fact, the choice of indices depends on the specific question(s) to be answer, as well as the specific aspect(s) or entity of biodiversity to be evaluated. In consequence, a set of indices cannot be recommended for all cases. Besides, traditional, most diversity indices have relied on three untrue assumptions: (i) all species are equal (only relative abundances establish the relative importance of species), (ii) all individuals are equal (whatever their size) and (iii) species abundances have been correctly assessed with appropriate tools and in similar units (Magurran, 2004).

In any case, although the choice of index(es) depends, to a great extent, on the specific questions and objectives of the study (there is lot of argumentative literature on this topic), three of the most commonly used indices are the Margalef's index for richness, the Shannon–Weaver's index for diversity and the Simpson's index for dominance.

Similarly, the use of indices for the quantification of the functional aspect of biodiversity is essential in understanding relationships between biodiversity, ecosystem functioning and environmental constraints (Mouchet et al., 2010). In particular, indices of functional diversity



can be divided into three different categories: functional richness, functional evenness and functional divergence.

The topic of the selection of the best indices for the quantification of both structural and functional biodiversity is not within the scope of this document. Nonetheless, we strongly encourage the use of different indices in order to cover as much as possible the different aspects of the term biodiversity: richness, abundance, phylogenetic relatedness, functional traits, etc.



RULE 11: When monitoring the impact of phytomanagement practices on biodiversity in the long-term, take into consideration ecological succession

When studying ecosystems over long times, ecologists have traditionally observed gradual processes of change in ecological communities, particularly plant communities. The series of progressive changes in the species that make up a community over time is called ecological succession.

When dealing with the phytomanagement of degraded and/or contaminated sites, in the long-term, a phenomenon of secondary succession (see above) should be expected. Frequently, not always, this ecological succession involves a progression from communities with lower species diversity to communities with higher species diversity, a fact that should be taken into account when quantifying temporal changes in species diversity. In theory, the community reaches increasing levels of complexity until it becomes stable as a climax community. However, nowadays, the idea of succession as a predictable process, composed of a series of stages and ending in a climax community, has been strongly questioned, among other reasons, due to the fact that ecosystems normally experience frequent disturbances that prevent a community from reaching an equilibrium state.

In any event, when measuring the impact of phytomanagement practices on plant diversity (most of the studies on succession have been carried out with plant communities) in the long-term, it is very useful to take into consideration the processes of ecological succession during the interpretation of data.



RULE 12: When monitoring the impact of phytomanagement practices on biodiversity, determine different aspects of both structural and functional biodiversity

Many studies on the impact of disturbances (e.g., agronomic practices, contamination, climate change, nitrogen deposition, etc.) on biological diversity are focused exclusively on structural biodiversity (usually, of only one or, at most, a few taxonomic groups). But phytomanagement often has a strong functional component related to the provision of functions and ecosystem services. Then, it is highly beneficial to include both types of biodiversity, i.e. structural and functional diversity, when promoting biodiversity under phytomanagement.

Then, apart from a selection as wide as possible of taxonomic groups, an analysis of functional groups, traits, guilds and so on must be included in phytomanagement initiatives.

Although the identification of possible links between structural and functional biodiversity is undoubtedly a difficult task, such identification is of much value from both an academic/scientific and management point of view. In this respect, statistical multivariate analysis tools, applied to the set of parameters used to quantify structural and functional diversity, are suitable for the establishment of hypotheses regarding the abovementioned link (here, it must be remembered that correlation does not imply causality).



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