1 Keep and promote biodiversity at polluted sites under

2 phytomanagement

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26 Abstract

27 The phytomanagement concept combines a sustainable reduction of pollutant linkages at risk-assessed 28 contaminated sites with the generation of both valuable biomass for the (bio)economy and ecosystem 29 services. One of the potential benefits of phytomanagement is the possibility to increase biodiversity in 30 polluted sites. However, the unique biodiversity present in some polluted sites can be severely impacted 31 by the implementation of phytomanagement practices, even resulting in the local extinction of endemic 32 ecotypes or species of great conservation value. Here we highlight the importance of promoting measures 33 to minimize the potential adverse impact of phytomanagement on biodiversity at polluted sites, as well as 34 recommend practices to increase biodiversity at phytomanaged sites without compromising its 35 effectiveness in terms of reduction of pollutant linkages and the generation of valuable biomass and 36 ecosystem services.

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38 Keywords: contaminated soil; metal; metallophytes; phytoremediation; trace elements.

40 1. Introduction

41 The notion of phytomanagement is based on the combination of (i) a sustainable reduction of pollutant 42 linkages at degraded sites with (ii) the generation of valuable products and essential ecosystem services. 43 In other words, its main purpose is to grow profitable plants to minimize pollutant-induced environmental 44 risks while maximizing economic and/or ecological revenues. It is often claimed that one of the potential 45 benefits of phytomanagement is the possibility to enhance biodiversity in the degraded site under 46 recovery. Pertinently, it must be strongly emphasized that some polluted sites, most relevantly mining 47 sites, can harbour a unique biodiversity that must be carefully preserved. In any event, protecting 48 biodiversity is of the utmost importance as human well-being depends upon biodiversity in many 49 different ways (Naeem et al., 2016). In consequence, under the current scenario of global change and 50 biodiversity loss, it is crucial to use as many tools as possible to preserve the fabric of life and the natural 51 capital on which our survival and well-being depend. Biodiversity is known to be critical for the supply of 52 ecosystem services and, then, it is not surprising that much research effort has been directed at 53 understanding how biodiversity impacts ecosystem functioning and resilience, and concomitantly the 54 sustainable provision of goods and ecosystems services. This aspect has special relevance within the 55 phytomanagement framework since, as described above, the main purpose of phytomanagement is to grow profitable plants in order to minimize pollutant-induced environmental risks while maximizing 56 57 economic and/or ecological revenues in terms of products and ecosystem services. However, when 58 implementing actions to promote such biodiversity in phytomanaged sites, in most cases, the only 59 initiative is to enhance the number of different plant species grown for phytomanagement purposes. We 60 must overcome such incomplete approach by widening our understanding of how the different taxonomic 61 groups can be positively or negatively affected by phytomanagement practices. In addition, the unique 62 biodiversity present in some polluted sites can be negatively affected by the implementation of 63 phytomanagement practices. In this review paper, the importance of promoting (i) measures to minimize 64 the potential adverse impact of phytomanagement on biodiversity; and (ii) practices to increase 65 biodiversity at phytomanaged sites, is highlighted.

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67 2. Phytomanagement: a sustainable gentle remediation option

As a result of a wide variety of anthropogenic activities and accidental spills, many soils are currentlypolluted with a myriad of potentially toxic compounds, such as trace elements (TEs), mineral oils,

polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, etc.
Unfortunately, the remediation of polluted soils is often a very expensive, environmentally-disruptive
activity, especially at large sites and/or in those soils simultaneously polluted with several contaminants
inducing adverse effects on biological receptors (Agnello et al. 2016).

Opportunely, in the last decades, various Gentle Remediation Options (GROs) have been developed as more cost-effective, environmentally-friendly and aesthetically-pleasing technologies for the remediation of large areas with polluted soils from mild up to medium levels of contamination (Vangronsveld et al. 2009; Kidd et al. 2015; Mench et al. 2018). Among them, phytoremediation and phytomanagement have shown their great potential, on the long term, for the sustainable remediation of polluted sites due to their capacity to combine an effective mitigation of pollutant-induced risks with the provision of valuable plant biomass and ecosystem services (Mench et al. 2018).

81 The term *phytoremediation* refers to a set of sustainable phytotechnologies focused on the use of 82 plant species to remediate polluted sites, mainly those affected by the presence of TEs via the 83 phytoextraction or phytostabilization options, which aim at (i) decreasing the available soil TEs, through 84 plant uptake and accumulation in the harvestable plant parts, or (ii) reducing the labile ("bioavailable") 85 TE pool usually by combining the growth of TE-excluding plants with the application of soil amendments 86 (Garbisu and Alkorta 2001; Alkorta et al. 2004a,b). However, the commercial application of 87 phytoextraction has been seriously hampered by its intrinsic limitations, e.g. the long time required to 88 effectively extract TEs from medium and highly polluted soils, root depth, lack of plants that can 89 accumulate more than one or two TEs, decrease of metal(loid) market prices, etc. In turn, one constraint 90 for the application of phytostabilization is that many risk-assessment regulations for soil remediation are 91 still based on total soil TEs, not on their bioavailable concentrations or site-specific risk assessment. 92 Paradoxically, the harmful effects of TEs on soil biota and, hence, soil health, are related to the 93 sensitivity/tolerance of living organism populations and the bioavailable pool rather than total metal(loid) 94 concentrations (Kumpiene et al. 2009, 2017), the bioavailable fraction being subject to uptake by soil 95 organisms, leaching, and transfer to other environmental media (Madejón et al. 2006).

96 In any event, for effective clean-up of TE-polluted soils, the combination of different
97 approaches, *e.g.* phytoextraction with hyperaccumulators, chelate-assisted phytoextraction,
98 phytostabilization, microbe-assisted phytoremediation (bioaugmentation), traditional breeding and/or

genetic engineering of phytoremediation plants, etc., appears necessary to increase the applicability ofphytoremediation in the future (Yang et al. 2020a).

101 To overcome the phytoremediation limitations, the concept of phytomanagement evolved (Fig. 102 1), combining a sustainable reduction in pollutant linkages with the generation of plant biomass (mainly 103 non-food crops) and ecosystem services (Mench et al. 2009; Robinson et al. 2009; Fässler et al. 2010; 104 Robinson and McIvor 2013; Cundy et al. 2016; Burges et al. 2018). The phytomanagement objective is to 105 grow profitable plants to control the bioavailable pool of soil pollutants (e.g., TEs), thereby minimizing 106 environmental risks, while maximizing economic and ecological revenues (Vangronsveld et al. 2009). In 107 this way, phytomanagement (commonly based on the interactions among plants, microorganisms and soil 108 amendments) is often considered a "holding strategy" for vacant sites until their remediation is 109 undertaken according to future land use (Mench et al. 2018). Most importantly, compared to other 110 remediation technologies, the requirements of phytomanagement for chemicals and energy are much 111 lower, as well as the total cost, making it a viable strategy for the remediation of large polluted areas 112 (Thewys et al. 2010; Kuppens et al. 2015; Giagnoni et al. 2020).

113 The production of valuable plant biomass (for timber, bioenergy, biofortified products, 114 ecomaterials, etc.) is considered essential for the commercial success of phytomanagement (Conesa et al. 115 2012). Energy crops, e.g. Miscanthus spp., Ricinus communis L. and Brassica napus L., can be grown for 116 biofuel production (Burges et al. 2018). Other plant species can be grown for the production of biochar, 117 raw materials (oil, paper, chemicals, essential oils, etc.), medicinal purposes, etc. (Pandey et al. 2016). 118 Likewise, the growth of fast growing trees opens the possibility to phytoextract some metals in excess 119 (e.g., Cd, Zn, Ni, U, Cs, and Sr) while producing biomass for bioenergy and products (e.g., timber, resin, 120 adhesives, etc.). Other phytomanagement options are aimed at removing the bioavailable metal(loid) 121 fraction, the so-called "bioavailable contaminant stripping (BCS)", while providing ecosystem services 122 and feedstocks for biomass-processing (bio)technologies (Herzig et al. 2014).

Since the 2010s, an increasing attention is developing on the capacity of phytomanagement options to provide a wide variety of co-benefits and ecosystem services, such as primary production, control of soil erosion, water runoff/drainage management, carbon sequestration, amenity and recreation, aesthetic value, habitat for animals and microorganisms, biodiversity, etc. (Evangelou et al. 2015; Kidd et al. 2015; Cundy et al. 2016; Simek et al. 2017; Touceda-González et al. 2017a; Xue et al. 2018). Strictly speaking, biodiversity *per se* is not an ecosystem service (Haines-Young and Potschin 2010); rather, 129 biodiversity supports the flow of vital ecosystem services that we depend upon (in other words, 130 biodiversity forms the biological infrastructure that supports the provision of ecosystem services). Indeed, 131 the ecosystem services (and, concomitantly, human well-being) depend essentially on the structures and 132 processes generated by living organisms and their interactions with, and processing of, abiotic materials 133 (Haines-Young and Potschin 2010; IPBES 2019). Although biodiversity has intrinsic value by itself (and, 134 then, it should be preserved in its own right), its utilitarian value has increasingly become the central 135 focus of the debates on the need to preserve our natural capital (Chang et al. 2007; Haines and Potskin 136 2010).

137 The phytomanagement capacity to promote biodiversity in polluted sites is of key importance, as 138 we are currently at a crucial juncture in human history, with biodiversity being lost at an accelerating pace 139 due to an increasingly affluent human population, climate change, uncontrolled development and habitat 140 destruction (Sandifer et al. 2015; IPBES 2019). Taking into consideration the links between biodiversity, 141 ecosystem functioning, ecosystem services and human well-being (Cardinale et al. 2006; Naeem et al. 142 2016), now more than ever, the importance of promoting biodiversity must be emphasized.

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144 **3. Unique biodiversity at polluted sites**

145 One of the recurrently mentioned potential benefits of phytomanagement is the possibility to increase 146 biodiversity in the polluted land in question. However, the unique biodiversity often present in many 147 polluted sites (in particular, long-term abandoned mine sites) can be severely impacted by the 148 implementation of phytomanagement practices, even resulting in the local extinction of endemic ecotypes 149 or species of great conservation value. Here we highlight the importance of promoting measures to 150 minimize the potential adverse impact of phytomanagement on biodiversity at polluted sites. After all, 151 some polluted sites, most relevantly mining sites, harbour a unique biodiversity that must be 152 painstakingly preserved. Actually derelict soils can provide an interesting biodiversity for a variety of 153 uses (Vincent et al. 2018).

In particular, there is a need to conserve metallophytes (*i.e.*, unique plant species that have evolved to survive on soils with high TE levels), which are nowadays increasingly under threat of extinction from mining activity (Whiting et al. 2004; Batty 2005; Baker et al. 2010; Paul et al. 2018). Indeed, regrettably, metalliferous ecosystems are presently threatened at a global scale by the growth of mining activities with concomitant extinction risks for metallophyte diversity (Whiting et al. 2004; Sélecket al. 2013).

Metallophytes are the consequence of powerful selective pressures over long evolutionary times 160 161 as a result of the presence of high total soil TEs (Ginocchio and Baker 2004). The intensity and duration 162 of the sustained evolutionary exposure to these high TE levels direct the degree of specialization of the 163 TE resistance trait. Thus, some populations of plant species can evolve TE resistance within a few years, 164 for example around metal smelters, if the selection pressure is high enough (Barrutia et al. 2011a). 165 Populations of pseudometallophytes present a greater capacity to withstand phytotoxicity induced by TE 166 excess, as compared with other populations of the same plant species from non-polluted sites (Whiting et 167 al. 2004; Barrutia et al. 2011a). But, as the duration of TE exposure increases, the mechanisms that allow 168 survival and growth in the presence of high TE levels become gradually more specialized, resulting in 169 true metallophytes or eumetallophytes that have developed evolutionary mechanisms to live and thrive on 170 metalliferous soils. As a matter of fact, true metallophytes have often diverged genetically and 171 morphologically to form new taxa endemic to their native metalliferous soils (Barrutia et al. 2011a). 172 Regrettably, their restricted geographic range is, partly, responsible for the current high rates of 173 population decline or, what is worse, irreversible extinction.

174 Plants growing in TE-polluted sites can be classified as (1) excluders: these plants limit TE 175 uptake and translocation and, then, maintain low TE concentrations in their aerial tissues; (2) indicators: 176 these plants accumulate TEs in their harvestable parts at concentrations similar to those present in the 177 polluted soil; and (3) accumulators/hyperaccumulators: these plants increase TE uptake, translocation 178 and accumulation in their aboveground biomass reaching levels that far exceed those present in the 179 polluted soil (van der Ent et al. 2013, 2015a; Malik et al. 2017; Massoura et al. 2014; Reeves et al. 180 2017a). Particularly, hyperaccumulators are exceptional plants that accumulate metal(loid)s in their 181 tissues to levels that can be hundreds or thousands of times greater than common ranges in other plant 182 species (van der Ent et al. 2013), and whose ecology is an active field of research, focusing on anti-183 herbivore defences, allelopathy and biotic interactions (Reeves et al. 2017b). Logically, it is important to 184 protect not only TE hyperaccumulators as nature's oddities but also TE excluders, indicators and 185 accumulators.

Apart from their intrinsic value as remarkable rare species, metallophytes are suitable candidatesfor the revegetation of mining sites, as well as for the implementation of phytoremediation

188 (phytoextraction/phytomining, phytostabilization) and phytomanagement initiatives (van der Ent et al. 189 2015b; Rosenkranz et al. 2019; Corzo Remigio et al. 2020). Thus, TE excluders and hyperaccumulators 190 have been extensively used for phytostabilization and phytoextraction purposes, respectively (Hernández-191 Allica et al. 2006; Epelde et al. 2008, 2009, 2010; Barrutia et al. 2009; Pardo et al. 2014; Garaiyurrebaso 192 et al. 2017). There is nowadays an increasing interest in the use of native plant species and populations 193 for the revegetation of TE polluted sites, as opposed to non-native, introduced species (Parraga-Aguado et 194 al. 2014; Chen et al. 2019). In this respect, a sturdy commitment to conservation of metallophyte 195 biodiversity is self-evident (Whiting et al. 2002).

Then, before starting any phytomanagement initiative, it is imperative to study the native vegetation of the polluted site in search of potential candidates (*e.g.*, metallophytes) for conservation purposes. If such candidates are identified, then, an area of the site (preferably, the area where the most interesting plant species have been identified) must be left unmanaged for conservation purposes (and, if needed, protection barriers must be installed).

In addition to protecting the natural environment of valued and treasured plant species (*i.e.*, *in situ* conservation in biotope "islands"), efforts must also be directed at conserving them *ex situ*, that is to say, in germplasm banks, seed gardens, arboreta, botanic gardens, etc. (Whiting et al. 2004), ideally maintaining the required degree of contaminant exposure (otherwise, contaminant-sensitive, non-adapted individuals might again become dominant in the plant population after a few cultivations). Additionally, when designing a strategy to preserve the valuable native vegetation at the polluted site, attention should also be paid to plant assemblages (*e.g.*, metalliferous distinctive plant communities).

208 Apart from the presence of unique metallophytes in TE-polluted sites, these degraded 209 environments can also harbour a valuable microbial diversity that can likewise be used for 210 phytoremediation and/or phytomanagement initiatives. For instance, TE resistant plant growth-promoting 211 rhizobacteria and endophytes, as well as TE-resistant mycorrhiza, can be isolated from TE-polluted sites 212 and, subsequently, be used to improve plant survival, growth and performance under the harsh conditions 213 usually present in many TE-polluted sites, particularly mining sites (Weyens et al. 2011; Ma et al. 2015; 214 Burges et al. 2016, 2017; Harrison and Griffin 2020). For instance, rhizobacterial inoculants (e.g., 215 Arthrobacter nicotinovorans SA40) have been shown to improve nickel phytoextraction by the 216 hyperaccumulator Alyssum pintodasilvae (Cabello-Conejo et al. 2014). Similarly, the inoculation of 217 ultramafic soils with *Microbacterium arabinogalactanolyticum* AY509224 increased soil Ni extractability

218 and uptake by Alyssum murale (Abou-Shanab et al. 2006). The inoculation with TE-resistant plant 219 growth-promoting bacteria has been reported to enhance the biomass of different plant species (e.g., 220 Brassica juncea, Ricinus communis, Helianthus annuus, and Sedum alfredii) growing in TE-polluted soils 221 (Dell'Amico et al. 2008; Jiang et al. 2008; Mastretta et al. 2009; Zaidi et al. 2006). Likewise, Kolbas et al. 222 (2015) reported the positive effects of endophytic bacteria for Cu phytoextraction by sunflower plants. 223 Truyens et al. (2015) found that inoculation of Agrostis capillaris plants with endophytes can be 224 beneficial for their establishment during phytoextraction and phytostabilisation of Cd polluted soils. The 225 co-inoculation of Paenibacillus mucilaginosus and Sinorhizobium meliloti in Cu-contaminated soil 226 planted with Medicago sativa improved alfalfa growth and decreased Cu accumulation in shoots, 227 compared to the uninoculated control (Ju et al. 2020). The inoculation of Pseudomonas vancouverensis 228 promoted As accumulation efficiency in Pteris vittata and Pteris multifidi (Yang et al. 2020b). However, 229 the effect of bacterial inoculants on plant growth and TE-accumulation has been shown to be plant 230 species-specific (Becerra-Castro et al. 2012).

231 Apart from the recognized conservation value of metallophytes present at TE-polluted sites, 232 plant species and, interestingly, microbial (bacteria, fungi) populations living in soils polluted with 233 organic compounds must also be considered for conservation purposes, owing to their intrinsic value as 234 well as their potential use for the rhizoremediation (*i.e.*, degradation of pollutants by rhizosphere bacteria) 235 (Barrutia et al. 2011b; Lacalle et al. 2018; Brereton et al. 2020) and bioremediation of organically 236 polluted soils (Garbisu et al. 2017; Anza et al. 2018; Meglouli et al. 2019; Villaverde et al. 2019). Since 237 bacterial and fungal strains isolated from organically-polluted soils can then be used for bioremediation 238 via bioaugmentation purposes, after thoroughly testing their degrading capabilities and potentials, it is 239 recommended to keep them in a microbial bank for possible future biotechnological applications.

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241 4. Managing biodiversity during phytomanagement

242 4.1. Phytomanagement under the current scenario of climate change

The negative consequences of climate change can nowadays be undoubtedly identified in the more
frequent alteration of natural and agricultural ecosystems, owing to, for instance, higher temperatures,
extreme droughts and storms, and an increased likelihood of heat waves and heavy precipitation episodes
(Alkorta et al. 2017). Not surprisingly, plant survival and growth are being significantly altered under

changing climatic conditions. Furthermore, increasing CO₂ concentrations in the atmosphere are currently
changing the physiology of plants, affecting, among other aspects, their growth rate.

Specifically, regarding the choice of plant species for phytomanagement in semi-arid and arid regions (*e.g.*, southern Europe) (Pulighe et al. 2019), and taking into account the critical importance of an adequate water regime for the success of revegetation programs, special attention should be paid to the selection of drought-resistant plant species and ecotypes, since the duration and frequency of extreme droughts is nowadays increasing in many semi-arid and arid regions (Risueño et al. 2020).

254 The possibility of irrigating phytomanagement crops is decidedly controversial, since water is an 255 increasingly scarce resource in many parts of the world. A proper sustainable management of water 256 resources is currently one of the greatest challenges for our society worldwide. Above all, we must first 257 ensure availability of good quality water for human consumption and agricultural purposes. Regrettably, 258 in the coming decades, the problem of water scarcity will probably get worse than it is now. Predictably, 259 an increase in the world human population will imply more water for human consumption and 260 agricultural production (agriculture accounts for around 70% of the water currently used in the world). 261 Then, it follows that the consumption of good quality water for irrigation of phytomanaged sites is, in 262 general, not considered a valid option, especially in semi-arid and arid regions. An alternative is the use 263 of wastewater for irrigation. An appealing, and currently attention-grabbing, option is the possibility of 264 treating such wastewater by means of rhizofiltration (*i.e.*, the use of plant roots and associated microbes to 265 absorb, concentrate and precipitate pollutants, especially TEs, from polluted effluents and waters) and/or 266 biodegradation, notably using constructed wetlands (CWs) and floating islands (Dushenkov et al. 1995; 267 Schröder et al. 2007; Zhang et al. 2007; Olguin et al. 2017).

268 Urban wastewater is known to contain nitrogen, phosphorus and other nutrients, leading to an 269 extra beneficial effect for plant growth through fertilization. Irrigation with wastewater is only 270 recommended for non-food and non-fodder crops, and then it would be an ideal option for 271 phytomanagement. Evidently, it would be beneficial to have an efficient urban wastewater treatment plant 272 closed to the site to be phytomanaged, so that the wastewater, directly or preferably after rhizofiltration in 273 a CW does not need to be transported a long distance. Interestingly, CWs can also be used to treat acid 274 mine drainage and then there is the possibility of reusing the treated water to eventually irrigate mine 275 tailings (Pat-Espadas et al. 2018).

276 Therefore, especially in semi-arid and arid regions of the world, for phytomanagement purposes, 277 it is recommended to select plant species that are resistant to water stress, extreme droughts and heat 278 waves for increasing the long-term success of the phytomanagement strategy (Risueño et al. 2020). For 279 instance, as water supply and its distribution during the crop cycle is a key limiting factor for crop 280 production in SW France, the sunflower and tobacco ability to stand more frequent heat waves and long 281 droughts is certainly an advantage (Kidd et al. 2015; Mench et al. 2018). Although hundreds of plant 282 species are suitable candidates for phytoremediation and/or phytomanagement purposes, there is 283 nowadays an urgent need to identify those which can successfully be used under the current scenario of 284 climate change.

285 The potential indirect effects of climate change on the soil biota present in phytomanaged sites 286 must be also considered, through the abovementioned climate change-induced alterations in plant growth 287 and physiology. Although higher levels of atmospheric CO_2 are *a priori* not expected to directly affect 288 soil microbial communities (*i.e.*, CO_2 concentrations in the soil are much higher than in the atmosphere), 289 higher atmospheric CO₂ concentrations can indirectly impact on soil microbial communities through 290 higher plant growth, increases in litter deposition and rhizodeposition (often resulting in a stimulation of 291 soil microbial biomass and activity), faster nutrient uptake and water use efficiency (Phillips et al. 2011; 292 Bardgett et al. 2013; Burns et al. 2013; Alkorta et al. 2017). Such climate change-induced variations of 293 rhizodeposition patterns, and concomitant changes in the composition and activity of rhizosphere 294 microorganisms, can modify TE bioavailability in soils (Rajkumar et al. 2013), thus potentially affecting 295 plant performance during phytomanagement.

296 The consequences of climate change (via higher atmospheric CO_2 concentrations, heat waves, 297 extreme droughts, higher temperatures, etc.) on beneficial plant-microorganism interactions (e.g., plant 298 growth-promoting rhizobacteria, endophytes, and mycorrhiza) are increasingly being studied (Compant et 299 al. 2005, 2010; Classen et al. 2015; Cavicchioli et al. 2019; Risueño et al. 2020). Plant growth-promoting 300 bacteria and fungi can positively affect water-stressed plants and, then, their inoculation should nowadays 301 be strongly considered for phytomanagement. On the other hand, climate-induced changes in soil 302 temperature and moisture can alter soil processes, such as organic matter decomposition and nutrient 303 cycling (Burns et al. 2013), supported, to a great extent, by the activity of soil microorganisms.

304 Some phytomanagement practices (*e.g.*, the application of organic amendments, low- or no-305 tillage practices, grassland implementation and afforestation) have great potential for carbon sequestration and, hence, climate change mitigation. The incorporation of trees in phytomanagement initiatives (*e.g.*, as
part of intercropping systems) has also acknowledged positive effects in this respect (Schoeneberger et al.
2012; Alam et al. 2014; Zeng et al. 2019a,b; Brereton et al. 2020).

In theory, a possible option for adaptation to climate change in phytomanagement is to incorporate to the planting scheme as many plant species as possible, and preferably from different vegetation types: grasses, shrubs and trees. Nevertheless, in many situations this is not a realistic, feasible option because the specific plant assemblages established for phytomanagement purposes are determined, to a great extent, on the future land use and on the particular non-food crops intended to be delivered to the local chains processing the harvestable biomass.

Moreover, the conservation of plant biodiversity (*e.g.*, the aforementioned metallophyte diversity) is crucial for adaptation to climate change as part of an "insurance policy": different species, varieties and ecotypes may be needed in the future as environmental conditions are altered by climate change.

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320 *4.2. Promotion of biodiversity under phytomanagement*

321 Under the inherent constraints inevitably derived from the phytomanagement goals, it is certainly possible 322 to promote biodiversity in phytomanaged sites by means of, for instance, growing as many plant species 323 and varieties/ecotypes from different vegetation types (grasses, shrubs and trees) as possible (Table 1). 324 Interestingly, the establishment of different plant species in phytomanaged sites can result in the 325 generation of a wider variety of valuable products and ecosystem services (Evangelou et al. 2015; Pandey 326 and Bauddh 2018).

327 Many additional benefits can be obtained when combining different plant species for the 328 phytoremediation and phytomanagement of polluted sites. For instance, the combination of Pteris vittata 329 with Morus alba and Broussonetia papyrifera not only increased the phytoextraction of trace elements 330 but alleviated phytotoxicity as well (Zeng et al. 2019b). In a similar study, the co-planting of P. vittata 331 with Arundo donax, M. alba and B. papyrifera resulted in an improvement of soil health (Zeng et al. 332 2019a). Furthermore, intercropping with Paspalum miliaceum and Axonopus affinis was efficient in 333 promoting gravevine growth in Cu-polluted soil by reducing metal bioavailability (De Conti et al. 2019). 334 Interestingly, the combination of tree, shrub and grass species in a metal polluted soil resulted in a more 335 efficient employment of water resources and a higher biodiversity of soil microorganisms (Parraga336 Aguado et al. 2014). In contrast, the co-planting of Odontarrhena chalcidica or Noccaea goesingensis

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with Lotus corniculatus for Ni removal led to reduced values of shoot biomass (Rosenkranz et al. 2019).

338 The beneficial effects of co-planting have also been reported for organically-polluted and mixed-339 polluted soils. Wang et al. (2013) reported an enhanced degradation of PAHs, in the presence of trace 340 elements, when S. alfredii was combined with Lolium perenne or Ricinus communis. In agreement with 341 these results, in their studies on intercropping with Medicago sativa and Festuca arundinacea, Sun et al. 342 (2011) observed higher PAH degradation values under intercropping vs. monoculture. Likewise, 343 intercropping with M. sativa, L. perenne and F. arundinacea improved the degradation of phthalic acid 344 esters. Finally, F. arundinacea was also co-planted with Salix miyabeana and M. sativa, finding out that 345 when crops were cultivated in pairs they showed an enhanced rhizosphere community in terms of the 346 presence of plant growth-promoting bacteria (Brereton et al. 2020).

347 Aboveground and belowground organisms are closely linked: plants provide organic carbon for 348 soil decomposers and resources for root-associated organisms; in turn, soil decomposers break down dead 349 plant material and regulate plant growth by determining the nutrient supply (Wardle et al. 2004). 350 Different plant species differ in the quantity and quality of litter and root exudates, thus affecting the 351 biomass, activity and diversity (mainly, composition) of soil microbial communities. A more diversified 352 vegetation leads to a higher number of ecological niches and, hence, biodiversity (Risueño et al. 2020). 353 Indeed, higher plant richness results in a higher variety of root exudates and types of litter, thus 354 stimulating biodiversity belowground (Wardle et al. 2004; Haichar et al. 2008). Nonetheless, Li et al. 355 (2015) found no relationship between plant and soil bacterial diversity in an early successional forest, and 356 a negative correlation in a late successional forest. Similarly, Kowalchuk et al. (2000) reported a negative 357 correlation between grassland plant and soil ammonia-oxidizing bacterial diversity. These contradictory 358 results point out to the vast complexity of the multiple links and interactions between aboveground and 359 belowground diversity (Wardle et al. 2004; De Deyn and Van der Putten 2005; Kardol and Wardle 2010), 360 which, at the moment, are far from being well understood. Phytomanagement, apart from increasing soil 361 microbial biomass and activity, can induce shifts in the bacterial community structure at both the total 362 community and functional group levels (Touceda-González et al. 2017a). In a study on the effectiveness 363 of dolomite and compost as amendments for enhancing Cu phytostabilization with Populus trichocarpa x 364 deltoides cv. Beaupré and Agrostis gigantea L., Cu stabilization and phytomanagement induced positive 365 changes in the microbial community of soil leachates, enriching this community with plant beneficial366 bacteria (Giagnoni et al. 2020).

367 The presence of phytopathogens and root herbivores in the rhizosphere can produce a negative 368 feedback on plant growth, whereas mycorrhizal fungi and plant-growth promoting rhizobacteria can have 369 a positive one on plant growth (Sessitsch et al. 2013; Sura-de Jong et al. 2015). In any case, the evidence 370 for positive or negative links between aboveground and belowground biodiversity is mixed, and not all of 371 the mechanisms by which aboveground organisms affect belowground diversity and vice versa 372 necessarily lead to correlations of species richness in both domains (Hooper et al. 2000). The common 373 perception that belowground biodiversity should follow similar patterns to those of plant diversity during 374 ecosystem development is challenged by Delgado-Baquerizo et al. (2020).

A higher richness of plant species can, for instance, be used to promote the biodegradation of aged polycyclic aromatic compounds in soil: oxygenated PAHs (some of which are more toxic than their related PAHs) can, however, accumulate in soils during such a plant-assisted remediation process (Bandowe et al. 2019).

Through an increase in plant diversity and, hence, in the number of ecological niches and possible habitats, it is also desirable to promote the aboveground and belowground diversity of animals (*e.g.*, arthropods: insects, arachnids, myriapods, etc.; earthworms; nematodes; mammals; birds; and so on), of course, always paying close attention to the potential risk of pollutant bioaccumulation and biomagnification (*e.g.*, TE biomagnification along the trophic chain) (Peterson et al. 2003). Interestingly, these animals can act as phytomanagement crop auxiliaries, helping to fight pests, pollinate the cultivated plants, etc. (Verkerk et al. 1998; Ferron and Deguine 2005).

Finally, intercropping systems have been extensively investigated for phytoremediation purposes (Sun et al. 2011; Ma et al. 2013; Wang et al. 2013; Alam et al. 2014; De Conti et al. 2019; Zeng et al. 2019a,b; Brereton et al. 2020) with additional benefits in terms of aboveground and belowground diversity. Likewise, as individual plant species repeatedly possess a limited range of TE phytoremediation capacities, functional complementarity principles could be of value for the phytoremediation of soils polluted with multiple TEs by means of using assemblages of species (Desjardins et al. 2018).

Biodiversity provides a wide range of values, some of them indirectly such as aesthetic value,
cultural value, spiritual value, scientific value, educational value, etc. Arguably, from an anthropocentric
point of view, the most important value of biodiversity comes from the ecosystem services it provides.

Biodiversity preserves the structure and integrity on which healthy ecosystems depend to provide the vitalecosystem services on which we rely on.

397 Among other values of biodiversity, the following two are often discussed when dealing with the 398 conservation of biodiversity and the human use of natural resources: (1) intrinsic value: as such, we have 399 the moral responsibility to preserve biodiversity (well-known nature writers such as Henry David 400 Thoreau, John Muir, Aldo Leopold, etc. have emphasized the intrinsic value of biodiversity); and (2) 401 utilitarian value: as such, focused on the commercial and subsistence benefits (e.g., food, medicines, raw 402 materials, energy, etc.) of biodiversity to humankind. Within this utilitarian perspective, the idea is to 403 protect biodiversity so that we can utilize it later for our own benefit. Obviously, this utilitarian value of 404 biodiversity is inextricably link to the phytomanagement concept. In any case, when designing a 405 phytomanagement initiative, it is unquestionably possible to promote biodiversity within the limits 406 imposed by the specific phytomanagement objectives (e.g., by means growing as many plant species as 407 possible) with the concomitant potential benefit of obtaining a wider variety of products and ecosystem 408 services.

409 Anyhow, the biodiversity concept is anything but simple. Among others, it includes the 410 following aspects: richness (or the number of species), evenness (relative abundances resulting in rare and 411 dominant species), composition (in terms of taxonomic groups), phylogenetic relatedness/distinctiveness, 412 and spatial and temporal distribution. Regarding species composition, biological species are certainly not 413 all equal: there are keystone species, foundation species, umbrella species, flagship species, charismatic 414 species, ecosystem engineers, invasive species, indicator species, chemical engineers, biological 415 regulators, etc., leading us to the difficult and arduous challenge to prioritize among them (Vane-Wright 416 et al. 1991).

Some authors proposed to assign more value to those species that lack close relatives, as 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 (Vane-Wright et al. 1991; Cadotte et al. 2010). Besides, some species appear to perform phylogenetically narrow processes (*e.g.*, nitrification, atmospheric nitrogen fixation) while others perform phylogenetically broad processes (*e.g.*, denitrification). The former show a lower degree of functional redundancy, compared to the latter. 424 To assess the influence of phytomanagement practices on biodiversity (such a broad concept) is 425 anything but easy. There are still many unanswered questions that research is yet to answer, e.g. What 426 number of species is a good number? What species composition is best? What degree of phylogenetic 427 distance is more adequate? How differently should we value the different types of species? Are 428 functionally redundant species less valuable than non-functionally redundant species? These questions 429 being answered, we must not take only richness into consideration when promoting biodiversity at 430 polluted sites under phytomanagement. To the best of our expertise and capacities, we must try to 431 consider other relevant aspects also included within the biodiversity concept.

432 To further complicate matters, biodiversity is difficult to quantify, at least partly, due to the 433 multitude of indices available to measure it (e.g., species richness, Shannon-Wiener entropy, Simpson's 434 index, Berger-Parker index, etc.). This is not surprising because of the abovementioned complexity of all 435 the aspects of biodiversity, which inevitably leads to the fact that no single perfect indicator for 436 biodiversity can be devised (Duelli and Obrist 2003). As a matter of fact, the choice of index often 437 depends on the question(s) to be answered, as well as on the specific aspect(s) or entity of biodiversity to 438 be evaluated. Paradoxically, most diversity indices have traditionally relied on three untrue assumptions: 439 (i) all species are equal; (ii) all individuals are equal; and (iii) species abundances have been correctly 440 assessed with appropriate tools and in similar units (Magurran 2004). In any case, although the choice of 441 index(es) depends, to a great extent, on the specific questions and objectives of the study, three of the 442 most commonly used indices are the Margalef's index for richness, the Shannon-Weaver's index for 443 diversity and the Simpson's index for dominance.

444 Similarly, the use of indices for quantifying functional biodiversity (functional richness, 445 functional evenness, and functional divergence) is essential to better understand the links between 446 biodiversity, ecosystem functioning and environmental constraints (Mouchet et al. 2010). Indeed, many 447 studies on the impact of disturbances (e.g., agronomic practices, contamination, climate change, nitrogen 448 deposition, etc.) on biological diversity are focused exclusively on structural biodiversity (usually, of only 449 one or a few taxonomic groups). But phytomanagement has a strong functional component related to the 450 provision of ecosystem services. Thus, it is highly beneficial to include both types of biodiversity, *i.e.* 451 structural and functional diversity, when promoting biodiversity under phytomanagement. Apart from a 452 selection, as wide as possible, of taxonomic groups, an analysis of functional groups, traits, guilds and so 453 on must be included in phytomanagement initiatives (Kumpiene et al. 2014; Durand et al. 2017; Touceda454 González et al. 2017a,b; Xue et al. 2018; Burges et al. 2020).

455 Although the identification of links between structural and functional biodiversity is undoubtedly 456 a challenging task, such identification is of much value from both an academic/scientific and management 457 point of view. Statistical multivariate analyses, applied to the group of variables used to measure 458 structural and functional diversity, are suitable tools for the establishment of hypotheses regarding the 459 abovementioned links.

The topic of the selection of the best indices to quantify both structural and functional biodiversity is not within the scope of this document. Nonetheless, we encourage the use of various indices for covering as much as possible the different aspects of the term biodiversity: richness, abundance, phylogenetic relatedness, functional traits, etc. Ideally, one should make the best efforts possible to evaluate the effect of phytomanagement practices on the various levels of biodiversity: genetic, species, populations and communities/ecosystems. However, biodiversity is not simply the sum of all ecosystems, species and genetic material, as it represents the variability within and among them.

467 In particular, for soil microorganisms, the assessment of genetic diversity is indispensable for 468 microbial ecologists since: (i) all the current definitions of "species" are inadequate for prokaryotes, 469 among other reasons due to the transfer of genes by horizontal gene transfer; and (ii) most 470 microorganisms cannot be cultivated and so we have no other choice than to study them by means of the 471 application of molecular biology techniques. In consequence, most soil microbial ecologists are nowadays 472 focused on the use of next generation sequencing techniques (e.g., metabarcoding, metagenomics) for the 473 quantification of soil microbial diversity. But next generation sequencing has still many technical 474 limitations and then we must be cautions when drawing conclusions about the effect of disturbances or 475 practices (e.g., phytomanagement) on soil microbial diversity.

476 Most studies on the effect of phytoremediation or phytomanagement practices on microbial 477 diversity are focused on soil microbial communities, especially rhizosphere microorganisms. In this 478 respect, more attention should be paid to plant microbiota and plant microbiomes (*e.g.*, in the 479 phyllosphere) under phytomanagement (Imperato et al. 2019).

480 Similarly, concerning genetic diversity, there are still many unanswered questions, such as, for481 example: The more genes the better? Are all genes equally important? Can we talk about "good" genes

482 (*e.g.*, genes involved in contaminant biodegradation pathways) and "bad" genes (*e.g.*, antibiotic resistance

genes)? How can be combined data from metagenomic, metatranscriptomic and metaepigenomic studies?

483

Likewise, 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?

Regarding the critical links between biodiversity and ecosystem functioning, one should take into consideration the concept of emergent properties, *i.e.* those new qualities that appear on higher integration levels and represent more than the sum of the low-level components (Reuter et al. 2005). For understanding these emergent properties, the interaction between the different elements must be closely studied (Reuter et al. 2005). 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 properties.

495 On the other hand, when promoting biodiversity under phytomanagement, it is important to 496 always include organisms from the different levels of the trophic chain. Instead, when evaluating their 497 effect on biodiversity, most phytomanagement initiatives only pay attention to aboveground botanical 498 diversity (richness, composition, vegetation structure) and, occasionally, include some belowground soil 499 biota, in many cases just microorganisms owing to their well-known key role in critical soil processes 500 and, hence, functions and ecosystem services (Xue et al. 2018; Burges et al. 2020). Nonetheless, for a 501 biodiversity assessment with more ecological relevance, it is desirable to include taxonomic groups from 502 the different levels of the trophic chain. As a matter of fact, we should study as many taxonomic groups 503 from the food web as possible (Garrouj et al. 2018; Ali et al. 2019; Prins et al. 2019).

504 Simplifying, the aboveground food web includes producers (plants), primary consumers 505 (herbivores) and secondary consumers (predators). Regarding consumers, there is an unresolved debate 506 regarding the benefits and disadvantages associated to the presence of animals in phytomanaged sites. 507 Actually, when dealing with the remediation of polluted sites, in many cases, animals are deliberately 508 excluded, in an attempt to avoid possible ecotoxic effects on exposed animals and, also, to minimize the 509 risk of bioaccumulation and biomagnification (Mann et al. 2011). But animals (e.g., arthropods, 510 earthworms, mammals, birds, etc.) can act as phytomanagement crop auxiliaries, helping to fight pests, 511 pollinate the cultivated plants, etc. (Verkerk et al. 1998; Ferron and Deguine 2005).

512 Pertaining to the soil ecosystem, an amazing diversity of soil organisms make up its food web: 513 bacteria, fungi, algae, protozoa, nematodes, micro-arthropods, earthworms, insects, small vertebrates 514 (mice, moles), etc. Like all food webs, the soil food web is fueled by primary producers such as plants, lichens, mosses, photosynthetic bacteria (e.g., cyanobacteria) and unicellular algae. The remaining 515 516 members of the soil biota obtain energy and carbon by consuming the organic compounds produced by 517 primary producers.

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4.3. Adaptive monitoring during phytomanagement

520 When dealing with long-term monitoring programs, such as the one for assessing the influence of 521 phytomanagement practices on biodiversity, as time passes, it is inevitable that (i) new analytical 522 techniques, methods and equipments might appear in the market; (ii) different approaches, concepts, 523 ideas, etc. might come up; (iii) changes in the ecosystem developmental stage will occur; (iv) unexpected 524 environmental threats might emerge; (v) budget fluctuations might threaten the initiative, and so forth 525 (Epelde et al. 2014). For that reason, we propose that the paradigm of adaptive monitoring (this paradigm 526 enables monitoring programs to evolve iteratively as new information emerges and research questions 527 change) should be incorporated to the long-term monitoring of the effect of phytomanagement practices 528 on biodiversity.

529 To this purpose, among other aspects, (i) well-formulated, clear and tractable questions must be 530 established at the beginning of the phytomanagement initiative; (ii) a rigorous statistical design must be 531 implemented from the onset of the study, notably accounting for the spatial variability of soil 532 contamination, contaminant exposure and pollutant linkage; and (iii) a conceptual model of the site under 533 phytomanagement must be created (Cundy et al. 2016).

534 As part of the adaptive monitoring program, periodically (the time period will depend on the 535 specific phytomanagement initiative), an expert judgment analysis must be organized to revise and, if 536 necessary, update the different aspects that make up the biodiversity monitoring program. Expert 537 judgment analyses often encourage the forging of partnership between researchers, policy-makers and 538 resource managers, an aspect of the utmost importance in phytomanagement (Cundy et al. 2013).

539 The accomplishment of economic, social and environmental benefits is a key aspect of 540 phytomanagement (Cundy et al. 2016). In particular, the provisioning of ecosystem services (carbon 541 sequestration, improvement of soil fertility, control of soil erosion, improvement of air quality, climate

and water regulation, production of atmospheric oxygen, provision of habitat, etc.) is a crucial componentof phytomanagement initiatives (Burges et al. 2018).

544 The provision of ecosystem services is underpinned by a variety of ecological processes and 545 functions which themselves are driven by biodiversity. Although changes in biodiversity can affect 546 ecosystem processes and, hence, the provision of ecosystem services, in some situations, biomass, species composition, functional traits, etc. are more important than biodiversity itself for the provisioning of those 547 548 services. Nonetheless, trade-offs between biodiversity and ecosystem services might arise in some 549 situations (Bandowe et al. 2019). Also, trade-offs and conflicts between the different ecosystem services 550 themselves might also emerge, and, then, it is desirable to select from the onset what specific ecosystem 551 services to promote, and implement measures that minimize conflicts.

552

553 Conclusion

554 Paraphrasing the three well-known M's of successful trading (Mind, Money management, and Method), 555 we can visualize the links between biodiversity and phytomanagement according to three M's of 556 successful phytomanagement: (1) Mind: for effective phytomanagement, we must use our mind and 557 creativity to design the best strategy for each specific site and casuistry (here, following the medical 558 aphorism "there are no diseases but sick people", we can state that "there is no pollution but polluted 559 areas"; all of them are different and require a site-specific assessment). In this respect, biodiversity 560 provides ideas, models and strategies (tested through millions of years of evolution) that we can learn 561 from; (2) Management: for successful phytomanagement, we must apply scientifically-based adaptive 562 management, especially under the current scenario of climate change. Biodiversity provides a myriad of 563 species, metabolic capabilities, functional traits, etc. which we can use in response to changing 564 conditions; and (3) Money: a fruitful phytomanagement will provide economic value through products 565 (crops for biomass-processing technologies) and ecosystem services which can help fuel our bioeconomy. 566 Interestingly, the promotion of biodiversity in phytomanaged sites can result in the generation of a wider 567 variety of valuable products and ecosystem services, while minimizing pollutant-induced environmental 568 risks. 569

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1019 Table	e 1: Ten examples of effects	of biodiversity und	ler phytomanagement.
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Plant species	Contaminants	Main finding	Reference
<i>Pteris vittata</i> co-planted with <i>Morus alba</i> and <i>Broussonetia papyrifera</i>	As, Cd, Pb and Zn	Co-planting alleviated toxicity and improved phytoextraction	Zeng et al. 2019b
Pteris vittata co-planted with Arundo donax, Morus alba and Broussonetia papyrifera	As, Cd, Pb, and Zn	Co-planting enhanced <i>P.</i> <i>vittata</i> growth and metal(oid) accumulation, and improve soil quality	Zeng et al. 2019a
Co-planting Sedum alfredii with Lolium perenne or Ricinus communis	Metals and PAHs	Co-planting <i>S. alfredii</i> with ryegrass or castor enhanced pyrene and anthracene dissipation	Wang et al. 2013
Intercropping: Medicago sativa with Festuca arundinacea	PAHs	Removal PAHs under intercropping was higher than under monoculture	Sun et al. 2011
<i>Odontarrhena chalcidica</i> or <i>Noccaea goesingensis</i> co- planted with <i>Lotus</i> <i>corniculatus</i>	Ni	Intercropping with <i>L.</i> <i>corniculatus</i> tended to decrease the shoot biomass of both species	Rosenkranz et al. 2019
The grass Piptatherum miliaceum, the shrub Helichrysum decumbens, and the trees Pinus halepensis and Tetraclinis articulata	Metal(loid)s	A diverse set of plant species with contrasting life forms may result in a more efficient employment of water resources and a higher biodiversity not only in relation to flora but also soil microbes	Parraga-Aguado et al. 2014
Medicago sativa, Lolium perenne and Festuca arundinacea	Phthalic acid esters (PAEs)	Intercropping with the three species was the most effective treatment for PAEs removal	Ma et al. 2013
Monocultures and polycultures of <i>Festuca</i> <i>arundinacea</i> , <i>Medicago</i> <i>sativa</i> and <i>Salix miyabeana</i>	Ag, As, Cd, Cr, Cu, Pb, Se and Zn	Co-cropping with the three species was the most robust scenario for remediation of multiple trace element contaminated soil	Desjardins et al. 2018
Grapevine was grown in monocropping, intercropping with <i>Paspalum plicatulum</i> and intercropping with <i>Axonopus affinis</i>	Cu	Intercropping with <i>P</i> . <i>plicatulum</i> and <i>A. affinis</i> was efficient in promoting the growth of grapevines at moderate and low levels of Cu contamination by reducing its bioavailability	De Conti et al. 2019
Co-cropping of Festuca arundinacea, Salix miyabeana and Medicago sativa	Trace elements and persistent organic pollutants (POPs)	The crops cultivated in pairs retained rhizosphere microbiome bacteria involved in plant growth promotion, POP tolerance and degradation, and improved nutrient acquisition	Brereton et al. 2020

1022 Figure legends

Figure 1: Evolution from phytoremediation to phytomanagement.

