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RESEARCH ARTICLE

# Plant and soil biodiversity is essential for supporting highly multifunctional forests during Mediterranean rewilding

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## Abstract

1. The multidimensional dynamics of biodiversity and ecosystem function during the rewilding of Mediterranean forests remain poorly understood, limiting our capacity to predict how future restoration efforts may help mitigate climate change.
2. Here, we investigated the changes in multiple dimensions of biodiversity and ecosystem services in a 120-year forest succession after harvest to identify potential trade-offs in multiple dimensions of ecosystem function, and further assess the link between above and below-ground biodiversity and function.
3. We found a positive influence of successional age on not only multiple dimensions of biodiversity and function but also some important trade-offs. Two ecosystem axes of function explained nearly 75.4% of functional variation during ecosystem rewilding. However, while the first axis increased with successional age promoting plant productivity and element stocks, the second axis followed a hump-shaped relationship with age supporting important reductions in nutrient availability and pathogen control in old forests. Our study further revealed a significant positive relationship between plant and soil biodiversity with multiple elements of multifunctionality as forests develop. Moreover, the influence of plant and soil biodiversity were especially important to support a high number of function working at high levels of functioning.
4. Our work provides new insights on the patterns and functional trade-offs in the multidimensional rewilding of forests and further highlights the importance of biodiversity for long-term Mediterranean rewilding.

**KEYWORDS**
biodiversity conservation, carbon sequestration, climate change, ecosystem sustainability, forest restoration, multiple ecosystems functions, trade-offs

## 1 | INTRODUCTION

Mediterranean forests are over of the most important global hotspots of biodiversity and are critical for supporting ecosystem function conservation and climate change mitigation (Cowling et al., 1996; Newbold et al., 2020). Over the past decades, forests have been exposed to anthropogenic pressure and rapid climate change, causing severe loss of biodiversity and function and threatening the sustainable development of local economies (Hanewinkel et al., 2013; Zhou, Zhou, Eldridge, et al., 2022). Rewilding is the process by which a disturbed ecosystem (e.g. after harvesting) transition towards a new natural state capable of supporting more biodiversity and valuable ecosystem services (Dandy & Wynne-Jones, 2019). Numerous international initiatives such as the Bonn Challenge (UNEP, 2011) and the New York Declaration on Forests have established ambitious targets for the rewilding of forests aiming at conserving biodiversity and function and promoting the restoration of degraded ecosystems (Bastin et al., 2019). Yet, despite the numerous ongoing restoration activities (Andrea, 2021; Mansourian et al., 2021), a holistic and multidimensional approach to evaluating our capacity to rewilding Mediterranean forests is largely lacking.

Mediterranean forests provide multiple services and functions (i.e. ecosystem multifunctionality, EMF) including carbon sequestration, wood production, soil fertility, plant and soil biodiversity preservation (Lucas-Borja et al., 2016; Manning et al., 2018; Zhou, Lucas-Borja, Eisenhauer, et al., 2022). To date, most previous work has focused on investigating the changes in averaging multifunctionality and individual functions during forest succession (e.g. Liu, He, et al., 2021; Liu, Zhu, et al., 2021; Lucas-Borja et al., 2016; Poorter, Craven, et al., 2021). For example, averaging EMF is known to increase with stand age in subtropical and Mediterranean forests (Lucas-Borja et al., 2016; Shi et al., 2021). Ecosystem dimensions are composed of groups of variables highly correlated with each other and representing important aspects of ecosystem function (e.g. productivity; Migliavacca et al., 2021). However, the changes in dimensions of above- and below-ground biodiversity and ecosystem functioning are far less studied. While averaging multifunctionality can provide useful information, it does not allow to identify potential trade-offs among independent dimensions of ecosystem function. Similarly, while plant richness is known to regulate EMF (Lucas-Borja & Delgado-Baquerizo, 2019), much less is known about how changes in multiple elements of soil biodiversity, such as bacteria, fungi, protists and invertebrates, correlate with multiple dimensions of ecosystem functions during the rewilding of Mediterranean forests after long-term succession. In addition, we ignore whether soil and plant biodiversity could help boost rewilding by supporting the number of functions that simultaneously exceeds a critical threshold.

The rewilding of nature needs to consider an integrative approach aiming to support multiple aspects of terrestrial ecosystems from ecosystem services, critical for human well-being, to the biodiversity of a myriad of below-ground and above-ground organisms. Moreover, rewilding needs to explore potential trade-offs in ecosystem services and plant and soil biodiversity with the goal of promoting long-term sustainable ecosystems (Bazzaz, 1979; Huang et al., 2018; Poorter, Rozendaal, et al., 2021; Wright et al., 2004). Failure to do so will impede us to better understand how biodiversity losses might affect ecosystem sustainability and future climate change mitigation. Yet, the temporal changes in the multiple dimensions of above- and below-ground biodiversity and ecosystem services, and the potential trade-offs during rewilding are virtually unknown in Mediterranean forests.

Here, we used a 120-year succession forest (*Pinus* sp.) experiment (after harvest) in a Mediterranean forest from Spain as our model system to investigate the successional changes in multiple dimensions of above- and below-ground biodiversity (sequencing-based diversity of bacteria, fungi, protists and invertebrates) and ecosystem services including soil nutrient availability, plant productivity, antibiotic resistance gene (ARG) control, pathogen control (as defined in Delgado-Baquerizo et al., 2020) and plant–fungal mutualism symbiosis proportion (see Table S1 for further rationale on these groups of functions) during Mediterranean rewilding. Our main goals were to (1) investigate the changes in plant–soil biodiversity and in the major axes of variation of multiple ecosystem functions during long-term succession, (2) identify potential functional trade-offs during forest rewilding and (3) determine the relationship between plant and soil microbial biodiversity with multiple dimensions of ecosystem functions during long-term forest rewilding. To address our research questions, we considered complementary multifunctional approaches such as averaging multifunctionality, multithreshold multifunctionality, multidimensional multifunctionality and individual groups of functions (see details below). Specifically, we aimed to test two hypotheses: (H1) Long-term forest rewilding would induce potential trade-offs among ecosystem services and (H2) both plant and soil biodiversity would support multiple ecosystem functions during Mediterranean rewilding.

## 2 | MATERIALS AND METHODS

### 2.1 | Site description

The experiment was conducted at the Los Palancares y Agregados (40°01′50″N; 1°59′10″W), located in Central-Eastern Spain. The study region has a Mediterranean climate with hot dry summers and humid winters. The mean annual temperature is 11.9°C, ranging from

−0.5°C in January to 30.5°C in July (Zhou, Lucas-Borja, Eisenhauer, et al., 2022). Mean annual precipitation is 595 mm, with an average of 99 mm occurred in summer. The soil at this site is an Entisol, with a pH ranging from 5.9 to 7.3 (Lucas-Borja & Delgado-Baquerizo, 2019). The study region has been listed as nature conservation area by the European Union's endangered habitats and the Government of Castilla La Mancha due to the vulnerability to global climate change and land use intensification. Dominant tree species consist of a mix of natural forests including *Quercus faginea Lam*, *Quercus ilex* L. and *Juniperus Thurifera* L. (Zhou, Lucas-Borja, Eisenhauer, et al., 2022).

## 2.2 | Experimental design and soil sampling

Experimental blocks were established within a Mediterranean pine forest succession, which was composed of five representative successional stages (20, 40, 80, 100 and 120 years; Lucas-Borja & Delgado-Baquerizo, 2019). In October 2014, we established four replicate plots in each successional stage (20 plots in total) that keep more than 500 m apart to account for spatial heterogeneity (Figure S1). Plots of five successional forests were >300 m apart from one another. Four replicate plots of each successional stage had similar climate scenarios (e.g. MAT, MAP), slope, aspect, elevation, plant diversity, and were at least 100 m apart from any forest edge. Soils from 0 to 10 cm soil layer were randomly sampled with a cylindrical core at five points in unaltered and undisturbed forest floor areas. Soils in the five cores were mixed as a composite sample and manually removed plant residues and then sieved through a 2-mm mesh. Each soil sample was divided into two parts, one was stored at −20°C for DNA extraction in the laboratory, and another was stored at 4°C for physical and chemical analyses.

## 2.3 | Soil properties

We used standardized protocols to measure soil properties (Maestre et al., 2012). Specifically, soil clay (particle size <2 μm) percentage was measured using laser diffraction (MasterSizer 2000; Malvern Corporation). Bulk density was measured by the soil samples that dried at 105°C for 96 h, and then calculated as the ratio of total dry weight to total soil volume. Soil pH was measured by a glass electrode (Model PHS-2; INESA Instrument) with a 1:2.5 (w:v) soil: water solution.

## 2.4 | Ecosystem services and functions

Five ecosystem services were included in this study: nutrient availability (e.g. N, P availability), plant productivity (e.g. litterfall, basal area), ARG control (inversed ARG abundance), pathogen control (opposite number of plant/animal pathogen proportions) and fungal symbiosis proportion (e.g. ectomycorrhizae and saprobes). Nutrient availability was used to estimate nutrient cycling function. Plant productivity was used as a proxy of climate regulation (e.g. C cycling regulation) function. ARG control, pathogen control and fungal symbiosis proportion were maintained healthy soil for the sustainability of forest ecosystems (Zhou, Lucas-Borja, Eisenhauer, et al., 2022).

### 2.4.1 | Nutrient availability

Total nitrogen (TN) was analysed by an elemental analyser (vario MICRO cube; Elementar, Germany). Soil organic matter (OM) content was analysed by the wet combustion method (Walkley-Black procedure) with potassium dichromate (Keeney & Nelson, 1982). Available phosphorus was analysed colorimetrically through the molybdate blue method, after the soils were extracted with 1 mol/L $NH_4F$ solution. Available nitrogen (AN) was determined in the supernatant using a Holland Skalar San~(++) continuous flow analyser (Quik Chem from method 10-107-064-D for $NH_4^+$-N and 10,107-04-1-H for $NO_3^-$-N, Germany). Soil exchangeable sodium (Na), calcium (Ca), potassium (K) and magnesium (Mg) were extracted with 1 mol/L $CH_3COONH_4$ solution and measured with atomic absorption spectrophotometry (Lucas-Borja & Delgado-Baquerizo, 2019). The concentrations of anions (chloride (Cl) and sulphate(S)) in the water extract (1:10, soil:water) were analysed by HPLC with a conductivity detector.

### 2.4.2 | Plant production

To measure litterfall, we placed twelve 0.5 × 0.5 m collection traps randomly at each plot in October 2014. Litterfall data were collected monthly, and samples were weighed after drying at 65°C for 48 h to constant weight. Litterfall was calculated as dry weight/box area. Plant biodiversity was quantified using species richness defined as the total number of observed species within each plot (Oksanen et al., 2015). We measured the trunk circumference at 1.3 m height above the ground to estimate basal area (the area of a breast-high cross section of all the trees per hectare). Plant cover was estimated from the densitometer readings along the center transects of each plot as well as the center of each nested subplot (Guyon & Battaglia, 2018).

### 2.4.3 | Antibiotic resistance gene control

The abundances of unique 285 ARGs encoding resistance to all the major categories of antibiotics were measured with high-throughput quantitative PCR (HT-qPCR) method (Hu et al., 2018). Thermal-cycling conditions were as follows: 95°C for 10 min, followed by 40 cycles of both 95 and 60°C for 30 s. All HT-qPCR reactions were performed in three technical replicates. We followed the five criteria of Hu et al. (2018) to treat the positive detection. The inversed abundance of ARGs was measured by calculating the inverse of it (−1 × total abundance of ARGs) (Delgado-Baquerizo et al., 2018).

### 2.4.4 | Pathogen control and plant–fungal symbiosis

We obtained the relative abundance of potential pathogens from amplicon sequencing by parsing the soil phylotypes using FungalTraits (Nguyen et al., 2016; Põlme et al., 2020). Only highly probable and probable guilds were used in these analyses. The plant or animal pathogen controls were obtained by calculating the inverse of these variables (−1 × total relative abundance of fungal plant/animal pathogens) (Delgado-Baquerizo et al., 2018).

## 2.5 | Soil biodiversity

We used Illumina MiSeq platform to measure soil biodiversity of bacteria, fungi, protists and invertebrates by amplicon sequencing. Powersoil DNA Isolation Kit was used to extract soil DNA. A portion of the eukaryotic 18S rRNA gene and bacterial 16S rRNA gene was sequenced using the Euk1391f/EukBr and 515F/806R, respectively (Ramirez et al., 2014). The combinations of QIIME, UNOISE3 and USEARCH were used to do the bioinformatic processing. Considering the zOTU approach is expected to provide similar results as those by OTU method, sequences were clustered into soil phylotypes (i.e. zOTUs) with a 100% identity level. We followed the methods used by Delgado-Baquerizo et al. (2018) and Guillou et al. (2013) to identify the representative sequences of zOTUs against the SILVA (16S rRNA gene) and PR2 (18S rRNA gene) databases (Guillou et al., 2013). The zOTU abundance tables of bacteria (16S rRNA gene), fungi (18S rRNA gene), protists (18S rRNA gene) and invertebrates (18S rRNA gene) were rarefied at 5000, 2000, 800 and 300 sequences per sample, respectively. We followed the method as described by Delgado-Baquerizo et al. (2018) to define protists as all eukaryotic taxa, except fungi, invertebrates (Metazoa) and vascular plants (Streptophyta). More details about the soil diversity measures method can be found in Delgado-Baquerizo et al. (2018). On average, bacterial communities were dominated by Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Planctomycetes, Proteobacteria, Verrucomicrobia; fungal communities were dominated by Ascomycota, Basidiomycota and Mucoromycota; protist communities were dominated by Aleveolata, Amoebozoa, Archaeplastida, Excavata, Rhizaria and Stramenopiles; and invertebrate communities were dominated by Annelida, Arthropoda, Nematoda, Rotifera and Tardigrada.

We used richness (i.e. number of soil phylotypes) as a metric of soil biodiversity, which is the most used and the simplest metric of biodiversity in microbial ecology. We averaged the relative abundance of soil phylotypes (zOTU abundance tables) of four soil replicates before calculating the richness of the concerned soil organisms. We then calculated the richness of the bacteria, fungi, protists and invertebrates using the averaged zOTU tables. Meanwhile, we acknowledge the potential limitation of sequencing approaches for quantifying the biodiversity of soil invertebrates; larger soil organisms are possibly underrepresented with this approach. More importantly, richness was highly correlated ($p < 0.001$) with Shannon diversity for the diversity of four groups of soil organisms.

## 2.6 | Ecosystem multifunctionality

EMF is a quantitative index to provide an easily interpretable and straightforward evaluation of the ability of ecosystems to sustain multiple ecosystem functions simultaneously (Maestre et al., 2012). In this study, we used three multifunctionality indexes: the averaging multifunctionality index, the multiple threshold method (Byrnes et al., 2014; Maestre et al., 2012), as well as the key the multidimensional multifunctionality approach. (1) Averaging multifunctionality: we first normalized (log-transform if needed) and standardized each ecosystem function measured using the *Z*-score transformation. These standardized ecosystem functions were then averaged to obtain a multifunctionality index (i.e. EMF). (2) Multithreshold multifunctionality: we used the standardized ecosystem functions data to facilitate possible comparisons in future related studies (Jing et al., 2020). The threshold gradient ranged from 5% to 99% at 1% intervals, and the relationships between EMF and stand age, plant biodiversity as well as soil biodiversity along the threshold gradient were assessed according to Byrnes et al. (2014). (3) Multiple dimensions of ecosystem function: the functional dimensions of multiple ecosystem functions were determined using unconstrained principal coordinate analyses (PCoAs) with Bray–Curtis distance (PCoA, as done in Zhang et al., 2019). The key axes of the multidimensional space of ecosystem services were confirmed as the concerned variables.

## 2.7 | Statistical and data analyses

Multiple regression model was applied to determine the relationships between principal components (dimensions) with EMF, stand age, plant and soil biodiversity, as well as between EMF with plant and soil biodiversity. The goodness of fit between concerned variables was estimated with linear or non-linear models. Considering the nonlinear methods may over-parameterize on diverse data, we conducted unconstrained PCoA with Bray–Curtis distance analysis (PCoA) on the multivariate space of the ecosystem functions/soil biodiversity. Specifically, multi-ecosystem functions/soil biodiversity were standardized using *Z*-transformation. We then extracted the explained variance of each component (dimensions) to represent the variation of the concerned variables. We performed the PCoA using the function PCoA() implemented in 'VEGAN' packages in R software. All EMF analyses were performed with 'MULTIFUNC', 'CORRPLOT', and 'NBCLUST' packages in R. Meanwhile, we used variation partitioning analysis to quantify the unique contribution of plant and soil biodiversity to EMF. A negative value in the variance explained for a concerned variable was interpreted as zero, indicating that the explanatory variables explained less variation than random normal variables (Delgado-Baquerizo et al., 2017). Variation partitioning analysis was conducted using the 'VEGAN' package in R. In addition, partial correlation analyses were conducted in SPSS to cross-validate the influence of plant and soil biodiversity on EMF controlling for change of stand age.

## 3 | RESULTS

### 3.1 | Multidimensional changes in ecosystem function during forest succession

Averaging multifunctionality increased with succession age (Figure S2). We also found that stand age was positively associated with high functional thresholds (over 50% of their maximum observed levels of functioning, Figures 2 and 3) but did not support a high number of functions working at low level of functioning. Our PCoA results showed that the first two axes of variation (functional dimensions) explained 75.4% of the multidimensional functional space variation (Figure 1a), with functional dimensions #1 and #2 explaining 56.5% and 18.9%, respectively. The first functional dimension was dominated by plant productivity and element stocks, whereas the second axis was dominated by nitrogen availability. Specifically, plant cover, basal area, OM, TN and AN contribute with positive loadings to functional dimension #1. Meanwhile, nitrogen availability was positively correlated with functional dimension #2. Moreover, EMF was positively correlated with functional dimension #1 but negatively correlated with functional dimension #2 (Figure 1c). Increased stand age followed a significant correlation with functional dimension #1, while showed a hump-shaped relationship with functional dimension #2 (Figure 1d). These results

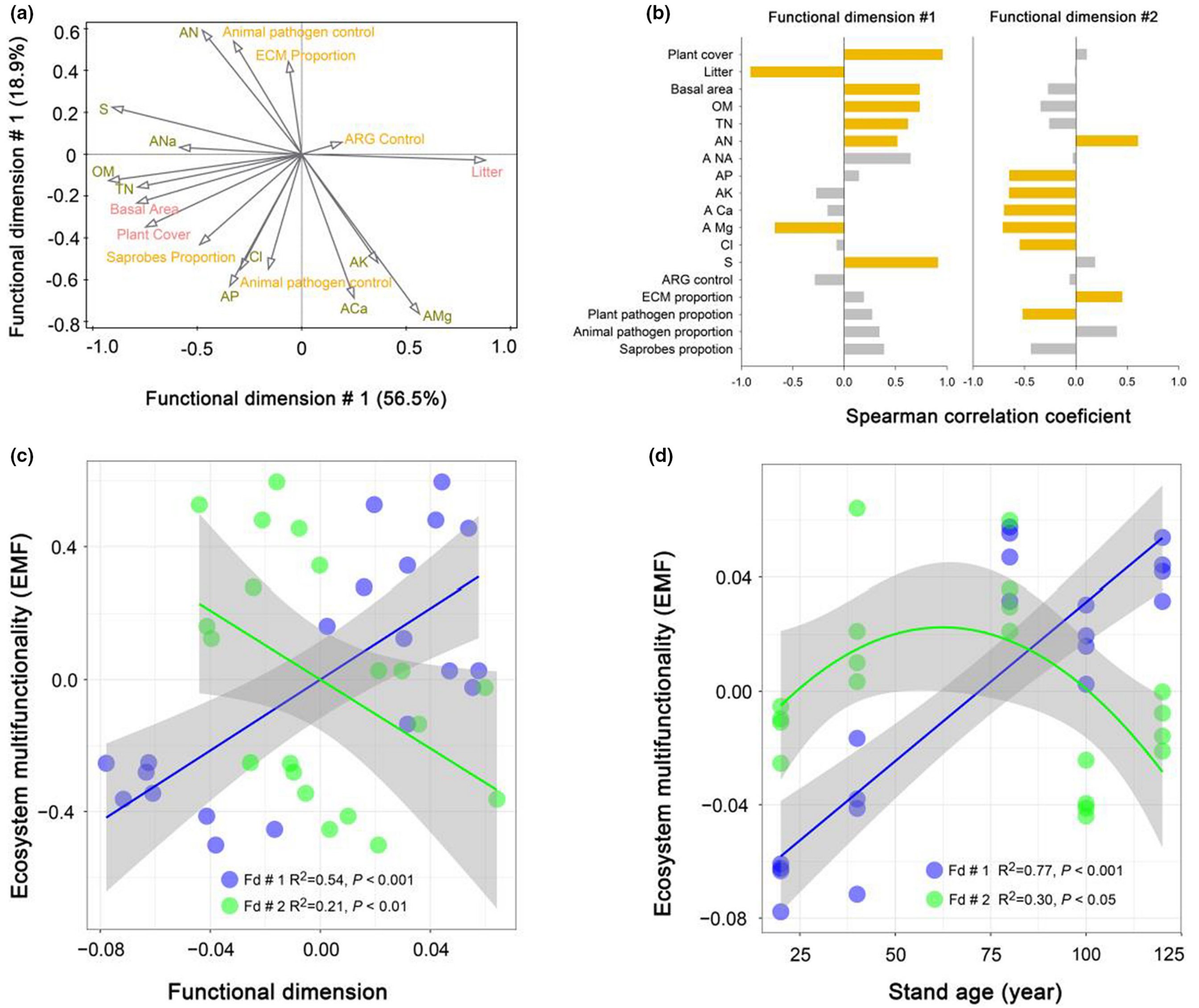


FIGURE 1 Unconstrained principal coordinate analyses with Bray–Curtis distance analysis of multiple ecosystem functions during forest succession (a). Bar plots of the Spearman correlation coefficients between multiple ecosystem functions with functional dimensions (b). Relationship between functional dimensions with ecosystem multifunctionality (c) and stand age (d). Orange bars represent the correlations that are considered significant ($p < 0.05$).

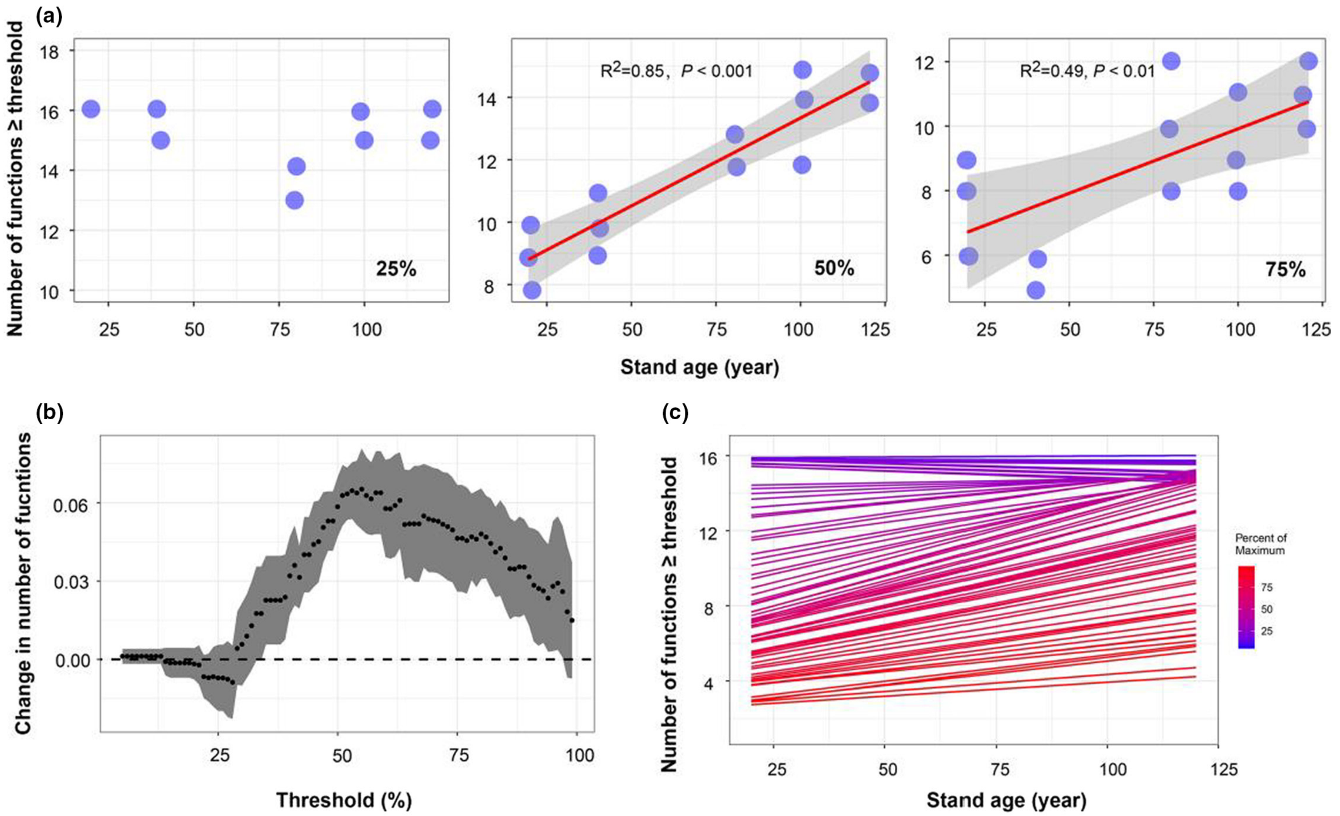


FIGURE 2 Forest rewilding promoted ecosystem multifunctionality (EMF). The relationship between stand age and EMF, defined as the number of functions reaching a threshold of some percentage of the maximum observed function. Panels show the relationship between stand age and EMF for three different thresholds (25%, 50% and 75% of maximum) in plots (a). The curve shows the slope changes of each coloured line with threshold levels and the grey area indicates the SE (b). The relationship between stand age and the number of functions at or above a threshold of some percentage of the maximum observed function. The effects of stand age on EMF are significant within the threshold ranges where the SE does not cross the zero line. The threshold method includes 18 functions. The number of functions working at different functional thresholds differs across samples as some samples support a larger number of functions working over a determined threshold (e.g. 25%). Colours indicate different thresholds as shown in the figure legend with cooler colours denoting lower thresholds and warmer colours denoting higher thresholds (c).

indicate potential trade-offs in ecosystem function in very old forest rewilding processes (Figure S3).

## 3.2 | Multidimensional changes in soil biodiversity during forest succession

Our results showed that forest succession significantly affected soil microbial community composition (Figure S4). Bacteria were dominated by Acidobacteria and Proteobacteria during all succession stages, with the proportion of Chloroflexi (4.85%) being especially important in early successional stages. Ascomycota (40.98%) and Basidiomycota (40.63%) dominated fungal communities during forest succession. The proportion of Ascomycota (43.38%) was larger than Basidiomycota (27.35%) in the first 20 years of forest succession but exhibited the opposite trend in very old forests (100–120 years). Rhizaria dominated the protist community during all stand ages. We also found changes in the community of soil invertebrates with Arthropoda dominating early successional stages and Nematoda being more dominant (in terms of proportion of 18s gene sequences) in older forests. Finally, the proportion of Annelida (34.76%) was especially high in the oldest forests (120 years).

Our results showed that the variation of microbial biodiversity during long-term forest succession could be explained by the first axis of a PCoA (biodiversity dimension #1) explaining 43.9% of variation, being especially representative for bacteria and invertebrates (Figure 4). Biodiversity dimension #1 was negatively correlated with the proportion of Acidobacteria, Chloroflexi, Aleveolata and Arthropoda but positively correlated with Proteobacteria, Nematoda, Rotifera and Tardigrda.

## 3.3 | Above- and below-ground biodiversity are positively correlated with multidimensional changes in ecosystem functions during forest rewilding

In general, plant and soil biodiversity were positively correlated with EMF. First, biodiversity dimension #1 was positively correlated with EMF and stand age. In addition, plant and soil biodiversity

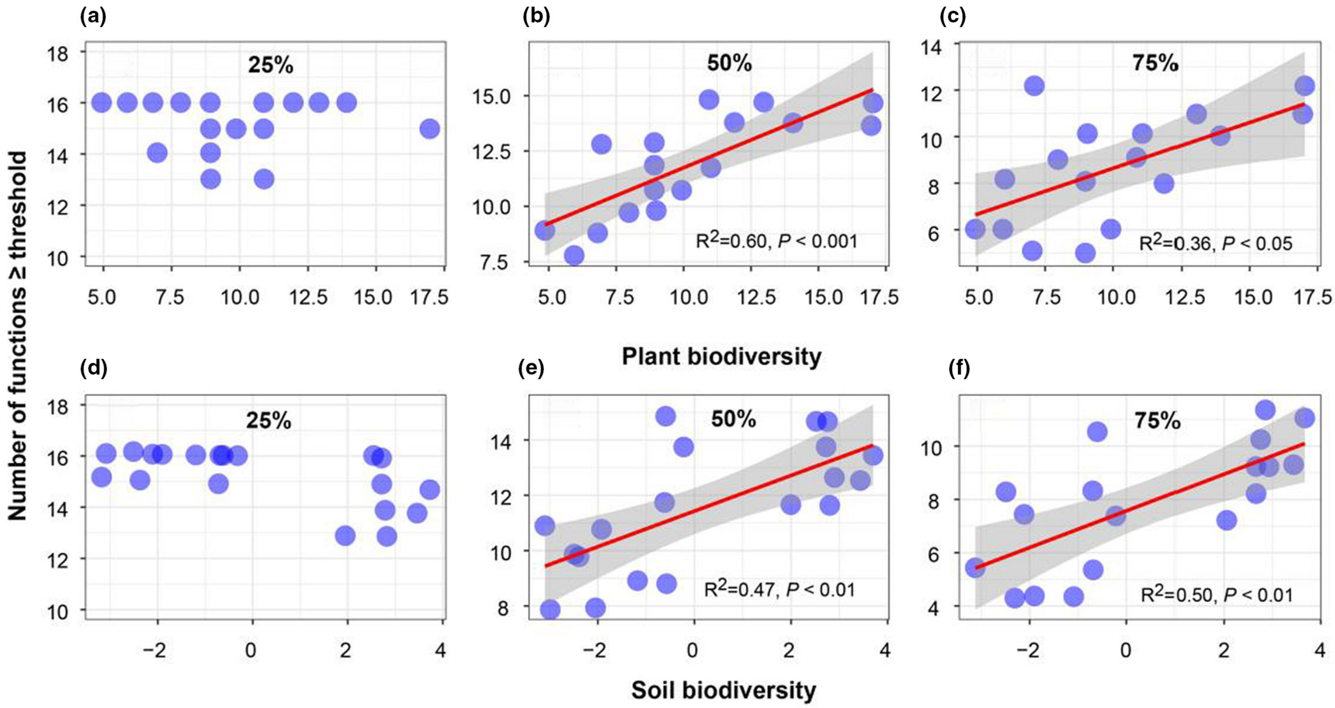


FIGURE 3 Plant and soil biodiversity drive changes in ecosystem multifunctionality (EMF) for the rewilding multifunctional Mediterranean forests. (a–c) Plant biodiversity drive changes in EMF. (d–f) Soil biodiversity drive changes in EMF. The relationships between the EMF and driving factors were drawn at three different thresholds (i.e. 25%, 50% and 75% maximum) levels in plots.

were positively correlated with averaging EMF (Figure 5, Figure S5). Importantly, stand age, plant and soil biodiversity were positively associated with a high number of functions working at high levels of functioning (over 50% of their maximum observed levels of functioning) (Figures 2 and 3), while this result was not observed for functions working at a low level of functioning (e.g. <25%). These results indicate that plant and soil biodiversity are important to support multiple functions simultaneously working at high levels of functioning during forest rewilding. The slope of the relationships between plant and soil biodiversity with EMF also confirmed that the effects of plant/soil biodiversity on EMF were significantly more important under high functional thresholds (average more than 30%, Figure S5). We further found that both plant and soil biodiversity was positively correlated with functional dimension #1 (Figure 5). In addition, the effects of plant biodiversity on EMF override impacts by soil biodiversity (Figure S7).

## 4 | DISCUSSION

Mediterranean forests are commonly acknowledged as hotspots of biodiversity and function. Understanding the dynamics of multiple dimensions of biodiversity and ecosystem function during the rewilding of Mediterranean forests is critical to better predict how forest ecosystems could help mitigate climate change in the near future (Chapin et al., 2002; Migliavacca et al., 2021). Here, we investigated how different aspects of multifunctionality changes during forest rewilding, and highlight the important role of plant and soil biodiversity in this process. First, we found that older forests support more ecosystem function, but that trade-offs are also present, and need to be considered during restoration processes. We provide solid evidence, that most of the variability in ecosystem functions could be captured by two key axes. Plant production and carbon sequestration increased with stand age, but nitrogen availability and pathogen control (the inverse of the proportion of soil-borne potential plant pathogens) decreased with time. We further highlight the role of biodiversity in supporting forest rewilding in Mediterranean forests and show that biodiversity is positively associated with highly functional ecosystems during forest succession. These findings are integral to the management of Mediterranean ecosystems during rewilding processes.

Two axes are needed to explain the functioning of Mediterranean forests during rewilding, while a single axis one accounts for most part of variation in soil biodiversity during rewilding, being positively correlated with soil age (Figure 2). Our results showed that functional dimension #1 explains 56.5% of the multidimensional functional variance and is dominated by plant productivity properties and soil nutrients, as indicated by the contributions of plant cover, litterfall, basal area, OM and TN. Most of those variables exhibit significantly positive correlations with functional dimension #1 (Figure 1b), suggesting the coupling between plant productivity and soil nutrients. Increased biomass by forest succession would stimulate more photosynthetically fixed C and litter inputs to the soil, increasing the soil OM accumulation and nutrient content (Bradford et al., 2016; Liu et al., 2019; Lucas-Borja et al., 2016). Stand age was positively

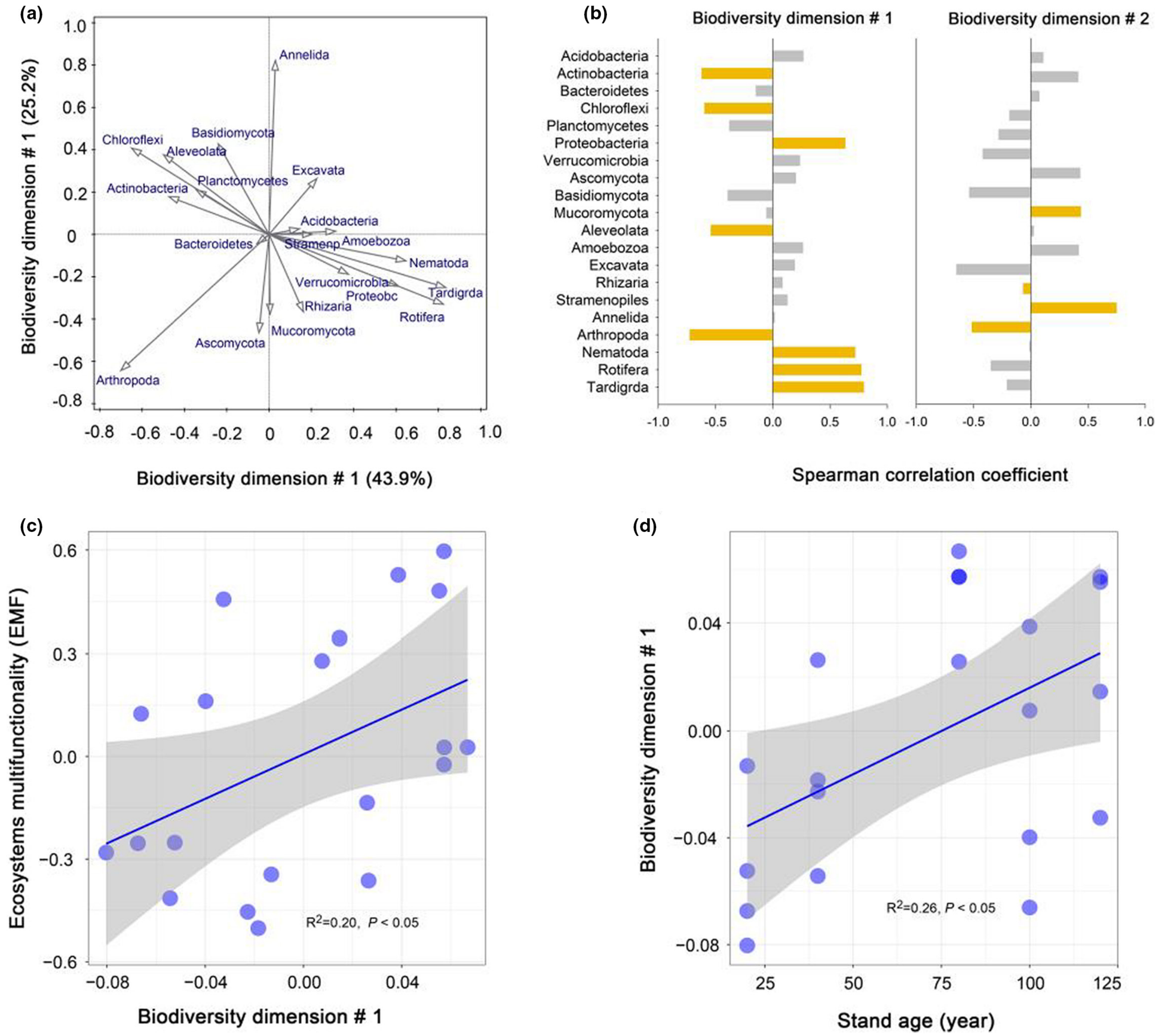


**FIGURE 4** Unconstrained principal coordinate analyses with Bray–Curtis distance analysis of dominated microbial biodiversity for the rewilding multifunctional Mediterranean forests (a). Bar plots of the Spearman correlations coefficient between dominated microbial groups with soil biodiversity dimensions (b). Relationship between biodiversity dimensions with ecosystem multifunctionality (c) and stand age (d). Orange bars represent the correlations that are considered significant ($p < 0.05$).

correlated with functional dimension #1, suggesting that forest succession could promote forest development, carbon sequestration and nutrient content of Mediterranean forest which is consistent with those in temperate and tropical forests (Heilmayr et al., 2020; Zhou et al., 2006). On the contrary, AN exhibits a hump-shaped relationship with stand age and contributes to positive loading with functional dimension #2 (Figure 1b, Figure S3). These results suggested an important functional trade-off in old Mediterranean forests. In particular, our findings suggest that as Mediterranean forests age, they not only increase their basal area and cover but also accumulate larger proportions of potential plant pathogens in their soils and support a reduced soil nitrogen availability.

Like the case in a biodiversity-ecosystem function experiment (Liu, He, et al., 2021; Liu, Zhu, et al., 2021; Ren et al., 2021), our design does not control the potential to identify the effects of forest succession on plant and soil biodiversity as well as EMF, but it does allow us to explore biodiversity drivers of ecosystem multiservices in plantation forests. Our research found that multiple ecosystem functions are highly positively correlated with plant and soil biodiversity during long-term forest succession (Figure 5). Plant biodiversity exhibited a significantly positive correlation with stand age, EMF and functional dimension #1. It has been shown that plant biodiversity may increase the heterogeneity of resources such as litter types and root exudates, largely leading to positive associations with EMF (Lucas-Borja & Delgado-Baquerizo, 2019). Meanwhile,

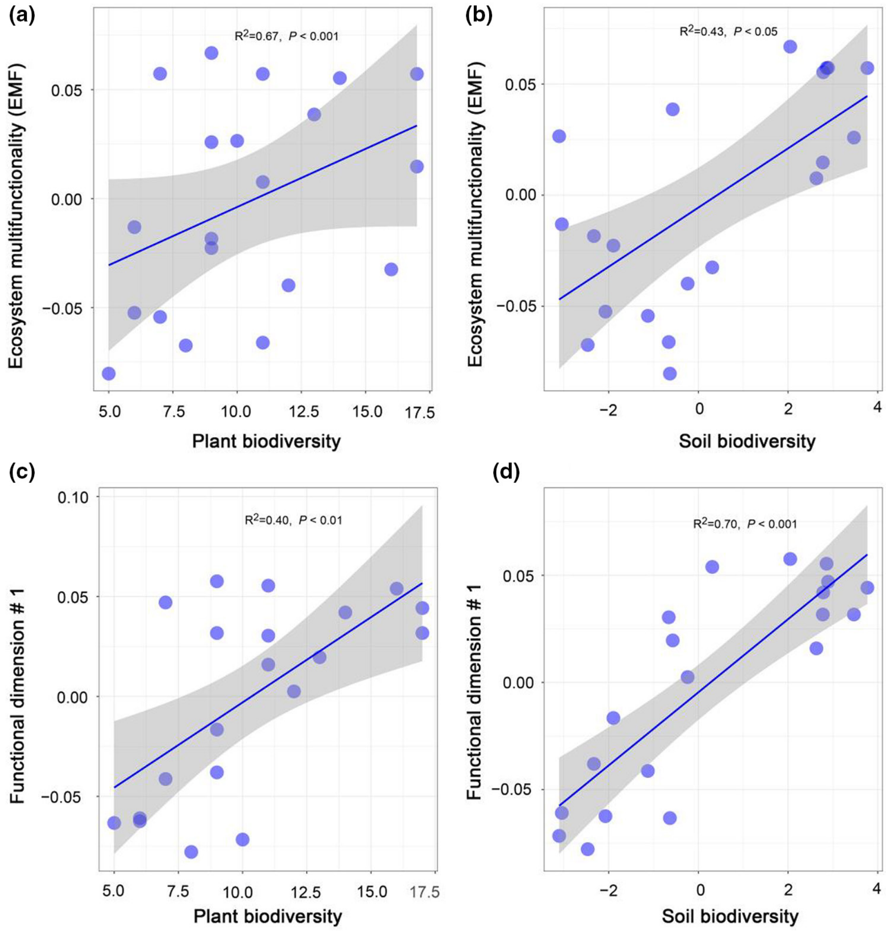


**FIGURE 5** Plant and soil biodiversity drive ecosystem multifunctionality for the rewilding multifunctional Mediterranean forests (a, b). Relationship between functional dimensions with plant biodiversity (c) and soil biodiversity (d).

plant biodiversity may also reduce disease and abundance of herbivores, stimulating soil nutrient cycling and then plant productivity (Cardinale et al., 2012; Haddad et al., 2011). In addition, our results were consistent when conducting additional partial correlations between plant and soil biodiversity with EMF after controlling for changes in stand age (Figure S6). This analysis further revealed that plant and soil biodiversity are essential for supporting highly multifunctional forests.

Consistent with a previous study conducted in subtropical forests (Shi et al., 2021), our results showed that soil microbial biodiversity could enhance the EMF during forest succession. Our study further indicates that higher proportions of Proteobacteria, Ascomycota, Nematoda, Rotifera and Tardigrada can contribute positively to support biodiversity dimension #1 and EMF. The enhanced microbial taxa by forest succession were fundamental for the maintenance of multiple functions and energy flow within the soil food web (Delgado-Baquerizo et al., 2020). Previous studies have found positive correlations between microbial biodiversity (i.e. bacteria and fungi) and EMF across environmental gradients (Delgado-Baquerizo et al., 2020), but the linkage between soil biodiversity and function is far less studied during forest rewilding processes. Niche differences in diverse microbial taxa during forest succession could fundamentally enhance the complementarity effect, promote more ecosystem functions simultaneously and support higher EMF (Fanin et al., 2018; Lefcheck et al., 2015). In addition, our results showed that the effect of plant biodiversity on EMF was greater than those by soil biodiversity. These changes may arise from the fact that plant biodiversity has different effects on the microbial taxa, which may offset the microbial effects as a whole (Figure S4). Finally, we provide important evidence that both biodiversity and function are key for supporting multifunctionality, and more importantly, ecosystems supporting a high number of functions working at high levels of functioning. This important result suggests that combined plant and soil biodiversity might help boost the functioning of ecosystems during restoration.

Forest rewilding has been a global priority in climate change mitigation and biodiversity conservation (Poorter, Rozendaal, et al., 2021). Our study provides compelling evidence that forest succession promotes multiple functions of Mediterranean forests, most of them captured by two key axes. With succession time increasing, the functional and soil biodiversity dimension #1 exhibited a significantly linear increasing trend. Meanwhile, our study also identified important functional trade-offs in old Mediterranean forests associated with reductions in nitrogen availability and pathogen control (second functional dimension), with a significant hump-shaped relationship between stand age and functional dimension #2 were observed. More importantly, our study demonstrated that both plant biodiversity and soil biodiversity were crucial to stimulate EMF, suggesting that maintaining plant/soil biodiversity is fundamental for mitigating future climate change in Mediterranean forests.

Our studies suggest that we should incorporate the multidimensional dynamics of biodiversity and ecosystem function during the rewilding of Mediterranean forests into the next-generation Earth systems models to improve predictions of future carbon-climate change feedback. These models do not consider either multiple dimensions of ecosystem functions or multithreshold multifunctionality, during long-term forest succession, which limits predictions of how Mediterranean ecosystems will respond to future climate change (Eyring et al., 2020; Migliavacca et al., 2021). Our study suggests that both multithresholds and multidimensional approaches are needed to support the management of ecosystem restoration in Mediterranean forests. More important, we provided evidence that biodiversity is critical for supporting highly functional forests during Mediterranean rewilding. Such knowledge is important to improve the predictability of the ecological consequences of forest succession under future changing climatic condition, and then support vibrant human cultures.

## AUTHOR CONTRIBUTIONS

Manuel Delgado-Baquerizo and Guiyao Zhou designed the study. Manuel Esteban Lucas-Borja collected the data. Guiyao Zhou and Shengen Liu analysed data. All authors contributed to the revision of the paper.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data used in this study were archived in figshare https://doi.org/10.6084/m9.figshare.21542154.v1 (Zhou, Lucas-Borja, Liu, et al., 2022).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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