



RESEARCH ARTICLE

Litter and soil biodiversity jointly drive ecosystem functions

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Abstract

The decomposition of litter and the supply of nutrients into and from the soil are two fundamental processes through which the above- and belowground world interact. Microbial biodiversity, and especially that of decomposers, plays a key role in these processes by helping litter decomposition. Yet the relative contribution of litter diversity and soil biodiversity in supporting multiple ecosystem services remains virtually unknown. Here we conducted a mesocosm experiment where leaf litter and soil biodiversity were manipulated to investigate their influence on plant productivity, litter decomposition, soil respiration, and enzymatic activity in the litterosphere. We showed that both leaf litter diversity and soil microbial diversity (richness and community composition) independently contributed to explain multiple ecosystem functions. Fungal saprobes community composition was especially important for supporting ecosystem multifunctionality (EMF), plant production, litter decomposition, and activity of soil phosphatase when compared with bacteria or other fungal functional groups and litter species richness. Moreover, leaf litter diversity and soil microbial diversity exerted previously undescribed and significantly interactive effects on EMF and multiple individual ecosystem functions, such as litter decomposition and plant production. Together, our work provides experimental evidence supporting the independent and interactive roles of litter and belowground soil biodiversity to maintain ecosystem functions and multiple services.

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KEYWORDS

ecosystem multifunctionality, fungal saprobes, litter decomposition, plant diversity, plant production, soil biodiversity

1 | INTRODUCTION

Plant and soil biodiversity are widely recognized to support multiple ecosystem functions from local (Bradford, 2014; Craven et al., 2016; Hautier et al., 2018; Jing et al., 2015; Tilman et al., 1996; Wagg et al., 2014) to global scale (Delgado-Baquerizo et al., 2020; Eisenhauer, 2022; Eldridge et al., 2023; Guerra et al., 2022; Maestre, Quero, et al., 2012). Plants and microbes can interact in many different ways from pathogenesis to symbiosis (Baldrian et al., 2023; Phillips et al., 2013; Tedersoo et al., 2020; Trivedi et al., 2020). However, one of the most fundamental ways in which plants and soil biodiversity interact across all ecosystems worldwide is through the process of litter decomposition (García-Palacios et al., 2021; Gessner et al., 2010; Semchenko et al., 2018), which regulates the entrance of carbon and plant nutrients into the soil with direct implications for supporting plant growth (Bardgett & van der Putten, 2014; Gessner et al., 2010). Litter decomposition is controlled by multiple biotic and abiotic variables (Bradford et al., 2016; Cornwell et al., 2010; Cotrufo et al., 2015; Swift et al., 1979), while we have limited experimental evidence on the unique contributions of plant and soil biodiversity (particularly fungal decomposers) and their interaction in supporting multiple ecosystem functions during litter decomposition (Averill et al., 2014; Phillips et al., 2013; Tedersoo et al., 2020). Understanding the complex interactions between litter and soil biodiversity is critical for predicting the consequences of plant and soil biodiversity loss on ecosystem functions (de Vries et al., 2013; Lange et al., 2015; Zhou et al., 2022).

Litter diversity is expected to promote soil microbial activity and function by increasing the diversity of resources for soil microbes as predicted by the hypothesis of “resource heterogeneity” (Gessner et al., 2010; Handa et al., 2014; Huys et al., 2022; Otsing et al., 2018; Vos et al., 2013). However, litter diversity per se cannot explain ecosystem functioning (Barantal et al., 2014). Put simply, without the decomposing role of the microbial community, litter would just accumulate on the soil being only subjected to abiotic decomposition (García-Palacios et al., 2013). More than 90% of global terrestrial plant production enters the soil as above- and belowground litter (García-Palacios et al., 2021; Gessner et al., 2010), which is then processed by soil organisms (Bardgett & van der Putten, 2014). Generally, the more diverse of microbial communities, the more tools the soil system has to derive the energy and resources from the decomposition of complex organic matter (Allison & Martiny, 2008; Bardgett & van der Putten, 2014; Jiao et al., 2022). Moreover, soil biodiversity involves not only species richness (number of taxa), but also community composition. Both these two components were previously reported to have important and independent role in driving organic matter decomposition and nutrient cycling (Delgado-Baquerizo, Reich, et al., 2017; Maestre, Castillo-Monroy, et al., 2012).

In some cases, microbial community composition was found to play an even more important role than richness in supporting ecosystem multifunctionality (EMF; Delgado-Baquerizo, Trivedi, et al., 2017; Li et al., 2021; Rivett & Bell, 2018). However, we lack quantitative evidence on the contribution of litter diversity and soil biodiversity (including species richness and community composition) in supporting multiple functions in littersphere (soil closely surrounded the litter), which hampers our understanding of the mechanisms behind this fundamental process between the above- and belowground worlds.

Here, we investigated the relative contribution of litter diversity [species richness, community composition (i.e., species combination) and chemical properties] and soil biodiversity (species richness and community composition) in explaining EMF. For this, we conducted a 4-month mesocosm experiment including combinations of litter from five vascular plants and lichen species (Tables S1 and S2) and a soil biodiversity gradient (dilution to extinction 10^0 to 10^{10}) using two different soils from Australia (Table S3). Understanding the nutrient recycling and biodiversity loss is critical for addressing the anthropogenic disturbance induced decline in EMF in Australia, one of the most arid regions in the world. We used *Microlaena stipoides* as our model plant to assess the consequence of the loss of above- and belowground biodiversity on plant production (above- and belowground plant biomass), leaf litter decomposition (mass loss), and soil organic matter decomposition in littersphere (soil respiration, soil enzyme activities and lignin and glucose degradation). We investigated: (1) whether leaf litter diversity and soil biodiversity have independent effects on plant above- and belowground production, litter decomposition, soil enzyme activities and soil lignin and glucose degradation, soil respiration and EMF; and (2) what predictors of the diversity within leaf litter or soil communities drive EMF and multiple individual functions.

2 | METHOD

2.1 | Experimental design

Soil samples from two semiarid ecosystems (Table S3) were sieved to <2 mm and divided in two portions: (1) soil for sterilization and (2) soil for microbial inoculum and experimental controls (non-sterilized original soils). The first portion was sterilized using a double dose of gamma radiation (50 kGy each) at ANSTO Life Sciences facilities. A dilution-to-extinction approach was used to prepare soil microcosms (Delgado-Baquerizo et al., 2016; Philippot et al., 2013). A parent inoculum suspension was prepared by mixing 25 g soil in 180 mL of sterilized phosphate buffer solution. The mixture was vortexed at high speed for 5 min. The sediment was then allowed to settle for 1 min. For each soil type, five dilutions from the supernatant were

used as the microbial inoculum to create a soil biodiversity gradient: undiluted (1; D0), 1/10 dilution (D1), 1/10³ dilution (D3), 1/10⁶ dilution (D6), and 1/10¹⁰ dilution (D10). The gradient of dilutions of the two soils were used to evaluate the influence of soil biodiversity on function. Three typical plant species (*Eucalyptus*, *Acacia*, and *Rytidosperma*) and two lichen species (*Diploschistes* and *Cladonia*) were used to create the leaf litter diversity gradient (1, 3, 5). A total of 120 microcosms with two soils with different litter diversity and soil biodiversity (5 soil biodiversity levels × 3 litter diversity levels × 4 replicates × 2 soil types) were prepared in pots (radius: 15 cm; height: 20 cm). Mesocosms were planted with a typical grass (*Microlaena stipoides*) from Australia. This grass is critical for the maintenance of livestock grazing in the country. Litter bags (4 g) were placed on the top of the soil at the beginning of the experiment covering the entire soil surface. The bottom side of the litter bag had a small mesh size (0.2 mm × 0.2 mm) to minimize the loss of fragments, and the upper side used a large mesh size (2 mm × 2 mm) to allow the mesofauna to freely access it. The amount of leaf litter in these bags aimed to represent real-field conditions in Australia. The moisture content in these microcosms was adjusted to 50% water holding capacity and maintained by adding sterile water when needed during an incubation period of 4 months. After incubation, an aliquot of soil was immediately frozen at -20°C and used for measuring microbial diversity (absolute abundance and community composition), and the rest stored at 4°C used for measuring soil properties and ecosystem functions.

2.2 | Testing microbial abundance and diversity in soil mesocosms

Quantitative PCR was used to quantify the absolute abundance of bacteria and fungi in soils as described in Delgado-Baquerizo et al. (2020). The diversity of soil bacterial and fungal community was measured using amplicon sequencing with the Illumina MiSeq platform (PE-300). Bacterial 16S rRNA gene and fungal ITS regions were sequenced using the 341F/805R and FITS7/ITS4 primer sets (Delgado-Baquerizo et al., 2020), respectively. The combinations of QIIME2 (Bolyen et al., 2019), UNOISE3 (Edgar, 2016) and USEARCH (Edgar, 2013) were used for bioinformatic processing. The high-quality sequences were clustered into zOTU (zero-radius OTU) with a 100% identity level. We calculated the richness of bacteria and fungi in each soil replicate from rarefied phylotypes tables (12,000 and 8600 sequences for bacteria and fungi, respectively). The fungal functional groups were identified using FungalTraits (Pöhlme et al., 2020) and the functional guilds with the “primary_lifestyle” were included in further statistical analysis.

2.3 | Ecosystem functions

Eleven ecosystem functions including plant production (above- and belowground plant biomass and plant height in the model

plant *Microlaena stipoides*), leaf litter decomposition (mass loss), and soil organic matter decomposition (soil lignin- and glucose-induced respiration, soil enzyme activities: phosphatase, betaglucosidase, N-acetylglucosaminidase, proteases and soil respiration) were measured. For determining plant aboveground and belowground biomass, all aboveground plant tissue and root tissue were sorted into target plant species, dried at 65°C for 48 h, and finally weighed. For calculating litter decomposition rate, the attached soil particles and in-growing roots were carefully removed from the litterbags, and the remaining litter was dried at 65°C to constant mass and weighed. In case of measuring soil respiration as well as lignin- and glucose-induced respiration (SIR), we used the MicroResp approach to measure absorbance at 570 nm after the 5 h incubation period at 20°C (Campbell et al., 2003). In this study, SIR glucose and lignin were determined as the difference of the soil respiration after adding glucose or lignin minus soil respiration (water). The enzyme activities were measured on 1 g of soil using fluorometry with 96-well microplates as described by Bell et al. (2013). Twelve replicate wells were set up for each sample and each standard concentration. The assay plate was incubated in the dark at 25°C for 3 h to mimic the average soil temperature (Trivedi et al., 2016).

2.4 | Ecosystem multifunctionality

EMF is a quantitative index to provide easily interpretable and straightforward evaluation of the ability of ecosystems to sustain multiple ecosystem functions simultaneously (Maestre, Quero, et al., 2012). In this study, we used the averaging multifunctionality index to represent EMF (Byrnes et al., 2014; Maestre, Quero, et al., 2012). Specifically, we first normalized (log-transform) and standardized each ecosystem function using the z-score transformation. The EMF index was then calculated by averaging the standardized ecosystem functions.

2.5 | Statistical analyses

General linear mixed models based on R packages “lme4” (Bates et al., 2014) and “lmerTest” (Kuznetsova et al., 2017) were used to test the effect of manipulated “litter diversity” and “soil microbial diversity” on ecosystem functions. The Akaike information criterion and Bayesian information criterion were estimated to assess the goodness of fit of the models. The conditional R_C^2 and marginal R_M^2 were used to represent the proportion of variance explained by “litter diversity” and “soil microbial diversity” without and with accounting for effects of “soil type.” We used variation partitioning modeling based on R packages “vegan” (Oksanen et al., 2013) to quantify the relative importance of three groups of factors as predictors of individual ecosystem function and multifunctionality. These three groups of predictors included: (1) soil biodiversity, (2) litter diversity and quality, and (3) soil type.

Soil biodiversity includes phylotypes richness and community composition (i.e., summarized using non-metric multidimensional scaling, Bray–Curtis distance) of bacteria, fungal saprobes, plant pathogen, mycorrhizal fungi, and overall fungal community. Litter diversity and quality include litter species richness, litter community composition, and community-weighted means of litter quality. The variation partitioning model was performed based on “vegan” package. Before this analysis, we used the “forward.sel” procedure to avoid redundancy and multicollinearity in variation partitioning analyses and included only significant predictors in the final variation partitioning model. Additionally, we used multiple regression models to assess the joint effects of soil biodiversity, litter diversity and quality, soil type, as well as the relative importance of individual variables on EMF and individual ecosystem function. The predictors included in this model were consistent with those in variation partitioning. All predictors and response variables were standardized before analyses using the z-score to interpret parameter estimates on a comparable scale. We used the R package “relaimpo” (Grömping, 2006) to estimate parameter coefficients for each predictor. Random forest modeling, a machine-learning algorithm that extends standard classification and regression tree methods, was applied to select the most important biomarkers of EMF and multiple individual ecosystem functions. The relative importance of biomarker was determined over 100 iterations. The optimal number of marker taxa was determined using 10-fold cross-validation implemented with the “rfcv” function of R package “randomForest” with five repeats (Breiman, 2001).

3 | RESULTS

3.1 | Microbial diversity as a function of experimental dilutions

We found that dilution induced significant but limited loss of richness in bacterial and fungal saprobes (Figure 1a–d). Specifically, dilution had significant effect on richness of bacterial community ($p = .0001$) and fungal saprobes ($p = .014$) but no effect on richness of overall fungal community and plant pathogens ($p > .05$). Dilution also caused a significant shift in microbial community composition (Figure 1e–h). Thus, dilution remarkably altered the community composition for most soil communities including bacterial community ($p = 1.646e-8$), fungal community ($p = 4.734e-8$), and fungal saprobes ($p = 2.297e-6$).

3.2 | Relationship between microbial diversity and function

Our results showed that experimental loss of soil microbial diversity significantly reduced most ecosystem functions including leaf litter decomposition, belowground plant production, respiration, and activity of betaglucosidase (Figure 2). However, the relationship between soil biodiversity and some other functions such as NAG was soil-type dependent (Figure S2). Most individual ecosystem functions were more related to soil biodiversity than to litter species richness. However, in the case of leaf litter decomposition, litter diversity was more important (Figure 2).

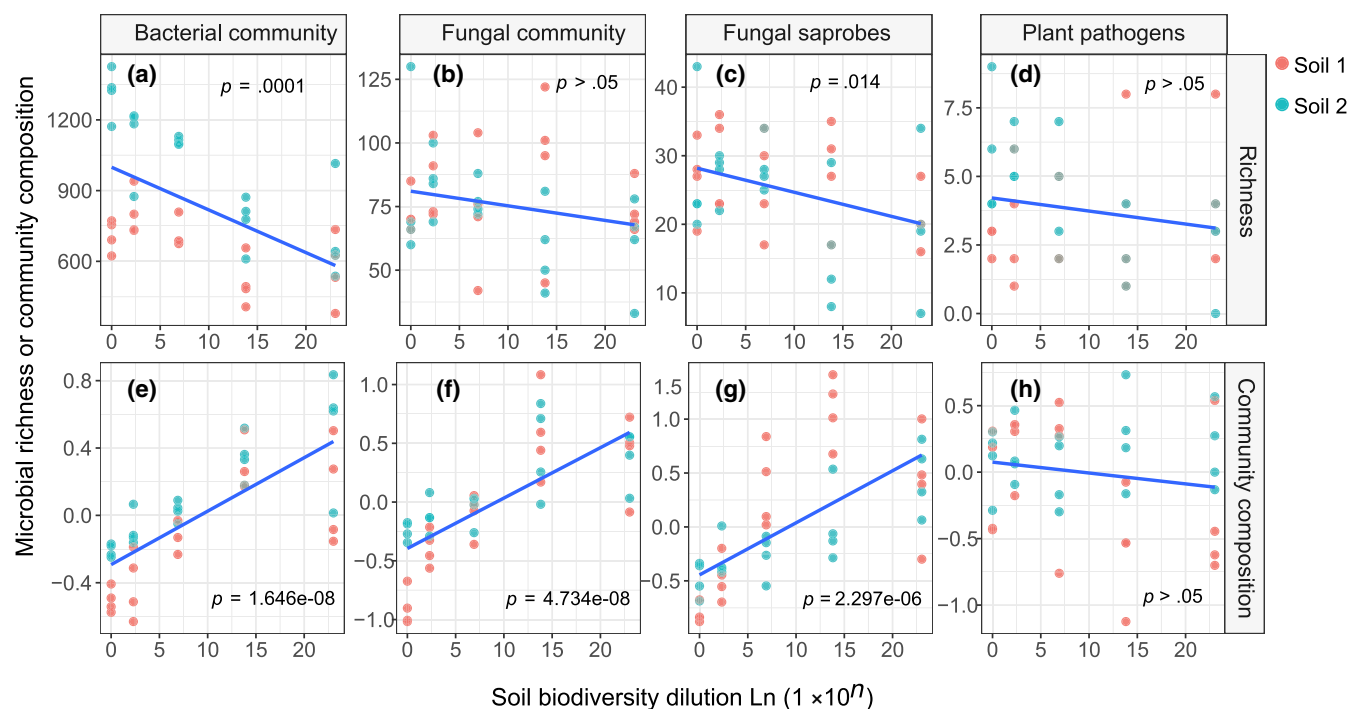


FIGURE 1 Relationships between microbial diversity and dilution treatment in two soil types. The regression line was reported only for significant relationships. (a–d) and (e–h) represent microbial richness and community composition, respectively. Significance levels are * $p < .05$, ** $p < .01$, *** $p < .001$.

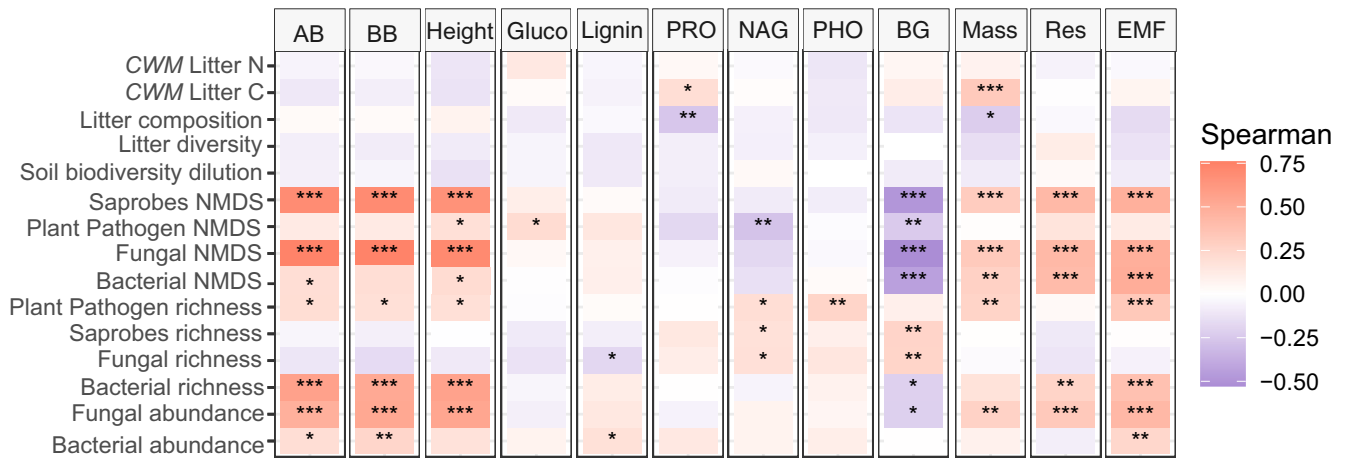


FIGURE 2 Spearman correlations between soil biodiversity, litter diversity and multiple ecosystem functions and multifunctionality. *, **, and *** indicate a significant relationship at $p < .05$, $p < .01$, and $p < .001$ level, respectively. Red and purple color indicate a positive and negative relationship. AB, BB, Height, Gluco, Lignin, PRO, NAG, PHO, BG, Mass, Res, and EMF represent plant aboveground biomass, plant belowground biomass, plant height, glucose in litterosphere soil, lignin in litterosphere, activity of soil proteases in litterosphere, activity of soil N-acetylglucosaminidase in litterosphere, activity of soil phosphatase in litterosphere, activity of soil betaglucosidase in litterosphere, mass loss of litter, soil respiration in litterosphere, and multifunctionality, respectively.

3.3 | Both litter diversity and soil microbial diversity contribute to explaining function

We then used variation partition model to quantify the relative importance of specific variables of leaf litter diversity (litter richness and community composition) and quality (e.g., C/N ratio), soil microbial diversity (richness and community composition), and soil type in predicting individual ecosystem functions and EMF (Figure 3). The results suggest that above- and belowground biodiversity both have unique roles in supporting specific ecosystem functions (Figure 3), and therefore jointly drive EMF. All measured variables could explain 3%–74.8% variation in multiple individual functions and EMF, with soil biodiversity was more important in predicting above-, belowground plant biomass, and EMF (Figure 3b,i,j). While leaf litter diversity was more associated with litter decomposition. Moreover, the most important predictor for specific functions was quite different. For example, the primary predictor for plant productions was fungal community composition (Table S5; $\text{adj}R^2 = .565-.568$), but that for soil respiration was bacterial community composition (Table S5; $\text{adj}R^2 = .150$). Nevertheless, leaf litter diversity had equal importance in driving litter decomposition as did by soil biodiversity.

We further grouped these 11 individual ecosystem functions into three major functions (litter decomposition, soil enzyme activity, and plant production), and used multiple regression models to assess the relationships between litter and soil microbial diversity and EMF (Figure 4). We found a function-dependent pattern among these relationships. For example, bacterial and fungal diversity had positive relationships with EMF (Figure 4a), leaf litter decomposition (Figure 4b), and plant production (Figure 4d), but were negatively related to enzyme activity (Figure 4c). Specifically, litter community composition (proportion of different litter species) showed consistently negative associations with all ecosystem functions

(Figure 4a–d). Moreover, all considered predictors could explain 30.1%–76.8% variation in EMF as well as three major groups of ecosystem functions (Figure 4). Among those important predictors, soil microbial diversity had important impacts on key functions but the contribution of fungal decomposer was consistently higher. All these findings suggest that both litter and soil biodiversity are important to support different aspects of soil function.

4 | DISCUSSION

Our study provides solid evidence of the unique contribution and critical interactions between litter diversity and soil biodiversity, specially decomposers, in supporting multiple ecosystem functions. This knowledge is important to better understand how losses in plant and soil biodiversity will impair ecosystem function in a world subjected to increasing species extinction.

Our findings confirmed that litter mass loss was more related to leaf litter composition and chemical properties than to litter richness during litter decomposition (Figure 2; Figures S1 and S2; Table S5). Although the debate on which of “quantity diversity” versus “quality diversity” exhibit higher importance in explaining litter decomposition is elusive (Cotrufo et al., 2015; Gessner et al., 2010; Kou et al., 2020), our result is in line with some previous studies which showed that litter community composition and functional traits play a more important role than litter species richness in driving litter decomposition processes (Meier & Bowman, 2008; Otsing et al., 2018; Wardle et al., 2006). One of the potential mechanisms is that litter quality has greater importance in regulating the nutrients-transfer among different litter species (e.g., N-transfer from the litter of nitrogen-fixing plants to that of rapidly decomposing plants; Handa et al., 2014), to meet the metabolic requirement

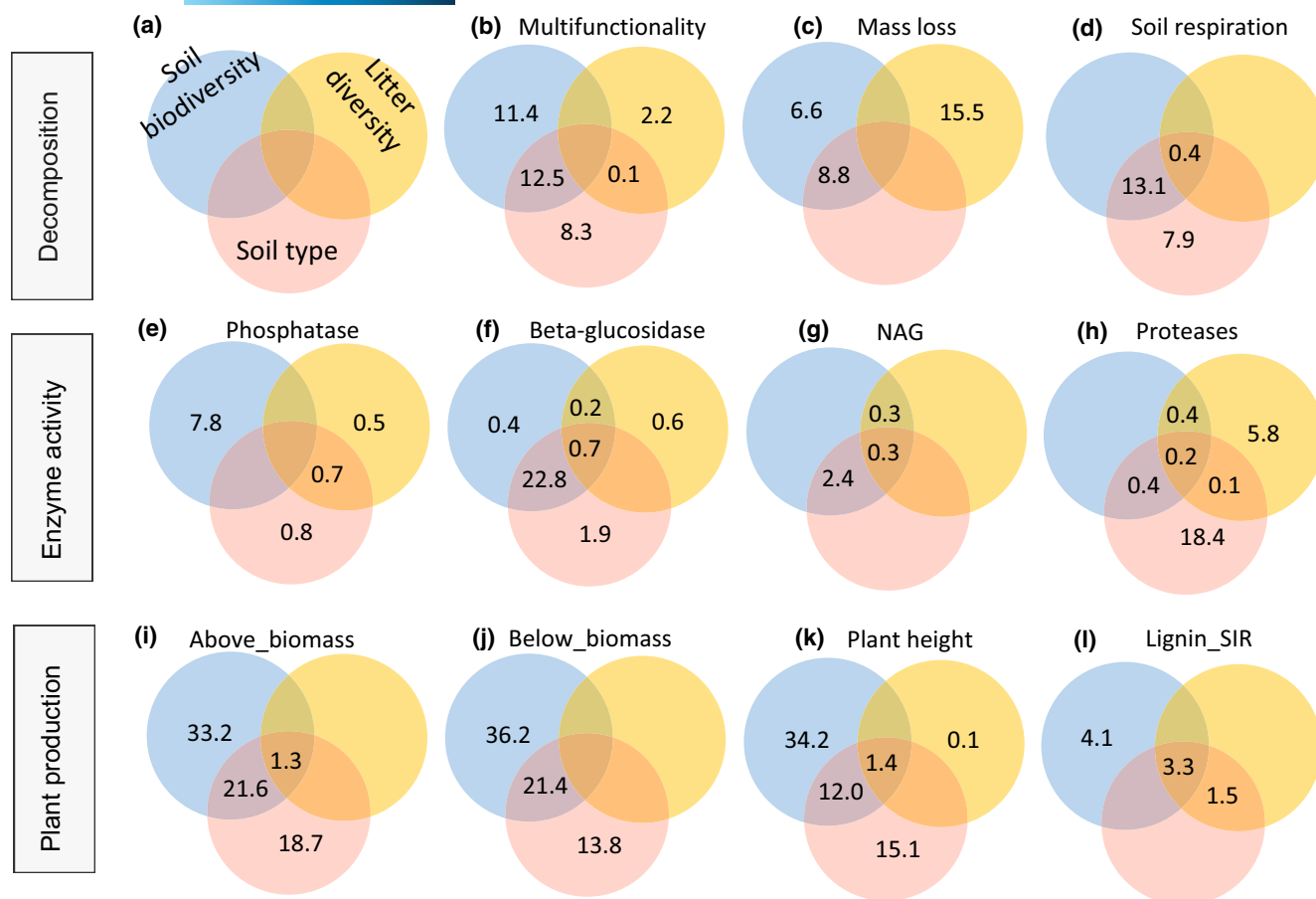


FIGURE 3 Venn diagrams of variance of ecosystem functions explained (%) by litter diversity (species richness, community composition, and community-weighted means of litter quality), soil biodiversity (microbial abundance, richness, and community composition), and soil type. (a-d), (e-h) and (i-l) represent decomposition, enzyme activity and plant production, respectively.

(i.e., stoichiometric ratio) of soil communities in litter decomposition (Delgado-Baquerizo, Reich, et al., 2017; Gessner et al., 2010). Also, our results of the closely relationships between leaf litter quality and soil protease activity in littersphere (Table S5) may directly support this hypothesis as soil protease is an important enzyme involved in C degradation (Trivedi et al., 2016). However, the biodiversity effects on litter decomposition were dependent not only on plant species but also on multiple trophic levels of soil communities (Handa et al., 2014). For example, litter diversity was also found to be a major driver regulating decomposition-associated functions via shaping fungal decomposer communities (Gessner et al., 2010; Kou et al., 2020; Xiao et al., 2020). This also supports our findings that fungal community particularly fungal decomposer (wood saprobes and soil saprobes) showed positive relationships with EMF and most individual functions including plant productions, leaf litter decomposition, nutrient cycling, and extracellular enzyme activities (Figure 2).

Nevertheless, our findings also showed that specific variables within litter diversity or soil microbial diversity could both predict unique variation in multifunctionality, with litter species composition and fungal community composition being the most important predictor of EMF (Figures 3 and 4; Table S5). In other words, greater soil biodiversity, especially in terms of fungal decomposers,

supported higher EMF, but this effect was independent of litter diversity. This pattern was also general across some specific ecosystem functions such as litter decomposition and betaglucosidase, indicating that plant and soil biodiversity can jointly interact to regulate multiple ecosystem functions (Bardgett & van der Putten, 2014; Yang et al., 2021). Moreover, we found divergent but significant interactions between litter diversity and soil microbial diversity on plant production, litter decomposition, and EMF (Figure 4a,b,d), but limited effect on individual function (Table S4). This result may also imply that different ecosystem functions were maintained by different composition of microbial community as supported by our random forest results (Figure S4) and previous studies (Delgado-Baquerizo et al., 2020; Eldridge et al., 2023).

In contrast, soil microbial diversity played a more important role in driving multifunctionality, phosphatase activity, above- and belowground plant production, and plant height (Figures 2-4; Table S5). Specifically, overall fungal community composition and saprobe community composition predicted much more variation ($\text{adj}R^2 = 21.6\% - 57.2\%$, Figure 3; Table S5) in these ecosystem functions than other fungal functional groups (e.g., EcM or plant pathogens) and bacterial diversity. Given that fungal community generally exhibit diverse functional guilds and life strategies (Bardgett & van

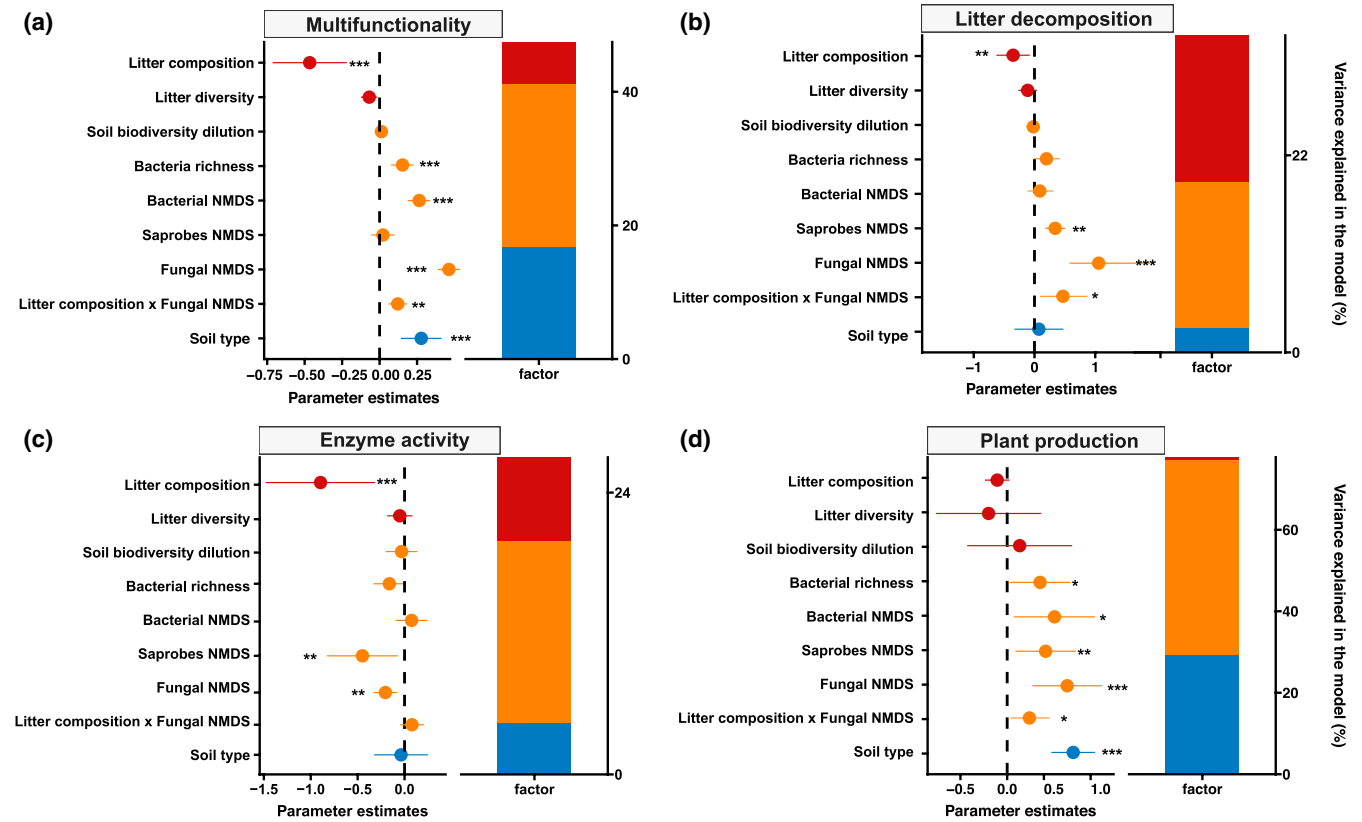


FIGURE 4 Drivers of ecosystem multifunctionality (a), litter decomposition (b), enzyme activity (c), and plant production (d). Multiple ranking regression reveals the relative importance of the most important predictors of ecosystem functions. The standardized regression coefficients of the models are shown for each predictor with their associated 95% confidence intervals. * $p < .05$, ** $p < .01$, *** $p < .001$. Bar graphs show the relative importance of each group of predictors, expressed as the percentage of explained variance. Microbial community composition was summarized using a non-metric multidimensional scaling (NMDS).

der Putten, 2014; Tedersoo et al., 2020), it is not surprising that higher fungal diversity could support higher ecosystem functions, particularly for plant production, than other organisms (Delgado-Baquerizo, Reich, et al., 2017; Liu et al., 2020; Yang et al., 2018). For instance, the fungal decomposer community plays a key role in the breakdown of plant litter (Gessner et al., 2010), providing a continuous source of nutrients to sustain plant production (Liu et al., 2022). These results also support previous observations and hypothesis that greater diversity of soil organisms can enhance litter break down, reduce nutrient leaching losses, and maintain resource turnover between above- and belowground communities (Delgado-Baquerizo et al., 2020).

Beyond litter diversity and soil microbial diversity, soil type could also predict a large variation in multifunctionality ($\text{adj}R^2 = 8.3\%$), soil respiration ($\text{adj}R^2 = 7.9\%$), PRO ($\text{adj}R^2 = 18.4\%$), and plant production ($\text{adj}R^2 = 13.8\% - 18.7\%$). This finding was in line with the result of the linear mixed modeling, which indicated that soil type was an important factor driving multiple individual ecosystem functions (Table S4). Our result also showed that above- and belowground biodiversity–ecosystem function relationships related to soil conditions (Figure S3). This may be associated with the fact that different soil habitats foster unique microbial communities according to their niche preference and

legacies (Delgado-Baquerizo et al., 2018), and thus support specific functions.

In conclusion, we showed that litter diversity and soil microbial diversity have significant unique and interactive effects on EMF and multiple individual ecosystem functions related to litter decomposition and plant production. Specifically, fungal decomposers play a more important role than other fungal functional groups (e.g., plant fungal pathogen) and bacterial community in supporting EMF. Although soil biodiversity loss and simplification of microbial community composition impaired multiple ecosystem functions, the relationships between biodiversity and EMF were greatly dependent on soil resource availability and community composition. These findings highlight the importance of considering the unique and interactive contributions from both above- and belowground biodiversity in sustaining multiple ecosystem functions and services.

AUTHOR CONTRIBUTIONS

Manuel Delgado-Baquerizo designed the research; Manuel Delgado-Baquerizo, Chanda Trivedi, Brajesh K. Singh, Juntao Wang, and Raúl Ochoa-Hueso set up the experiment and conducted laboratory work; Shengen Liu, Catarina S. C. Martins, Brajesh K. Singh, Juntao Wang, and Guiyao Zhou analyzed the data; Shengen Liu and Manuel

Delgado-Baquerizo wrote the first draft, and the rest of co-authors helped to improve the final draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.23732391>.

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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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