Consistent effects of biodiversity loss on multifunctionality across contrasting ecosystems

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Understanding how loss of biodiversity affects ecosystem functioning, and thus the delivery of ecosystem goods and services, has become increasingly necessary in a changing world. Considerable recent attention has focused on predicting how biodiversity loss simultaneously impacts multiple ecosystem functions (i.e., ecosystem multifunctionality), but how these effects vary across ecosystems remains unclear. Here, we used two 19-year plant diversity manipulation experiments, each established across a strong environmental

gradient, to show that although the effects of plant and associated fungal diversity loss on individual functions frequently differed among ecosystems, the consequences of biodiversity loss for multifunctionality were relatively invariant. However, the context-dependency of biodiversity effects also worked in opposing directions for different individual functions, meaning that similar multifunctionality values across contrasting ecosystems could potentially mask important differences in the effects of biodiversity on functioning among ecosystems. Our findings **highlight that understanding of the relative contribution of species or functional groups to individual ecosystem functions among contrasting ecosystems and their interactions (i.e., complementarity *versus* competition) is critical for guiding management efforts aimed at maintaining ecosystem multifunctionality and the delivery of multiple ecosystem services.**

Species that co-occur in natural ecosystems perform many individual functions, which in turn underpin the multiple goods and services that ecosystems provide to humans[1-3]. Because species vary in their contributions to different functions[4], it has been increasingly recognized that biodiversity may be important for the simultaneous maintenance of multiple ecosystem functions (hereafter 'multifunctionality')[5]. The concept of multifunctionality stems from the idea that the effects of biodiversity were stronger when multiple functions were considered compared to single functions. However, recent studies have demonstrated that ecosystem multifunctionality does not depend of the number of functions considered[6,7], suggesting that biodiversity can only affect multifunctionality *via* non-additive effects on individual ecosystem functions (i.e., through numerical values in mixtures being unequal to the sum of values based on the component monocultures). Although the concept of multifunctionality may be useful for assessing multiple aspects of functioning associated with biodiversity loss[8,9], our understanding about the underlying mechanisms remains limited.

Complementarity effects (i.e., synergistic effects of biodiversity through resource partitioning or facilitation) have been hypothesized to explain the positive effects of increasing species richness on ecosystem functions[10], and the greater ability of plant communities with higher diversity to capture resources and convert them into new biomass[11,12]. Most available empirical evidence of these mechanisms is derived either from short-term studies[13,14] (≤ 5 years) or from artificial biodiversity manipulation experiments in which plant diversity has been varied through random selection from species pools[15,16]. Although such studies have greatly improved our theoretical understanding of the role of biodiversity in ecosystem functioning, it is less clear as to how generalizable such findings are to natural ecosystems[17,18]. In particular, there is a dearth of studies on how long term manipulations of biodiversity in natural ecosystems impact on ecosystem multifunctionality and on the extent to which these effects vary across contrasting ecosystems.

The contribution of plant diversity to ecosystem functions may differ among ecosystems or along environmental gradients due to changes in ecosystem abiotic properties (notably soil fertility and water availability), plant functional traits, and shifts in species identity and relative abundance[5,19-22]. For instance, the loss of plant species with resource-acquisitive traits exerts greater control on plant biomass and nutrient recycling in productive ecosystems than does the loss of resource-conservative species[23]. Yet, despite growing evidence that the effects of biodiversity loss on single functions can vary according to environmental context[24,25], most biodiversity-multifunctionality studies have been conducted within a single context (but see[5,22]). While some studies have found stronger effects of biodiversity loss on ecosystem multifunctionality than have others[26-28], our understanding about whether, how and why these effects vary across contrasting ecosystems or along environmental gradients remains limited.

Further, recent studies have also shown that biodiversity at multiple trophic levels can have important impacts on multiple ecosystem functions[6,29], and that focusing only on plants may greatly underestimate the impact of biodiversity on multifunctionality in natural ecosystems[30]. For instance, a substantial proportion of plant carbon (C) is allocated belowground to roots and associated fungi, thereby influencing belowground organic matter (OM) turnover, C sequestration and nutrient dynamics[31-33]. Despite increasing evidence that root-associated fungi are major drivers of terrestrial biogeochemical cycling[32,33], it remains little understood whether and how simultaneous changes in the diversity of autotrophs (*i.e.*, plants) and decomposers (*i.e.*, soil fungi) may impact multifunctionality, and the extent to which these effects can vary across contrasting environmental contexts. Such an understanding is needed to better predict how multifunctionality in natural ecosystems may respond to changes in diversity across trophic levels caused by global change drivers and other environmental factors.

To evaluate the effect of biodiversity loss on ecosystem multifunctionality at two different trophic levels (plants and soil fungi) among contrasting ecosystems, we utilized an ongoing pair of experiments (each established in 1996) that had been running for 19 years at the time of measurement. The experiments include removal of plant species and functional groups to represent biodiversity loss on each of 30 forested islands that represent highly contrasting ecosystems in northern Sweden (M&M). The islands were formed after the most recent glaciation and are subjected to the same extrinsic factors other than disturbance history caused by wildfire[34]. Larger islands burn more frequently because they have a larger area to intercept lightning strikes[35]; several large islands have burned in the past century, whereas some small islands have not burned in the past 5000 years. The 30 islands include three classes of each of ten islands (hereafter 'island size class'): 10 'large' islands (>1.0 ha; on average, mean time since fire 585 years), 10 medium islands (0.1 to 1.0 ha, 2180 years), and 10 small islands (<0.1 ha, 3250 years). Previous work in this study system has shown that as the time since fire increases, net primary productivity, soil fertility and nutrient recycling decrease[34], leading to humus layers that can exceed 1 m in depth on the smallest islands due to accumulation of organic matter from plant and fungal residues[31] (Supplementary Table 1).

The removal experiments established on these islands are the longest running biodiversity manipulation experiments in existence that include repetitions of the same treatments across contrasting ecosystems. The first of these experiments consists of full factorial removals of three dominant ericaceous dwarf shrub species (*Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Empetrum hermaphroditum*) on each of the 30 islands. The other consists of full factorial removals of three plant functional groups (tree roots, mosses and dwarf shrubs) on each island. Both experiments in combination yield a total of 420 plots[23] (Supplementary Fig. 1). We used these experiments to determine effects of simultaneous changes in the diversity of plants and associated fungal communities on 15 separate individual ecosystem functions and properties (used as proxy of functions) that underpin several supporting, provisioning and regulating ecosystem services[1] (Supplementary Table 2). Consistent with previous studies[6,26-30,36], we consider individual 'functions' to consist of each of the physiological, geochemical and biological variables that provide information about the cycling of elements, energy flow and process rates in the ecosystem. The functions selected were related to nutrient cycling (nitrogen (N) and phosphorus (P) stocks, availability and flux), C cycling (C stock, organic matter quality and decomposition), plant productivity (dwarf shrub, moss and root biomass) and soil functioning (activity of and biomass of soil organisms), with higher levels indicating a greater ecosystem functioning. Higher levels of these functions have been linked to greater delivery of ecosystem services upon which humans depend[1-3].

From these experiments we determined how plant species and functional group removals (*i.e.*, plant diversity loss) on each island influenced the 15 selected functions and assessed their relative contribution to multifunctionality[27,28] across ecosystems. We also employed threshold approaches which allow testing of whether diverse plant communities are more likely to support multiple functions at high levels[8,26], and tested whether ecosystem multifunctionality depended of the number of functions considered[7]. We then characterized the effects of plant removals on soil fungal community and whether fungal diversity could in turn explain variation in multifunctionality beyond what could be explained by the loss of plant diversity. From this data set we were able to assess the extent to which plant and fungal diversity effects on both individual ecosystem functions and multifunctionality varied among contrasting ecosystems.

Results and discussion

Importance of environmental context for the effects of plant diversity loss on ecosystem functioning. Analyses of the 15 ecosystem functions indicated that the majority (but not all) of them were negatively affected by species removals. For instance, shrub biomass and the activity of soil organisms were systematically lower when the number of species was decreased (Fig. 1a). Yet, our analyses indicate that not only did the loss of plant species affect the individual functions, but that these effects often varied across island size classes (Supplementary Table 3). In particular, the relative contributions of *Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Empetrum hermaphroditum* to average ecosystem multifunctionality (standardized value of all functions) were higher in large, medium and small islands, respectively (Fig. 1c). Because *V.*

myrtillus is a fast growing species with acquisitive traits and *E. hermaphroditum* is a slow growing species with conservative traits (with *V. vitis-idaea* being intermediate)[50], this result shows that acquisitive species have their highest impacts on multifunctionality in productive ecosystems and that conservative species have their greatest impacts in less productive systems. This supports the prediction of the 'species sorting theory' stipulating that if different ecosystems vary in resources supply, then the identity of the species that maximizes ecosystem functions will vary for each ecosystem[37]. Further, this highlights that the loss of any particular species can have important consequences on overall functioning in one ecosystem, and minimal impacts in another.

The importance of the environmental context was even more apparent for the loss of plant functional groups, as revealed by many significant interactive effects between island size and particular removal treatments for several of these functions (Supplementary Table 3). For instance, tree root removals had negative, neutral, or positive effects on soil exchangeable P in large, medium and small islands, respectively (Supplementary Table 4). This indicates that changes in the composition of plant communities - both within and between functional groups - among island size classes do alter not only biogeochemical cycles and net primary productivity[34], but also the relative contribution of functional groups to individual ecosystem functions (Fig. 1b). This highlights that understanding of the relative contribution of species and functional groups to single functions among contrasting ecosystems is critical for guiding management efforts aimed at maintaining ecosystem multifunctionality. Further, our results reinforce that although many studies have provided compelling evidence for positive effects of biodiversity on ecosystem productivity, functioning and stability, this is not found for all studies and for all functions[9,38].

Consistent effects of biodiversity loss on multifunctionality across contrasting ecosystems. In contrast with the results on individual ecosystem functions, we found that negative effects of plant removals on ecosystem multifunctionality were largely independent of island size class (Fig. 2, Supplementary Table 5). This is because even if the relative contributions of species or functional groups varied across island size classes (Fig. 1c, d), the non-additive effects resulting from species interactions (e.g., through complementarity and/or competition) were relatively similar for many single functions across contrasting ecosystems. However, the relative consistency of multifunctionality values across contrasting ecosystems is also due to the context-dependent nature of biodiversity loss that can work in opposing directions for different functions. For instance, while soil mineral N and exchangeable N were unaffected by the number of plant functional groups present for large islands, these two functions increased and decreased respectively with increasing functional group richness for medium islands (Fig. 1b). Further, the maximum level reached by each function may vary with island size because of a different overall functioning (*e.g.*, fast nutrient cycling in large islands *vs*. conservative nutrient cycling in small islands[34]). Consequently, we caution that similar multifunctionality values across contrasting ecosystems may mask large differences in their functioning. This further reinforces the conclusion that explicit recognition of the relationships between plant diversity and individual ecosystem functions is necessary to more completely understand the influence of biodiversity on ecosystem multifunctionality[28].

Do the effects of biodiversity loss on multifunctionality depend of the number functions considered? The lack of an interactive effect of island size class and plant diversity on average multifunctionality occurred consistently even when considering different levels of ecosystem multifunctionality (see Supplementary Figs. 3-5 for more details), or when the number of functions considered to calculate multifunctionality was altered (Supplementary Fig. 6). In line with theoretical predictions of Gamfeldt and Roger[7], our long-term study provides experimental evidence that ecosystem multifunctionality does not depend on the number of functions considered. This is because even if slopes vary depending on which functions are included in any one combination of functions, the average slope stays constant across the full range of number of functions[7], and this across highly contrasting ecosystems. This indicates that any effect of biodiversity on multifunctionality results from non-additive effects of coexisting species or functional groups on individual ecosystem functions, and further underscores that a better understanding of species interactions is crucial to predict the role of biodiversity loss on ecosystem multifunctionality across contrasting ecosystems.

Complementarity *versus* competition drive the effects of biodiversity on multifunctionality. The net positive effects on multifunctionality when two or more species are present in the community suggest that complementarity is an important mechanism by which plant diversity enhances overall ecosystem functioning (Figs. 1, 2). This indicates that even if species that are best suited to the local conditions may vary among ecosystems[37], multiple coexisting species often results in higher individual ecosystem functions than a single species alone. Such effects can arise from niche differences (i.e., occupation of distinct niches at the local scale) among species or functional groups in their responses to limiting factors such as soil resource availability (e.g., by sharing different resources). Niche difference is often considered to be the main driver of long-term species coexistence by preventing competitive exclusion[39,40]. Here, the results of our long-term biodiversity experiment highlight that niche difference is also an important mechanism controlling the capacity of plant communities at maximizing multiple functions at high levels across contrasting ecosystems. This is likely because complementarity effects on some individual functions have the potential to drive changes in other functions (Fig. 1a, b). For instance, a better exploitation of soil nutrients resulting from higher resource partitioning and/or differences in resource use can drive increases in plant biomass, with further positive consequences on the activity of soil organisms.

However, our data also highlight that some species in combination can reduce multifunctionality, perhaps as a consequence of competitive interactions. Contrary to the expectations that competitive interactions among species should be higher in large islands because traits of coexisting species converge in more productive environments[40,41], we observed that decreases in multifunctionality depended on the interaction between species resource-use strategy and island size. For instance, the presence of *Vaccinium myrtillus* (a resource-acquisitive species) had negative effects in 3 species-mixtures on unproductive small islands, while *Empetrum hermaphroditum* (a resource-conservative species) had negative effects on medium islands which are more productive (Fig. 1c). This indicates that the balance between complementarity and competition is strongly impacted by resource availability. The negative effects of competitive interactions on multifunctionality were dependent nonetheless of different functions in contrasting ecosystems (Fig. 1a): N availability and soil P per area were the main functions negatively affected by the presence of *Empetrum hermaphroditum* and *Vaccinium myrtillus* in medium and small islands, respectively. This reinforces the conclusion that the relative contribution of species or functional groups to individual ecosystem functions is strongly context-dependent, but more importantly, emphasizes that both positive and negative non-additive effects on individual ecosystem functions may constrain the effects of biodiversity on ecosystem multifunctionality.

Biodiversity at different trophic levels is required to maintain ecosystem multifunctionality. Biodiversity loss at one trophic level (horizontal diversity) can have bottom-up or cascade impacts on the diversity and functioning of organisms at other trophic levels[42,43]. Because many ecosystem functions are driven by a multitude of complex interactions among organisms (e.g., plant and soil organisms), understanding how biodiversity affects ecosystem functioning requires assessing the role of diversity across trophic levels (vertical diversity)[44]. In particular, because root-associated fungi and decomposers are major drivers of terrestrial biogeochemical cycling in our study system[31,32], it is likely that ecosystem multifunctionality depends on simultaneous shifts in both plant and fungal communities. Here, our results reveal that fungal communities were affected by plant species and functional group removal (Supplementary Fig. 7 and Table 5) and particularly by shrub removal for all island size classes (Fig. 3a). According to a dispersion model, the number of fungal species negatively impacted by the removal of shrubs was three times higher than the number of fungal species positively affected (Fig. 3b); most of those that were impaired were ericoid, root-associated and/or saprotrophic fungi (Supplementary Table 6). This resulted in a significant decrease in fungal biomass and diversity (Supplementary Table 7 and Supplementary Fig. 8), especially on small (late successional) islands (Fig 3c, Supplementary Fig. 9).

We found that ecosystem multifunctionality was positively correlated with soil fungal diversity independently of island size class (Fig. 4), even when controlling for the variation in plant richness and environmental factors (Supplementary Fig. 11 and Supplementary Tables 8-9). Although fungal species and their contribution to ecosystem functioning differ between large and small islands[31,32], this highlights that diverse fungal communities are more likely to support high levels of functioning for multiple functions. These results confirm that microbial diversity is an important contributor to ecosystem multifunctionality[6,29,45] and reveal that not only do these effects occur over and above those exerted by plant diversity, but also that they are consistent across different island size classes (see Supplementary Figs. 11-13 for more details on multifunctionality at different thresholds). This may be because the context-dependent nature of fungal biodiversity loss can work in opposing directions for different functions, as we found for plant diversity. Further, this relative consistency could have arisen through complementarity effects being overall relatively similar among island size classes for many individual functions when fungal diversity increased due to comparable roles across classes of niche differences[46] and/or facilitative interactions[47] among fungal taxa that promote decomposition and soil nutrient acquisition processes, and other functions linked to these processes. Further, the effects of fungal diversity were still present when decreasing the number of functions considered to calculate multifunctionality (Supplementary Fig. 14). This confirms our previous observations with primary producers (Supplementary Table 6), and reinforces the conclusion that multifunctionality does not depend on the number of functions considered[7].

In line with the multiple studies showing that biodiversity loss usually impairs ecosystem functions[3,11,48], variance partitioning analysis of our data revealed that plant and fungal diversity both have a significant role in influencing some individual functions (Fig. 5). These effects were often affected by environmental context (see also Supplementary Table 3), supporting the idea that the loss of any particular species or functional groups can have important consequences on ecosystem functions in one ecosystem while only have weak effects in another. However, although fungal and especially plant diversity are important drivers of multifunctionality (plant diversity had a more important effect on multifunctionality than it does on all but one of the individual functions), any variation in this effect among island size classes (*i.e.*, interactive effects of island size class and removal treatments) was negligible (Fig. 5). The relative consistency of the effect of biodiversity loss on multifunctionality across ecosystems for both plants and fungi highlights that regardless of ecosystem type, the delivery of a broad range of functions requires the maintenance of species diversity at different trophic levels[6,29,30,45]. This further emphasizes that although the contribution of different species or functional groups to individual functions strongly varies across the island chronosequence, preserving biodiversity across highly contrasting ecosystems is indispensable to sustain ecosystem multifunctionality.

Conclusion

Synthetic biodiversity manipulation experiments and observational studies in natural ecosystems have both substantially advanced our understanding of biodiversity-ecosystem functioning relationships, but they each have different strengths and weaknesses[49]. Here we use a long-running ‘removal experiment’, which captures elements of both approaches through experimental manipulation of multiple natural ecosystems, to shed new light about how biodiversity may affect ecosystem multifunctionality in contrasting environments. It highlights that although effects of biodiversity loss on individual functions can vary with ecosystem type because of differences in soil fertility, productivity or biological communities[23-25], the effects of biodiversity loss for both autotrophs (*i.e.*, plants) and heterotrophs (*i.e.*, soil fungi) on ecosystem multifunctionality are relatively invariant among contrasting ecosystems. From a management perspective, these results emphasize the importance of conserving biodiversity for maintaining multifunctionality for both producer and consumer trophic levels, and in highly contrasting ecosystems that vary greatly in both abiotic and biotic properties. Further, ecosystems with greater species or functional groups richness nearly always had higher ecosystem multifunctionality, reinforcing the view that preserving biodiversity is likely to be consistently important for maintaining ecosystem functioning across very different natural ecosystems[50]. This also underscores the necessity for biodiversity conservation as a major policy priority for maintaining the delivery of ecosystem goods and services essential for human well-being across contrasting ecosystems.

Methods

Experimental design. The study was conducted on a group of forested islands that collectively represent a chronosequence situated in the two adjacent Swedish lakes, Lake Hornavan and Lake Uddjaure (65°55′ N to 66°09′ N; 17°43′ E to 17°55′ E) in northern Sweden[35,51]. Island archipelagos are ideal model systems for understanding the effects of biodiversity loss, because each island operates as an independent replicate ecosystem, thus allowing us to address questions at ecologically meaningful spatial scales. All islands were formed from unconsolidated granite boulders deposited by glacial eskers following the retreat of land ice about 9000 year ago. The mean annual precipitation is 750 mm and the monthly mean temperature varies between -14°C in January to 13°C in July. The only major extrinsic factor that differs among islands is the history of lightning ignited wildfire, with larger islands having burned more frequently than smaller islands because of their larger area to intercept lightning. As such, some large islands have burned in the last 60 years, while some small islands have not burned for the last 5000 years. For this study, we considered 30 islands which include ten each of three size classes known to differ markedly in successional age[35,51]: 10 large islands (>1.0 ha, mean time since fire 585 years), 10 medium islands (0.1 to 1.0 ha, 2180 years), and 10 small islands (<0.1 ha, 3250 years)[31,32] (Fig. S1).

Vegetation on the islands undergoes a distinct succession with increasing time since fire[34]. The three tree species present in the system are *Pinus sylvestris*, *Betula pubescens* and *Picea abies*, which have their greatest biomass on large, medium and small islands, respectively. The understorey shrub layer consists of the three dwarf shrub species *Vaccinium myrtillus, Vaccinium vitis-idaea* and *Empetrum hermaphroditum*, which have their greatest biomass on large, medium and small islands, respectively. The ground layer vegetation consists of two species of feather mosses, *Pleurozium schreberi* and *Hylocomium splendens*, whose biomasses increase slightly with decreasing island size[52]. All major plant species occur on all islands but their relative abundance varies greatly among them; the species that dominate on the large islands (*i.e.*, *P. sylvestris* and *V. myrtillus*) have traits associated with resource acquisition, support more biomass and produce litter of higher quality than do species that dominate on smaller islands (*i.e.*, *P. abies* and *E. hermaphroditum*), which have functional traits that are associated with greater resource conservation[53]. As such, the islands enter a state of ecosystem retrogression[54] as time since fire increases and island size decreases, and this is characterized by less primary productivity, lower rates of nutrient cycling and litter decomposition, and lower supply rates of available soil nutrients[34] (Supplementary Table 1). Because of the important changes in plant community composition as well as productivity, soil fertility and functioning between large, medium and small islands, we refer to the three island size classes as 'contrasting ecosystems' throughout the manuscript.

In this experiment, we used a removal experiment approach. Removal experiments are a type of biodiversity manipulation that is recognized as a powerful tool for investigating the effects of local, non-random losses of biotic components of biodiversity on ecological processes in natural ecosystems[55,56]. In August 1996, fourteen plots were established on each of the 30 islands (420 plots in total), each representing a different removal treatment. The removal treatments consists of two parts which can be considered as two separate experiments: (i) a plant functional group removal experiment' that involves a full factorial combination of three different plant functional group removals (8 treatments in total), *i.e.*, tree root removal (performed by root trenching), ericaceous shrub removal (performed manually), and feather moss removal (performed manually), and (ii) a plant species removal experiment that involves a full factorial combination of three ericaceous dwarf shrub species removals (8 treatments in total; 2 in common with the functional group removal experiment), *i.e.*, removal of *V. myrtillus*, removal of *V. vitis-idaea* and removal of *E. hermaphroditum*. The three plant functional groups represent >99% of all plant biomass present on the islands, while the three shrub species represent >97% of all dwarf shrub biomass present[23]. This design allows assessment of the contributions of all possible interactions among functional groups and among major species within a functional group to ecosystem functioning at local spatial scales across the island size gradient. Removal treatments are implemented on each plot each year (in its 19st year at the sampling date), and we have shown that legacy effects of removal disturbances had mostly ceased by 2003 (*i.e.*, the 7th year)[23]. All plots are 55 × 55 cm, but only the inner 45 cm × 45 cm are measured; plots are located at similar distances from the island shore irrespective of island size, to minimize the potential of confounding factors covarying with island size such as edge effects and macroclimate[34].

Field sampling. Over August 3-15 2015, five soil cores per plot were sampled (2100 soil cores in total) using a stainless steel cylinder (diameter of 0.03 m) to a depth of 10 cm. The brown part of the moss layer was set as the soil surface (0 cm depth) and the litter layer of the soil profile (which is usually negligible) was included in the soil cores when present. Mineral soil was discarded when necessary (usually in large islands, corresponding to less than 1% of all soil cores). One soil core was taken in the center of the plot for community sequencing and characterization of the soil organic matter chemistry and four soil cores at 20 cm from the plot center were collected in each corner for measurements of soil abiotic parameters, nematodes, substrate-induced respiration (SIR) and phospholipid fatty acids (PLFAs). When the humus layer was too shallow (as was the case for some large islands), additional soil cores were taken to allow sufficient soil quantities to perform chemical and biological analyses. Directly after collection, two of the four cores not used for pyrosequencing were passed through a 2-mm sieve and the other two cores were kept intact for nematode extraction. All soil cores were stored either refrigerated (4°C) or frozen (-20°C) within a few hours after sampling for further analyses. For the core used for pyrosequencing, living roots and rhizomes with a diameter of >2 mm, cones, stones and mineral parts were removed in the laboratory, and the remaining soil was homogenized, freeze dried and milled to a fine powder before sub-sampling for further analyses. Fungal community sequencing and bioinformatics are described in details in the Supplementary Methods 1.

Ecosystem functions. We selected 15 different ecosystem functions and properties (used as proxies of functions) that underpin several supporting, provisioning and regulating ecosystem services[1], and all of which are fundamental to soil biogeochemical processes and ecosystem productivity[27,57-59]. Consistent with previous studies, we consider individual 'functions' to consist of each of the physiological, geochemical and biological variables that provide information about the cycling of elements, energy flow, and process rates in the ecosystem, and that collectively contribute to 'ecosystem functioning'[6,26-29,36]. These ecosystem functions and properties fall into 5 different categories (3 functions per category); these categories relate to N cycling, P cycling, C cycling, plant productivity and soil functioning (Table S2). The 15 functions (described in Supplementary Table 2) are: (1) soil N per area, (2) soil mineral N, (3) soil exchangeable N, (4) soil P per area, (5) soil mineral P, (6) soil exchangeable P, (7) soil C per area, (8) decomposition rates, (9) soil organic matter alkyl:O-alkyl ratio, (10) shrub biomass, (11) moss biomass, (12) root biomass, (13) potential microbial activity, (14) active microbial biomass, and (15) nematode density. The 15 selected functions and properties that we selected are broadly in line with several previous studies that have investigated the role of biodiversity on ecosystem multifunctionality [6,26-29,36], and the rationale for their selection is presented in Supplementary Table 2. All the field and laboratory measurements to assess the 15 ecosystem functions are described in the Supplementary Methods 2.

Ecosystem multifunctionality. For the calculation of multifunctionality, the values for each of the 15 functions must be standardized before averaging to remove the effects of differences in measurement scale between functions. Our null hypothesis was that increasing the number of plant species or functional groups does not affect the level of multifunctionality. It is important to note that the absolute values of multifunctionality and their distributions are sensitive to the standardization method used. Some authors have used the scaled mean minus the standard deviation of all functions[60], the z-transformation[61,62], or a standardization by a maximum observed value[9,27]. Here, we chose to standardize each of the 15 functions to a common scale by dividing by the maximum observed level of functioning (eqn 1)[9]. We tackled the outlier issue by removing extreme values following the outlier labelling rule with a tuning parameter[63] of $g = 2$.

(eqn 1)

$$f(x) = \frac{x_i}{\max(x)}$$

where x refers to the mean observed value of the function in plot removal treatment i. This transformation puts responses on the scale of low values and 1. Average multifunctionality (MF_a) was then calculated as the mean of standardized values across all individual functions (eqn 2). All the functions were equally weighted[8]. This averaging approach creates a metric that is intuitively interpretable, *i.e.*, the proportion of maximum multifunctionality possible that is achieved in each experimental plot. However, it is important to note that values at an intermediate level, for example, 0.6, could be due to mean values of all values having a value of 0.6, or it could be due to different functions having values of 0.9 and 0.3. Thus, we caution that similar multifunctionality values for different ecosystems may mask a very different functioning among them.

(eqn 2)

$$\mathrm{MF}_a = \frac{1}{F}\sum_{i=1}^{F} g(r_i(f_i))$$

where F is the number of functions being measured, f_i is a measure of function i, r_i is a mathematical function that sets f_i to be positive and g is a transformation that standardizes measures of all 15 functions to the same scale. We also calculated the net effect of per species or functional group addition on average multifunctionality (standardized value of all functions) separately for each island size class. The values of multifunctionality in three-species plots were compared to two-species plots (in which the species of interest was absent), two-species plots were compared to one-species plots, and one-species plots were compared to the treatment in which all species were removed.

We then used the multiple threshold multifunctionality (MF_t) approach (eqn 3)[8] to evaluate whether the effect of biodiversity loss on ecosystem multifunctionality differed across the full range of possible thresholds (from 5 to 100% at 1% intervals) between the different island size classes. This method evaluates the relationships between diversity and the total number of functions that have values greater than or equal to a threshold defined as a given percentage of the maximum observed rate of each individual function. We defined a set proportion of a maximum value for each function (here defined as the mean of the seven highest values) to serve as our threshold value and calculated the threshold index for each individual plot[8].

(eqn 3)

$$\mathrm{MF}_t = \sum_{i=1}^{F}(r_i(f_i) > t_i)$$

where F is the number of functions being measured, f_i is the value for function i in the plot, r_i is a mathematical function that sets f_i to be positive and t_i is the threshold value corresponding to the proportion of the maximum value for each function. To assess the capacity of biodiversity to maintain multiple functions at high levels, we identified the threshold of maximum diversity effect (*Tmde*) which is the value of the threshold where diversity has its strongest effect, and identified the realized maximum effect of diversity (*Rmde*) which is the strength of the linear relationships between diversity and multifunctionality (*i.e.*, slope) where diversity has its strongest effect[8]. These two indicators allow us to assess whether the effect of biodiversity loss on the thresholds reached differs across contrasting ecosystems, and how many species are needed to increase the number of functions. We also note that defining the maximum threshold at an unachievable high value inevitably leads to finding no biodiversity effects because no biodiversity level can reach the threshold whereas defining the maximum threshold at a low value inevitably leads to finding no biodiversity effect because all biodiversity levels will reach the threshold. Consequently, we emphasize that care is needed with this approach to avoid over-interpreting biodiversity effects at low or high thresholds[9].

Analyses of fungal communities. To allow meaningful comparisons of fungal OTU richness among samples, we compared richness estimates (α-diversity) using individual-based rarefaction for each plot in order to standardize the number of sequences[64]. We rarefied the number of sequences to 300 as a compromise between a meaningful number of reads and the minimum number of sequences found across all samples (three samples with 20, 161 and 163 sequences were excluded from this analysis). We then used linear mixed models (LMMs) for the 'functional group removal' experiment to test for the effect of island size class as the main plot factor, and plant functional group removals (*i.e.*, tree removal, shrub removal and moss removal) as subplot factors, on the estimated fungal species richness. Island identity was included as a random effect to account for the natural variation among ecosystems.

Using the regularized-logarithm transformation (rlog) that stabilizes the variance across the mean for 'count data'[65], we calculated the overall similarity among removal treatments for the functional group removal experiment across the 30 islands. The resulting heatmap and clustering distance were presented using the 'Poisson' distance, which takes the inherent variance structure of counts into consideration when calculating the distances between samples [66]. To examine the estimation of dispersion values for each OTU, we employed a differential expression analysis for the shrub removal factor because this factor had the strongest impact on the microbial community structure. The '$\log_2$ Fold Change' in the MA-plots represent the effect size estimate, that is how much the OTU's expression has changed due to the treatment effect (e.g., when shrubs are present or absent). Because of the multiple testing that this involved, Benjamini-Hochberg corrections were used to avoid false discovery rates[67], and only 'adjusted *P*-values' were considered significant. A Venn diagram was used to examine the number of 'depleted' or 'enriched' OTUs that were unique or common across the different island size classes.

Finally, to examine the differences in fungal community composition among island size classes and plant removals, we ran non-metric multidimensional scaling (nMDS) using Bray-Curtis dissimilarity matrices calculated on the relative abundance of OTUs. Those OTUs with less than 0.01% as their maximum relative abundance were removed prior to the analyses. We then used permutational multivariate analysis of variance (PERMANOVA, $n = 9999$) to test for statistical significance of the effects of plant removal treatments on fungal community composition for each island size class. For the species removal experiment, island size class as the main plot factor and shrub species removals (*i.e.*, *V. myrtillus* removal, *V. vitis-idaea* removal and *E. hermaphroditum* removal) as subplot factors were considered as fixed factors in the linear mixed models (LMMs). For the functional group removal experiment, island size class as the main plot factor, and functional group removals (*i.e.*, tree removal, shrub removal and moss removal) as subplot factors were considered as fixed factors in the LMMs. Permutations were constrained within islands. Island identity was included in the model as random factor to account for the natural variation among ecosystems.

Ecosystem multifunctionality – diversity relationship. We first used LMMs to test for statistical significance of island size class and plant removals in explaining the observed variation for each individual ecosystem function as well as the multifunctionality index, for both the species removal experiment and functional group experiments, exactly as described above for the analyses of fungal community composition. We then performed a second series of LMMs to evaluate the relationship of ecosystem multifunctionality with both plant and fungal diversity. Because fungal diversity was not directly experimentally manipulated, we tested for the relationship of multifunctionality with fungal diversity after correcting for potentially covarying and confounding factors such as plant diversity and key environmental variables that were measured in all plots (*i.e.*, ^{14}C age, water content, ^{15}N, ^{13}C, C:N ratio). Of these environmental variables, ^{14}C age represents time since last fire, water content is an essential factor controlling biogeochemical processes, ^{13}C and ^{15}N abundances have been interpreted as measures of the contribution of microbial components to soil organic matter (SOM) and N transfer from mycorrhizal fungi to plants, and C:N ratio is an important indicator of N accumulation and fertility in natural ecosystems[31,68,69]. These environmental variables were all centered and standardized prior to the analyses[70]. Different models, each involving different combinations of environmental variables added as co-factors, were then compared to the most parsimonious model. Akaike's Information Criterion (AIC) and *P*-values for the effects of both plant and fungal diversity were determined for each model selected. We also employed an alternative approach to directly assess the relationship between fungal species richness and ecosystem multifunctionality, which involved using the residuals of the model between multifunctionality and plant diversity to identify effects of fungal richness on multifunctionality that were independent of the effects of plant diversity. As for the first approach using LMMs, we corrected for potential confounding factors that may have co-varied with both fungal diversity and multifunctionality by adding them to the model (*i.e.*, ^{14}C age, water content, ^{15}N, ^{13}C, and C:N ratio).

We employed a third approach as proposed by Mori et al.[6] to confirm the importance of plant and fungal diversity in explaining ecosystem multifunctionality. Instead of averaging the standardized values of individual functions, we included the identity of functions (resulting in fifteen categories) as a random effect in the LMM. Similar to the previous models, AIC and *P*-values for both plant and fungal diversity were determined for each model selected. In this analysis, we also examined the diversity-functioning relationship of each individual function using standardized values. This approach allows examination of the responses of individual functions and enables the visualization of all possible tradeoffs among contrasting ecosystem functions[28]. Finally, we used variance partitioning to quantify the proportion of total variance explained by each factor (*i.e.*, island size class, plant diversity, fungal diversity and their interactions) for each of the 15 individual ecosystem functions and for ecosystem multifunctionality, using the pamer.fnc function in the 'LMERConvenienceFunctions' package[71].

We then used the threshold multifunctionality approach to evaluate whether the effect of plant and fungal species richness on ecosystem multifunctionality differed from low to high thresholds (20, 40, 60 and 80%)[8] for each of the three island size classes. We used generalized linear mixed models (GLMM) using a quasi-poisson error distribution and island identity as a random effect to evaluate the effect of diversity and island size class at each of the four different thresholds selected. We also conducted a sensitivity analysis by individually removing each island from the data set and re-running the above analyses at each of the four thresholds[9]. From each threshold model, we extracted the regression coefficient corresponding to the richness effect and compared that coefficient to the coefficient from the corresponding threshold model run on the entire data set. Finally, to assess the role of biodiversity in maintaining multiple functions at high levels, we employed a multiple threshold multifunctionality approach that allows plotting the effect of diversity on multifunctionality across the full range of possible thresholds[8]. We also verified that our results were not substantially affected by the selection and number of ecosystem functions. To do this we reduced the number of ecosystem functions from 15 to 12, 10 and 8 by randomly removing functions to see if the selection of functions affected the outcome for each island size class. We used R software 3.0.2 (http://www.r-project.org/), with 'DESeq2', 'ggplot2', 'glmmADMB', 'lme4', 'lmerTest', 'LMERConvenienceFunctions', 'multcomp', 'multifunc', and 'vegan' libraries for all data analyses.

Data availability. The datasets generated and analyzed during the current study and associated R codes are available from figshare: https://figshare.com/s/2cecc28232be7505a493.

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Author contributions

D.A.W. acquired the necessary funding and designed the experiment. N.F., D.A.W., P.K., M.J.G., M-C.N, M.F., M.C., J.A.B. collected and analyzed the data. N.F. wrote the first draft of the manuscript with substantial improvements by D.A.W. and P.K. All authors contributed to manuscript completion and revision.

Additional information

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Competing interests

The authors declare no competing financial interests.

Figure legends

Figure 1 | The influence of plant species and functional group removals on individual ecosystem functions. For each of the three island size classes, panels depict the responses of the 15 ecosystem functions to biodiversity loss for (**a**) the number of shrub species present following experimental removals, and (**b**) the number of plant functional groups present following removals. Each line depicts the moving average of standardized values of the 15 functions according to the diversity of species or functional groups (from 0 to 3). Points representing the experimental plots for each relationship were not represented to improve figure clarity. Histograms (mean ± SE, n =10) characterize the net effect on average multifunctionality (standardized value of all functions) per addition of each species [*Vaccinium myrtillus* (Vm), *Vaccinium vitis-idaea* (Vv), and *Empetrum hermaphroditum* (Eh)] or functional group [Trees (T), Shrubs (S), and Mosses (M)] when one, two or three species (**c**) or functional groups (**d**) are present. The highest value for each level of diversity is shown in darker shading. Island size classes are represented in the histograms in colors: large = red, blue = medium, green = small. More details on how biodiversity loss affects individual functions are provided in Table S3.

Figure 2 | The influence of plant species and functional group removals on ecosystem multifunctionality. For each of the three island size classes, panels depict the responses of ecosystem multifunctionality (based on 15 ecosystem functions; Table S2) to all possible combinations of (**a**) shrub species removal treatments [*Vaccinium myrtillus* (-Vm), *Vaccinium vitis-idaea* (-Vv), and *Empetrum hermaphroditum* (-Eh)], and (**b**) functional group removal treatments [Trees (-T), Shrubs (-S), and Mosses (-M)]. Boxplots characterize the lower quartile (Q1), median (Q2), upper quartile (Q3) and the interquartile range (IQR = Q3–Q1), which covers the central 50% of the data; the whiskers represent 95% of the data. Dark circles within each boxplot represent the mean (*m*). Different letters indicate significant differences between treatments ($P < 0.05$). Island size classes are represented in colors: large = red, blue = medium, green = small.

Figure 3 | The effects of plant removals on fungal community structure and diversity. (**a**) Heat-map and clustering analysis for operational taxonomic unit (OTU) counts (‘Poisson’ distance) after 19 years of plant functional group removals [Trees (-T), Shrubs (-S), and Mosses (-M)] on small, medium and large sized islands. Darker colors indicate higher values of community similarity, and clustering analysis (brown vs blue) represent the treatments in which shrubs are present or absent, respectively. (**b**) Enrichment (+36 OTUs, in green) and depletion (-

97 OTUs, in red) of fungal species when the shrubs are removed. Each point represents an individual OTU. The horizontal axis represents the mean abundance of OTUs over all samples, and the position along the vertical axis represents the log of the abundance fold change when shrubs are removed compared with when they are present. Some representative examples of OTUs belonging to the ericoid mycorrhizal fungi are also shown in the presence and absence of shrubs. (**C**) Venn diagram representing the numbers of differentially enriched and depleted OTUs shared between each island size class. Island size classes are represented in colors: large = red, blue = medium, green = small.

Figure 4 | The relationship between fungal species richness and ecosystem multifunctionality. Relationships are shown between fungal species richness and residuals of the model between multifunctionality and plant diversity for (**a**) the species removal experiment, and (**b**) the functional group removal experiment. Each point represents a separate experimental plot. Bands around the regression line represent the 95% confidence level interval. Results of the models between island size class and fungal diversity are given is the lower right corner. Island size classes are represented in colors: large = red, blue = medium, green = small.

Figure 5 | Importance of biodiversity and island size class (*i.e.*, environmental context) on 15 individual ecosystem functions and overall ecosystem multifunctionality, as revealed by variance partitioning analysis. The depicted results show the percentage of overall variation explained by island size class, plant diversity, fungal diversity and their interactions for (**a**) the species removal experiment, and (**b**) the functional group removal experiment.

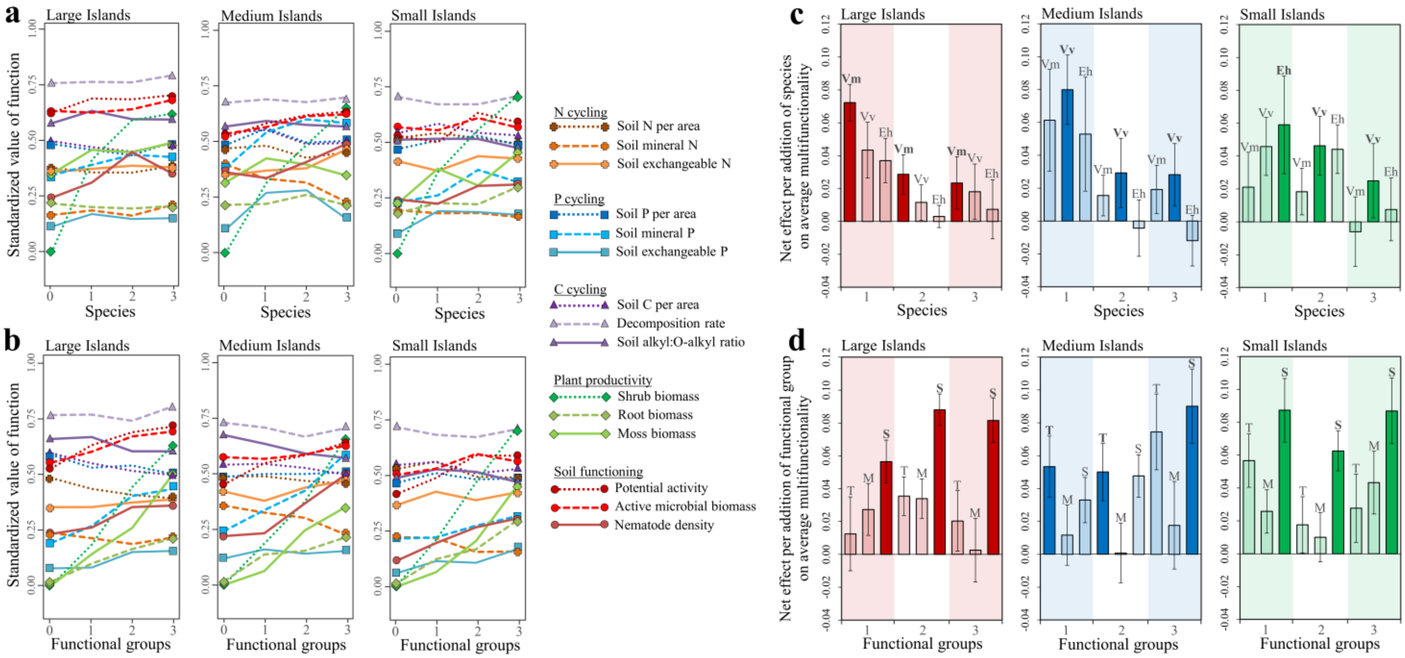

a
b
c
d
Large Islands
Medium Islands
Small Islands
Standardized value of function
Species
Functional groups
0.00
0.25
0.50
0.75
1.00
0
1
2
3
N cycling
Soil N per area
Soil mineral N
Soil exchangeable N
P cycling
Soil P per area
Soil mineral P
Soil exchangeable P
C cycling
Soil C per area
Decomposition rate
Soil alkyl:O-alkyl ratio
Plant productivity
Shrub biomass
Root biomass
Moss biomass
Soil functioning
Potential activity
Active microbial biomass
Nematode density
Net effect per addition of species on average multifunctionality
Net effect per addition of functional group on average multifunctionality
-0.04
-0.02
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Vm
Vv
Eh
T
M
S

Figure 1

Figure 2

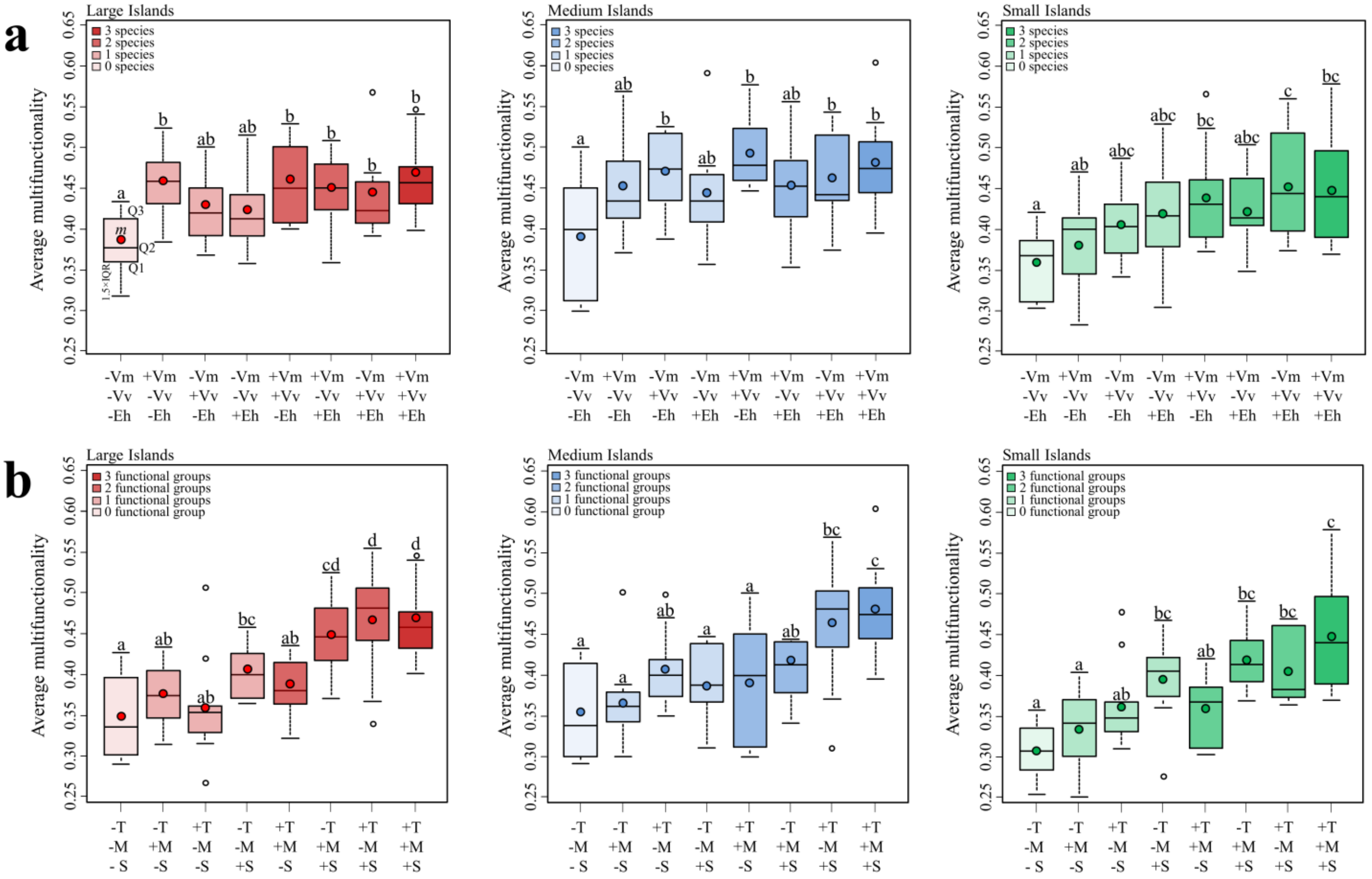
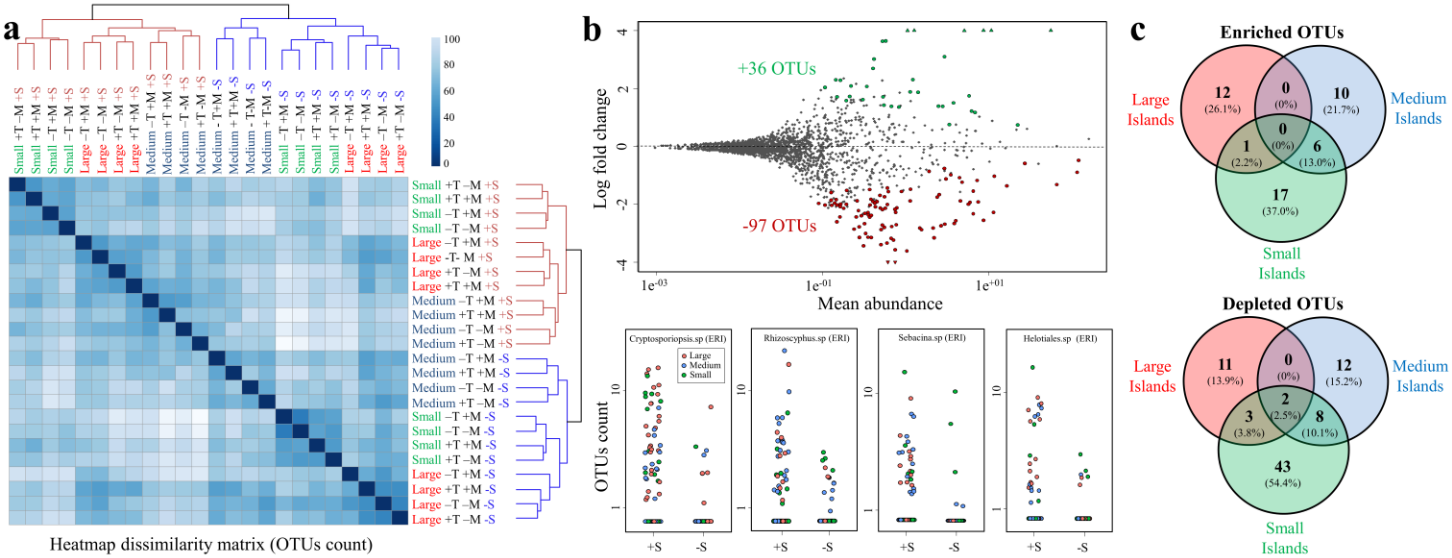

a
Large Islands
3 species
2 species
1 species
0 species
Average multifunctionality
0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65
a b ab ab b b b b
m
Q3
Q2
Q1
1.5×IQR
-Vm -Vv -Eh
+Vm -Vv -Eh
-Vm +Vv -Eh
-Vm -Vv +Eh
+Vm +Vv -Eh
+Vm -Vv +Eh
-Vm +Vv +Eh
+Vm +Vv +Eh
Medium Islands
a ab b ab b ab b b
Small Islands
a ab abc abc bc abc c bc
b
Large Islands
3 functional groups
2 functional groups
1 functional groups
0 functional group
a ab ab bc ab cd d d
-T -M - S
-T +M -S
+T -M -S
-T -M +S
+T +M -S
-T +M +S
+T -M +S
+T +M +S
Medium Islands
a a ab a a ab bc c
Small Islands
a a ab bc ab bc bc c

Figure 3



Heatmap dissimilarity matrix (OTUs count)

Figure 4

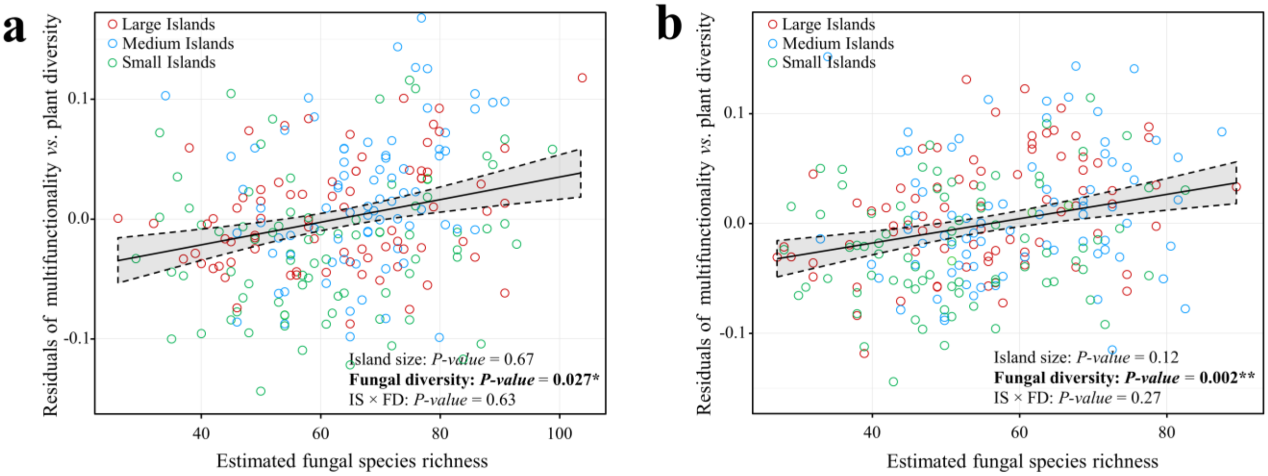
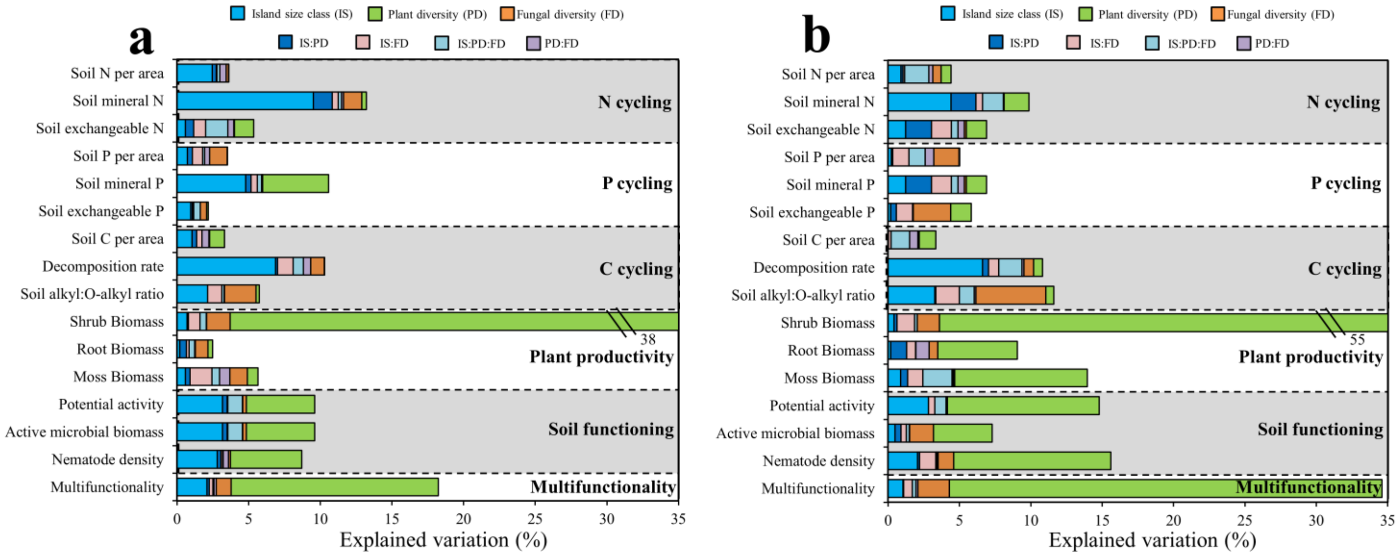

a
Large Islands
Medium Islands
Small Islands
Residuals of multifunctionality *vs.* plant diversity
0.1
0.0
-0.1
40
60
80
100
Estimated fungal species richness
Island size: *P-value* = 0.67
Fungal diversity: *P-value* = 0.027*
IS × FD: *P-value* = 0.63
b
Large Islands
Medium Islands
Small Islands
Residuals of multifunctionality *vs.* plant diversity
0.1
0.0
-0.1
40
60
80
Estimated fungal species richness
Island size: *P-value* = 0.12
Fungal diversity: *P-value* = 0.002**
IS × FD: *P-value* = 0.27

Figure 5


a
b
Island size class (IS)
Plant diversity (PD)
Fungal diversity (FD)
IS:PD
IS:FD
IS:PD:FD
PD:FD
Soil N per area
Soil mineral N
Soil exchangeable N
Soil P per area
Soil mineral P
Soil exchangeable P
Soil C per area
Decomposition rate
Soil alkyl:O-alkyl ratio
Shrub Biomass
Root Biomass
Moss Biomass
Potential activity
Active microbial biomass
Nematode density
Multifunctionality
N cycling
P cycling
C cycling
Plant productivity
Soil functioning
Multifunctionality
38
55
0
5
10
15
20
25
30
35
Explained variation (%)