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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

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

34

35 Species that co-occur in natural ecosystems perform many individual functions, which in turn
36 underpin the multiple goods and services that ecosystems provide to humans¹⁻³. Because species
37 vary in their contributions to different functions⁴, it has been increasingly recognized that
38 biodiversity may be important for the simultaneous maintenance of multiple ecosystem functions
39 (hereafter ‘multifunctionality’)⁵. The concept of multifunctionality stems from the idea that the
40 effects of biodiversity were stronger when multiple functions were considered compared to single
41 functions. However, recent studies have demonstrated that ecosystem multifunctionality does not
42 depend of the number of functions considered^{6,7}, suggesting that biodiversity can only affect
43 multifunctionality *via* non-additive effects on individual ecosystem functions (i.e., through
44 numerical values in mixtures being unequal to the sum of values based on the component
45 monocultures). Although the concept of multifunctionality may be useful for assessing multiple

46 aspects of functioning associated with biodiversity loss^{8,9}, our understanding about the underlying
47 mechanisms remains limited.

48 Complementarity effects (i.e., synergistic effects of biodiversity through resource
49 partitioning or facilitation) have been hypothesized to explain the positive effects of increasing
50 species richness on ecosystem functions¹⁰, and the greater ability of plant communities with
51 higher diversity to capture resources and convert them into new biomass^{11,12}. Most available
52 empirical evidence of these mechanisms is derived either from short-term studies^{13,14} (≤ 5 years)
53 or from artificial biodiversity manipulation experiments in which plant diversity has been varied
54 through random selection from species pools^{15,16}. Although such studies have greatly improved
55 our theoretical understanding of the role of biodiversity in ecosystem functioning, it is less clear
56 as to how generalizable such findings are to natural ecosystems^{17,18}. In particular, there is a dearth
57 of studies on how long term manipulations of biodiversity in natural ecosystems impact on
58 ecosystem multifunctionality and on the extent to which these effects vary across contrasting
59 ecosystems.

60 The contribution of plant diversity to ecosystem functions may differ among ecosystems
61 or along environmental gradients due to changes in ecosystem abiotic properties (notably soil
62 fertility and water availability), plant functional traits, and shifts in species identity and relative
63 abundance^{5,19-22}. For instance, the loss of plant species with resource-acquisitive traits exerts
64 greater control on plant biomass and nutrient recycling in productive ecosystems than does the
65 loss of resource-conservative species²³. Yet, despite growing evidence that the effects of
66 biodiversity loss on single functions can vary according to environmental context^{24,25}, most
67 biodiversity-multifunctionality studies have been conducted within a single context (but see^{5,22}).
68 While some studies have found stronger effects of biodiversity loss on ecosystem

69 multifunctionality than have others²⁶⁻²⁸, our understanding about whether, how and why these
70 effects vary across contrasting ecosystems or along environmental gradients remains limited.

71 Further, recent studies have also shown that biodiversity at multiple trophic levels can
72 have important impacts on multiple ecosystem functions^{6,29}, and that focusing only on plants may
73 greatly underestimate the impact of biodiversity on multifunctionality in natural ecosystems³⁰.
74 For instance, a substantial proportion of plant carbon (C) is allocated belowground to roots and
75 associated fungi, thereby influencing belowground organic matter (OM) turnover, C
76 sequestration and nutrient dynamics³¹⁻³³. Despite increasing evidence that root-associated fungi
77 are major drivers of terrestrial biogeochemical cycling^{32,33}, it remains little understood whether
78 and how simultaneous changes in the diversity of autotrophs (*i.e.*, plants) and decomposers (*i.e.*,
79 soil fungi) may impact multifunctionality, and the extent to which these effects can vary across
80 contrasting environmental contexts. Such an understanding is needed to better predict how
81 multifunctionality in natural ecosystems may respond to changes in diversity across trophic levels
82 caused by global change drivers and other environmental factors.

83 To evaluate the effect of biodiversity loss on ecosystem multifunctionality at two different
84 trophic levels (plants and soil fungi) among contrasting ecosystems, we utilized an ongoing pair
85 of experiments (each established in 1996) that had been running for 19 years at the time of
86 measurement. The experiments include removal of plant species and functional groups to
87 represent biodiversity loss on each of 30 forested islands that represent highly contrasting
88 ecosystems in northern Sweden (M&M). The islands were formed after the most recent glaciation
89 and are subjected to the same extrinsic factors other than disturbance history caused by wildfire³⁴.
90 Larger islands burn more frequently because they have a larger area to intercept lightning
91 strikes³⁵; several large islands have burned in the past century, whereas some small islands have
92 not burned in the past 5000 years. The 30 islands include three classes of each of ten islands

93 (hereafter ‘island size class’): 10 ‘large’ islands (>1.0 ha; on average, mean time since fire 585
94 years), 10 medium islands (0.1 to 1.0 ha, 2180 years), and 10 small islands (<0.1 ha, 3250 years).
95 Previous work in this study system has shown that as the time since fire increases, net primary
96 productivity, soil fertility and nutrient recycling decrease³⁴, leading to humus layers that can
97 exceed 1 m in depth on the smallest islands due to accumulation of organic matter from plant and
98 fungal residues³¹ (Supplementary Table 1).

99   The removal experiments established on these islands are the longest running biodiversity
100 manipulation experiments in existence that include repetitions of the same treatments across
101 contrasting ecosystems. The first of these experiments consists of full factorial removals of three
102 dominant ericaceous dwarf shrub species (*Vaccinium myrtillus*, *Vaccinium vitis-idaea* and
103 *Empetrum hermaphroditum*) on each of the 30 islands. The other consists of full factorial
104 removals of three plant functional groups (tree roots, mosses and dwarf shrubs) on each island.
105 Both experiments in combination yield a total of 420 plots²³ (Supplementary Fig. 1). We used
106 these experiments to determine effects of simultaneous changes in the diversity of plants and
107 associated fungal communities on 15 separate individual ecosystem functions and properties
108 (used as proxy of functions) that underpin several supporting, provisioning and regulating
109 ecosystem services¹ (Supplementary Table 2). Consistent with previous studies^{6,26-30,36}, we
110 consider individual ‘functions’ to consist of each of the physiological, geochemical and
111 biological variables that provide information about the cycling of elements, energy flow and
112 process rates in the ecosystem. The functions selected were related to nutrient cycling (nitrogen
113 (N) and phosphorus (P) stocks, availability and flux), C cycling (C stock, organic matter quality
114 and decomposition), plant productivity (dwarf shrub, moss and root biomass) and soil functioning
115 (activity of and biomass of soil organisms), with higher levels indicating a greater ecosystem

116 functioning. Higher levels of these functions have been linked to greater delivery of ecosystem
117 services upon which humans depend¹⁻³.

118 From these experiments we determined how plant species and functional group removals
119 (*i.e.*, plant diversity loss) on each island influenced the 15 selected functions and assessed their
120 relative contribution to multifunctionality^{27,28} across ecosystems. We also employed threshold
121 approaches which allow testing of whether diverse plant communities are more likely to support
122 multiple functions at high levels^{8,26}, and tested whether ecosystem multifunctionality depended of
123 the number of functions considered⁷. We then characterized the effects of plant removals on soil
124 fungal community and whether fungal diversity could in turn explain variation in
125 multifunctionality beyond what could be explained by the loss of plant diversity. From this data
126 set we were able to assess the extent to which plant and fungal diversity effects on both
127 individual ecosystem functions and multifunctionality varied among contrasting ecosystems.

128

129 **Results and discussion**

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

139 *myrtillus* is a fast growing species with acquisitive traits and *E. hermaphroditum* is a slow
140 growing species with conservative traits (with *V. vitis-idaea* being intermediate)⁵⁰, this result
141 shows that acquisitive species have their highest impacts on multifunctionality in productive
142 ecosystems and that conservative species have their greatest impacts in less productive systems.
143 This supports the prediction of the ‘species sorting theory’ stipulating that if different ecosystems
144 vary in resources supply, then the identity of the species that maximizes ecosystem functions will
145 vary for each ecosystem³⁷. Further, this highlights that the loss of any particular species can have
146 important consequences on overall functioning in one ecosystem, and minimal impacts in
147 another.

148   The importance of the environmental context was even more apparent for the loss of plant
149 functional groups, as revealed by many significant interactive effects between island size and
150 particular removal treatments for several of these functions (Supplementary Table 3). For
151 instance, tree root removals had negative, neutral, or positive effects on soil exchangeable P in
152 large, medium and small islands, respectively (Supplementary Table 4). This indicates that
153 changes in the composition of plant communities - both within and between functional groups -
154 among island size classes do alter not only biogeochemical cycles and net primary productivity³⁴,
155 but also the relative contribution of functional groups to individual ecosystem functions (Fig. 1b).
156 This highlights that understanding of the relative contribution of species and functional groups to
157 single functions among contrasting ecosystems is critical for guiding management efforts aimed
158 at maintaining ecosystem multifunctionality. Further, our results reinforce that although many
159 studies have provided compelling evidence for positive effects of biodiversity on ecosystem
160 productivity, functioning and stability, this is not found for all studies and for all functions^{9,38}.

161

162 **Consistent effects of biodiversity loss on multifunctionality across contrasting ecosystems.** In
163 contrast with the results on individual ecosystem functions, we found that negative effects of
164 plant removals on ecosystem multifunctionality were largely independent of island size class
165 (Fig. 2, Supplementary Table 5). This is because even if the relative contributions of species or
166 functional groups varied across island size classes (Fig. 1c, d), the non-additive effects resulting
167 from species interactions (e.g., through complementarity and/or competition) were relatively
168 similar for many single functions across contrasting ecosystems. However, the relative
169 consistency of multifunctionality values across contrasting ecosystems is also due to the context-
170 dependent nature of biodiversity loss that can work in opposing directions for different functions.
171 For instance, while soil mineral N and exchangeable N were unaffected by the number of plant
172 functional groups present for large islands, these two functions increased and decreased
173 respectively with increasing functional group richness for medium islands (Fig. 1b). Further, the
174 maximum level reached by each function may vary with island size because of a different overall
175 functioning (e.g., fast nutrient cycling in large islands vs. conservative nutrient cycling in small
176 islands³⁴). Consequently, we caution that similar multifunctionality values across contrasting
177 ecosystems may mask large differences in their functioning. This further reinforces the
178 conclusion that explicit recognition of the relationships between plant diversity and individual
179 ecosystem functions is necessary to more completely understand the influence of biodiversity on
180 ecosystem multifunctionality²⁸.

181

182 **Do the effects of biodiversity loss on multifunctionality depend of the number functions**
183 **considered?** The lack of an interactive effect of island size class and plant diversity on average
184 multifunctionality occurred consistently even when considering different levels of ecosystem
185 multifunctionality (see Supplementary Figs. 3-5 for more details), or when the number of

186 functions considered to calculate multifunctionality was altered (Supplementary Fig. 6). In line
187 with theoretical predictions of Gamfeldt and Roger⁷, our long-term study provides experimental
188 evidence that ecosystem multifunctionality does not depend on the number of functions
189 considered. This is because even if slopes vary depending on which functions are included in any
190 one combination of functions, the average slope stays constant across the full range of number of
191 functions⁷, and this across highly contrasting ecosystems. This indicates that any effect of
192 biodiversity on multifunctionality results from non-additive effects of coexisting species or
193 functional groups on individual ecosystem functions, and further underscores that a better
194 understanding of species interactions is crucial to predict the role of biodiversity loss on
195 ecosystem multifunctionality across contrasting ecosystems.

196

197 **Complementarity *versus* competition drive the effects of biodiversity on multifunctionality.**

198 The net positive effects on multifunctionality when two or more species are present in the
199 community suggest that complementarity is an important mechanism by which plant diversity
200 enhances overall ecosystem functioning (Figs. 1, 2). This indicates that even if species that are
201 best suited to the local conditions may vary among ecosystems³⁷, multiple coexisting species
202 often results in higher individual ecosystem functions than a single species alone. Such effects
203 can arise from niche differences (i.e., occupation of distinct niches at the local scale) among
204 species or functional groups in their responses to limiting factors such as soil resource availability
205 (e.g., by sharing different resources). Niche difference is often considered to be the main driver of
206 long-term species coexistence by preventing competitive exclusion^{39,40}. Here, the results of our
207 long-term biodiversity experiment highlight that niche difference is also an important mechanism
208 controlling the capacity of plant communities at maximizing multiple functions at high levels
209 across contrasting ecosystems. This is likely because complementarity effects on some individual

210 functions have the potential to drive changes in other functions (Fig. 1a, b). For instance, a better
211 exploitation of soil nutrients resulting from higher resource partitioning and/or differences in
212 resource use can drive increases in plant biomass, with further positive consequences on the
213 activity of soil organisms.

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

232

233 **Biodiversity at different trophic levels is required to maintain ecosystem multifunctionality.**

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

250 We found that ecosystem multifunctionality was positively correlated with soil fungal
251 diversity independently of island size class (Fig. 4), even when controlling for the variation in
252 plant richness and environmental factors (Supplementary Fig. 11 and Supplementary Tables 8-9).
253 Although fungal species and their contribution to ecosystem functioning differ between large and
254 small islands^{31,32}, this highlights that diverse fungal communities are more likely to support high
255 levels of functioning for multiple functions. These results confirm that microbial diversity is an
256 important contributor to ecosystem multifunctionality^{6,29,45} and reveal that not only do these

257 effects occur over and above those exerted by plant diversity, but also that they are consistent
258 across different island size classes (see Supplementary Figs. 11-13 for more details on
259 multifunctionality at different thresholds). This may be because the context-dependent nature of
260 fungal biodiversity loss can work in opposing directions for different functions, as we found for
261 plant diversity. Further, this relative consistency could have arisen through complementarity
262 effects being overall relatively similar among island size classes for many individual functions
263 when fungal diversity increased due to comparable roles across classes of niche differences⁴⁶
264 and/or facilitative interactions⁴⁷ among fungal taxa that promote decomposition and soil nutrient
265 acquisition processes, and other functions linked to these processes. Further, the effects of fungal
266 diversity were still present when decreasing the number of functions considered to calculate
267 multifunctionality (Supplementary Fig. 14). This confirms our previous observations with
268 primary producers (Supplementary Table 6), and reinforces the conclusion that multifunctionality
269 does not depend on the number of functions considered⁷.

270 In line with the multiple studies showing that biodiversity loss usually impairs ecosystem
271 functions^{3,11,48}, variance partitioning analysis of our data revealed that plant and fungal diversity
272 both have a significant role in influencing some individual functions (Fig. 5). These effects were
273 often affected by environmental context (see also Supplementary Table 3), supporting the idea
274 that the loss of any particular species or functional groups can have important consequences on
275 ecosystem functions in one ecosystem while only have weak effects in another. However,
276 although fungal and especially plant diversity are important drivers of multifunctionality (plant
277 diversity had a more important effect on multifunctionality than it does on all but one of the
278 individual functions), any variation in this effect among island size classes (*i.e.*, interactive
279 effects of island size class and removal treatments) was negligible (Fig. 5). The relative
280 consistency of the effect of biodiversity loss on multifunctionality across ecosystems for both

281 plants and fungi highlights that regardless of ecosystem type, the delivery of a broad range of
282 functions requires the maintenance of species diversity at different trophic levels^{6,29,30,45}. This
283 further emphasizes that although the contribution of different species or functional groups to
284 individual functions strongly varies across the island chronosequence, preserving biodiversity
285 across highly contrasting ecosystems is indispensable to sustain ecosystem multifunctionality.

286

287 **Conclusion**

288 Synthetic biodiversity manipulation experiments and observational studies in natural ecosystems
289 have both substantially advanced our understanding of biodiversity-ecosystem functioning
290 relationships, but they each have different strengths and weaknesses⁴⁹. Here we use a long-
291 running ‘removal experiment’, which captures elements of both approaches through experimental
292 manipulation of multiple natural ecosystems, to shed new light about how biodiversity may affect
293 ecosystem multifunctionality in contrasting environments. It highlights that although effects of
294 biodiversity loss on individual functions can vary with ecosystem type because of differences in
295 soil fertility, productivity or biological communities²³⁻²⁵, the effects of biodiversity loss for both
296 autotrophs (*i.e.*, plants) and heterotrophs (*i.e.*, soil fungi) on ecosystem multifunctionality are
297 relatively invariant among contrasting ecosystems. From a management perspective, these results
298 emphasize the importance of conserving biodiversity for maintaining multifunctionality for both
299 producer and consumer trophic levels, and in highly contrasting ecosystems that vary greatly in
300 both abiotic and biotic properties. Further, ecosystems with greater species or functional groups
301 richness nearly always had higher ecosystem multifunctionality, reinforcing the view that
302 preserving biodiversity is likely to be consistently important for maintaining ecosystem
303 functioning across very different natural ecosystems⁵⁰. 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 fire³⁴. 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 size⁵². 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 conservation⁵³. As such, the islands enter a state of ecosystem retrogression⁵⁴ 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 nutrients³⁴ (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 present²³. 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 macroclimate³⁴.

366

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,

375 substrate-induced respiration (SIR) and phospholipid fatty acids (PLFAs). When the humus layer
376 was too shallow (as was the case for some large islands), additional soil cores were taken to allow
377 sufficient soil quantities to perform chemical and biological analyses. Directly after collection,
378 two of the four cores not used for pyrosequencing were passed through a 2-mm sieve and the
379 other two cores were kept intact for nematode extraction. All soil cores were stored either
380 refrigerated (4°C) or frozen (-20°C) within a few hours after sampling for further analyses. For
381 the core used for pyrosequencing, living roots and rhizomes with a diameter of >2 mm, cones,
382 stones and mineral parts were removed in the laboratory, and the remaining soil was
383 homogenized, freeze dried and milled to a fine powder before sub-sampling for further analyses.
384 Fungal community sequencing and bioinformatics are described in details in the Supplementary
385 Methods 1.

386

387 **Ecosystem functions.** We selected 15 different ecosystem functions and properties (used as
388 proxies of functions) that underpin several supporting, provisioning and regulating ecosystem
389 services¹, and all of which are fundamental to soil biogeochemical processes and ecosystem
390 productivity^{27,57-59}. Consistent with previous studies, we consider individual ‘functions’ to consist
391 of each of the physiological, geochemical and biological variables that provide information about
392 the cycling of elements, energy flow, and process rates in the ecosystem, and that collectively
393 contribute to ‘ecosystem functioning’^{6,26-29,36}. These ecosystem functions and properties fall into
394 5 different categories (3 functions per category); these categories relate to N cycling, P cycling, C
395 cycling, plant productivity and soil functioning (Table S2). The 15 functions (described in
396 Supplementary Table 2) are: (1) soil N per area, (2) soil mineral N, (3) soil exchangeable N, (4)
397 soil P per area, (5) soil mineral P, (6) soil exchangeable P, (7) soil C per area, (8) decomposition
398 rates, (9) soil organic matter alkyl:O-alkyl ratio, (10) shrub biomass, (11) moss biomass, (12) root

399 biomass, (13) potential microbial activity, (14) active microbial biomass, and (15) nematode
400 density. The 15 selected functions and properties that we selected are broadly in line with several
401 previous studies that have investigated the role of biodiversity on ecosystem multifunctionality
402 ^{6,26-29,36}, and the rationale for their selection is presented in Supplementary Table 2. All the field
403 and laboratory measurements to assess the 15 ecosystem functions are described in the
404 Supplementary Methods 2.

405

406 **Ecosystem multifunctionality.** For the calculation of multifunctionality, the values for each of
407 the 15 functions must be standardized before averaging to remove the effects of differences in
408 measurement scale between functions. Our null hypothesis was that increasing the number of
409 plant species or functional groups does not affect the level of multifunctionality. It is important to
410 note that the absolute values of multifunctionality and their distributions are sensitive to the
411 standardization method used. Some authors have used the scaled mean minus the standard
412 deviation of all functions⁶⁰, the z-transformation^{61,62}, or a standardization by a maximum
413 observed value^{9,27}. Here, we chose to standardize each of the 15 functions to a common scale by
414 dividing by the maximum observed level of functioning (eqn 1)⁹. We tackled the outlier issue by
415 removing extreme values following the outlier labelling rule with a tuning parameter⁶³ of $g = 2$.

416

417 (eqn 1)

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

418 where x refers to the mean observed value of the function in plot removal treatment i . This
419 transformation puts responses on the scale of low values and 1. Average multifunctionality (MF_a)
420 was then calculated as the mean of standardized values across all individual functions (eqn 2). All

the functions were equally weighted⁸. 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)

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

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

443 maximum value for each function (here defined as the mean of the seven highest values) to serve
444 as our threshold value and calculated the threshold index for each individual plot⁸.

445

446 (eqn 3)

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

447 where F is the number of functions being measured, f_i is the value for function i in the plot, r_i is a
448 mathematical function that sets f_i to be positive and t_i is the threshold value corresponding to the
449 proportion of the maximum value for each function. To assess the capacity of biodiversity to
450 maintain multiple functions at high levels, we identified the threshold of maximum diversity
451 effect ($Tmde$) which is the value of the threshold where diversity has its strongest effect, and
452 identified the realized maximum effect of diversity ($Rmde$) which is the strength of the linear
453 relationships between diversity and multifunctionality (*i.e.*, slope) where diversity has its
454 strongest effect⁸. These two indicators allow us to assess whether the effect of biodiversity loss
455 on the thresholds reached differs across contrasting ecosystems, and how many species are
456 needed to increase the number of functions. We also note that defining the maximum threshold at
457 an unachievable high value inevitably leads to finding no biodiversity effects because no
458 biodiversity level can reach the threshold whereas defining the maximum threshold at a low value
459 inevitably leads to finding no biodiversity effect because all biodiversity levels will reach the
460 threshold. Consequently, we emphasize that care is needed with this approach to avoid over-
461 interpreting biodiversity effects at low or high thresholds⁹.

462

463 **Analyses of fungal communities.** To allow meaningful comparisons of fungal OTU richness
464 among samples, we compared richness estimates (α -diversity) using individual-based rarefaction

465 for each plot in order to standardize the number of sequences⁶⁴. We rarefied the number of
466 sequences to 300 as a compromise between a meaningful number of reads and the minimum
467 number of sequences found across all samples (three samples with 20, 161 and 163 sequences
468 were excluded from this analysis). We then used linear mixed models (LMMs) for the ‘functional
469 group removal’ experiment to test for the effect of island size class as the main plot factor, and
470 plant functional group removals (*i.e.*, tree removal, shrub removal and moss removal) as subplot
471 factors, on the estimated fungal species richness. Island identity was included as a random effect
472 to account for the natural variation among ecosystems.

473   Using the regularized-logarithm transformation (rlog) that stabilizes the variance across
474 the mean for ‘count data’⁶⁵, we calculated the overall similarity among removal treatments for the
475 functional group removal experiment across the 30 islands. The resulting heatmap and clustering
476 distance were presented using the ‘Poisson’ distance, which takes the inherent variance structure
477 of counts into consideration when calculating the distances between samples⁶⁶. To examine the
478 estimation of dispersion values for each OTU, we employed a differential expression analysis for
479 the shrub removal factor because this factor had the strongest impact on the microbial community
480 structure. The ‘log₂ Fold Change’ in the MA-plots represent the effect size estimate, that is how
481 much the OTU’s expression has changed due to the treatment effect (e.g., when shrubs are
482 present or absent). Because of the multiple testing that this involved, Benjamini-Hochberg
483 corrections were used to avoid false discovery rates⁶⁷, and only ‘adjusted *P*-values’ were
484 considered significant. A Venn diagram was used to examine the number of ‘depleted’ or
485 ‘enriched’ OTUs that were unique or common across the different island size classes.

486   Finally, to examine the differences in fungal community composition among island size
487 classes and plant removals, we ran non-metric multidimensional scaling (nMDS) using Bray-
488 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.

500

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

513 contribution of microbial components to soil organic matter (SOM) and N transfer from
514 mycorrhizal fungi to plants, and C:N ratio is an important indicator of N accumulation and
515 fertility in natural ecosystems^{31,68,69}. These environmental variables were all centered and
516 standardized prior to the analyses⁷⁰. Different models, each involving different combinations of
517 environmental variables added as co-factors, were then compared to the most parsimonious
518 model. Akaike's Information Criterion (AIC) and *P*-values for the effects of both plant and
519 fungal diversity were determined for each model selected. We also employed an alternative
520 approach to directly assess the relationship between fungal species richness and ecosystem
521 multifunctionality, which involved using the residuals of the model between multifunctionality
522 and plant diversity to identify effects of fungal richness on multifunctionality that were
523 independent of the effects of plant diversity. As for the first approach using LMMs, we corrected
524 for potential confounding factors that may have co-varied with both fungal diversity and
525 multifunctionality by adding them to the model (*i.e.*, ¹⁴C age, water content, ¹⁵N, ¹³C, and C:N
526 ratio).

527   We employed a third approach as proposed by Mori et al.⁶ to confirm the importance of
528 plant and fungal diversity in explaining ecosystem multifunctionality. Instead of averaging the
529 standardized values of individual functions, we included the identity of functions (resulting in
530 fifteen categories) as a random effect in the LMM. Similar to the previous models, AIC and *P*-
531 values for both plant and fungal diversity were determined for each model selected. In this
532 analysis, we also examined the diversity-functioning relationship of each individual function
533 using standardized values. This approach allows examination of the responses of individual
534 functions and enables the visualization of all possible tradeoffs among contrasting ecosystem
535 functions²⁸. Finally, we used variance partitioning to quantify the proportion of total variance
536 explained by each factor (*i.e.*, island size class, plant diversity, fungal diversity and their

537 interactions) for each of the 15 individual ecosystem functions and for ecosystem
538 multifunctionality, using the `pamer.fnc` function in the ‘LMERConvenienceFunctions’ package⁷¹.

539 We then used the threshold multifunctionality approach to evaluate whether the effect of
540 plant and fungal species richness on ecosystem multifunctionality differed from low to high
541 thresholds (20, 40, 60 and 80%)⁸ for each of the three island size classes. We used generalized
542 linear mixed models (GLMM) using a quasi-poisson error distribution and island identity as a
543 random effect to evaluate the effect of diversity and island size class at each of the four different
544 thresholds selected. We also conducted a sensitivity analysis by individually removing each
545 island from the data set and re-running the above analyses at each of the four thresholds⁹. From
546 each threshold model, we extracted the regression coefficient corresponding to the richness effect
547 and compared that coefficient to the coefficient from the corresponding threshold model run on
548 the entire data set. Finally, to assess the role of biodiversity in maintaining multiple functions at
549 high levels, we employed a multiple threshold multifunctionality approach that allows plotting
550 the effect of diversity on multifunctionality across the full range of possible thresholds⁸. We also
551 verified that our results were not substantially affected by the selection and number of ecosystem
552 functions. To do this we reduced the number of ecosystem functions from 15 to 12, 10 and 8 by
553 randomly removing functions to see if the selection of functions affected the outcome for each
554 island size class. We used R software 3.0.2 (<http://www.r-project.org/>), with ‘DESeq2’,
555 ‘ggplot2’, ‘glmmADMB’, ‘lme4’, ‘lmerTest’, ‘LMERConvenienceFunctions’, ‘multcomp’,
556 ‘multifunc’, and ‘vegan’ libraries for all data analyses.

557

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

560

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727

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734

735 **Author contributions**

736 D.A.W. acquired the necessary funding and designed the experiment. N.F., D.A.W., P.K.,
737 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
738 the manuscript with substantial improvements by D.A.W. and P.K. All authors contributed to
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740

741 **Additional information**

742 **Supplementary information** is available for this paper.

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745

746 **Competing interests**

747 The authors declare no competing financial interests.

748

749 **Figure legends**

750 **Figure 1 | The influence of plant species and functional group removals on individual**
751 **ecosystem functions.** For each of the three island size classes, panels depict the responses of the
752 15 ecosystem functions to biodiversity loss for **(a)** the number of shrub species present following
753 experimental removals, and **(b)** the number of plant functional groups present following
754 removals. Each line depicts the moving average of standardized values of the 15 functions
755 according to the diversity of species or functional groups (from 0 to 3). Points representing the
756 experimental plots for each relationship were not represented to improve figure clarity.
757 Histograms (mean $\pm$ SE, $n = 10$) characterize the net effect on average multifunctionality
758 (standardized value of all functions) per addition of each species [*Vaccinium myrtillus* (Vm),

759 *Vaccinium vitis-idaea* (Vv), and *Empetrum hermaphroditum* (Eh)] or functional group [Trees (T),
760 Shrubs (S), and Mosses (M)] when one, two or three species (c) or functional groups (d) are
761 present. The highest value for each level of diversity is shown in darker shading. Island size
762 classes are represented in the histograms in colors: large = red, blue = medium, green = small.
763 More details on how biodiversity loss affects individual functions are provided in Table S3.

764

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

776

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

783 97 OTUs, in red) of fungal species when the shrubs are removed. Each point represents an
784 individual OTU. The horizontal axis represents the mean abundance of OTUs over all samples,
785 and the position along the vertical axis represents the log of the abundance fold change when
786 shrubs are removed compared with when they are present. Some representative examples of
787 OTUs belonging to the ericoid mycorrhizal fungi are also shown in the presence and absence of
788 shrubs. (C) Venn diagram representing the numbers of differentially enriched and depleted OTUs
789 shared between each island size class. Island size classes are represented in colors: large = red,
790 blue = medium, green = small.

791

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

799

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

805 **Figure**

809 **Figure 5**