

Impact of plant functional group and species removals on soil and plant nitrogen and phosphorus across a retrogressive chronosequence

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1 **Impact of plant functional group and species removals on soil and plant**
2 **nitrogen and phosphorus across a retrogressive chronosequence**

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14 **Running title:** Biodiversity loss effects on soil nutrients

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Summary

1. In the prolonged absence of catastrophic disturbance, ecosystem retrogression occurs, which is characterized by declining soil nitrogen (N) and phosphorus (P) availability, increasing plant and soil N to P ratios, and reduced plant biomass and productivity. It is, however, largely unknown as to how the effects of plant communities on soil nutrients change during retrogression or might contribute to declining nutrient availability as retrogression proceeds.

2. We studied a well-characterized system of 30 lake islands in northern Sweden that collectively represent a 5000-year post-fire retrogressive chronosequence. For each island, we established an experiment that involved full factorial removal of three plant functional groups (tree roots, dwarf shrubs and mosses), and of three species of dwarf shrub (*Vaccinium myrtillus*, *V. vitis-idaea* and *Empetrum hermaphroditum*). After 19 years, we took various measures of soil N and P availability, and measured foliar N and P for each dwarf shrub species, for each plot in the experiment.

3. Although plant removal effects (and particularly removal of tree roots, shrubs, and *Vaccinium* species) on belowground N and P measures sometimes changed during retrogression, this seldom happened in a way that explains the decline in nutrient availability and increase in N to P ratios that characterizes ecosystem retrogression. The only exceptions were that the positive effects of tree roots on soil mineral N and P, and of *V. myrtillus* on soil mineral P, declined during retrogression.

4. Plant removal effects on community-level measures of shrub N and P varied greatly across the chronosequence, but these effects again did not align with the changes in soil nutrient availability or N to P ratios that characterize ecosystem retrogression.

5. *Synthesis.* Our results suggest that retrogression, and associated changes in nutrient availability and soil N to P ratios, is driven mainly by longer-term pedogenic processes as opposed to shorter-term effects of plant communities on soil N and P availability. More generally, they illustrate the

value of long-term and large-scale experimental manipulations of plant communities for showing how effects of biodiversity loss on ecosystem properties vary across contrasting ecosystems.

Key-words: Boreal forest, chronosequence, environmental gradient, nitrogen, phosphorus, plant species effects, removal experiment, retrogression, succession

Introduction

Following catastrophic ecological disturbances, primary or secondary succession occurs, and for the first few hundred years this involves ecosystem development to a maximal biomass phase. This development is usually characterized by the increasing availability of nutrients for plants, and accumulation of plant biomass and soil organic matter (Odum 1969; Walker & del Moral 2003). However, in the long term absence of major disturbances this maximal biomass phase does not persist, and in the time scale of millennia and beyond, a decline or retrogressive phase occurs in which there is a reduction in soil fertility, plant productivity, and eventually standing plant biomass (Vitousek 2004; Wardle *et al.* 2004; Peltzer *et al.* 2010). This long term decline has now been characterized for several contrasting chronosequences worldwide with greatly differing macroclimate and geology, and that span from the boreal zone to the tropics (Wardle *et al.* 2004; Selman & Hart 2010; Laliberté *et al.* 2012; Wardle *et al.* 2015; Laliberté *et al.* 2017). An important driver of this retrogression is a long-term decline in plant-available nutrients, through net loss of phosphorus (P) from parent material and occlusion of phosphorus which remains (Walker & Syers 1976; Vitousek 2004; Turner & Laliberté 2015), and in some instances through the binding of nitrogen (N) into chemical forms that are less accessible for plant uptake (Northup *et al.* 1995; Clemmensen *et al.* 2015).

Most studies on declines in soil fertility during retrogression have focused on changes that occur during pedogenesis and alterations in the chemical forms of major nutrients (Walker &

Syers 1976; Vitousek 2004; Turner *et al.* 2007; Peltzer *et al.* 2010). However, there are plausible reasons as to why vegetation changes could also conceivably contribute to the reduced nutrient availability that is observed during retrogression. It is well known that plant species composition can be a major driver of soil fertility especially in low diversity systems or when individual plants dominate the ground under them, due to high variation among species in the quality and quantity of their litter inputs (Hobbie 1992), their impacts on associated soil microbial communities (Griffiths *et al.* 1992), and their depletion of nutrients in the soil (Huston & De Angelis 1994). Further, plant community composition and functional trait spectra often show large changes as retrogression proceeds (Richardson *et al.* 2004; Hayes *et al.* 2014), with a shift towards plants that produce litter that is more defended and decomposes more slowly (Crews *et al.* 1995; Wardle *et al.* 2009; Lagerström *et al.* 2013), and that support associated fungal communities that produce recalcitrant necromass (Clemmensen *et al.* 2013). As such, it is plausible that shifts towards plants with more resource-conservative strategies during retrogression could feed back to reduced availability of soil nutrients. However, to demonstrate this convincingly would require formal experimentation, and such experimental tests are currently lacking.

In the present study, we focused on an ongoing long term plot-based experiment established across a well-characterized retrogressive chronosequence involving 30 forested lake islands in the boreal forested zone of northern Sweden (Wardle *et al.* 1997; 2012). These islands vary in time since their most recent wildfire, and collectively represent a post-fire chronosequence of 5000 years duration (Wardle *et al.* 2004). With increasing time since fire soil nutrient availability declines, leading to changes in the plant community towards species with more resource-conservative functional traits, and impairment of decomposition processes, plant productivity and standing plant biomass (Wardle *et al.* 2003; 2012; Kumordzi *et al.* 2015; Clemmensen *et al.* 2015). The experiment we used involves full factorial manipulations (by experimental removals) of three plant functional groups (tree roots, dwarf shrubs, and mosses), and of three dominant dwarf shrub species (*Vaccinium myrtillus*, *Vaccinium vitis-idaea* and

Empetrum hermaphroditum), on all 30 islands (Wardle & Zackrisson 2005; Fanin *et al.* 2018, 2019; Kardol *et al.* 2018). Here, *V. myrtillus* is the most resource-acquisitive of the three species and it declines during retrogression, while *E. hermaphroditum* is the most resource-conservative and it increases during retrogression. The experiment was initiated in 1996 and here we report on results obtained from it 19 years after set-up. This experiment is the longest running plant diversity manipulation experiment in existence across contrasting ecosystems, and it provides unique opportunities for assessing how plant communities affect soil nutrient availability as retrogression proceeds.

For this experiment we took measurements in every plot of total soil N and P, soil mineral N and P, absorption of mineral N and P by ionic resin capsules, and total foliar N and P of each dwarf shrub species present. Because we know from earlier work in this chronosequence that during retrogression there is a shift towards plant species and associated fungal communities that are more resource-conservative (Wardle *et al.* 2003; 2012; Lagerström *et al.* 2013; Clemmensen *et al.* 2015), we predicted that our experimental plant removals would have increasingly positive effects on N and P availability as retrogression proceeds. Specifically, we sought to test each of three hypotheses. First, for the plant functional group removals, we hypothesized that removal of each of the three groups would have stronger positive effects on available soil N and P levels with increasing time during retrogression. Second, for the species removal experiment, we hypothesized that across the chronosequence, removal of the most resource-conservative shrub species (*E. hermaphroditum*) which increases during retrogression would have stronger positive effects on available soil N and P than would the most acquisitive species (*V. myrtillus*) which declines during retrogression. Third, we hypothesized that the increasingly positive effects of plant removals on soil N and P availability as retrogression proceeds would be mirrored aboveground in terms of increased foliar N and P concentrations in the shrub community. By testing these three hypotheses in combination, we sought to better understand how plant communities may drive the availability of nutrients during retrogression.

Materials and methods

STUDY SYSTEM, EXPERIMENTAL SET-UP AND FIELD SAMPLING

The study system consists of 30 islands in lakes Hornavan and Uddjaure in northern Sweden (65°55'N to 66°09'N; 17°43'E to 17°55'E). The mean annual precipitation is 750 mm, and the mean temperature is +13 °C in July and -14 °C in January. All islands were formed following the retreat of land ice about 9000 years ago. The only major extrinsic factor that varies 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 (Wardle *et al.* 1997, 2003).

Previous studies on these islands have shown that as they become smaller and time since fire increases, they enter a state of 'ecosystem retrogression' (Peltzer *et al.* 2010) in which there is a reduction in soil N and P availability, an increase in soil N to P and C to P ratios, and a decline in plant biomass and ecosystem productivity (Wardle *et al.* 2004; 2012) (Table S1).

Consistent with our previous work in this study system (e.g. Wardle & Zackrisson 2005; Fanin *et al.* 2018, 2019; Kardol *et al.* 2018), we divided the 30 islands into three size classes with 10 islands per class: large (> 1.0 ha), medium (0.1–1.0 ha) and small (< 0.1 ha), with a mean time since last major fire of 585, 2180 and 3250 years, respectively. The use of size classes in this way enables simple statistical testing of interactive effects of island size with other factors such as experimental treatments. All islands have burned at least 60 years ago (and up to 5350 years ago) and so have not been subjected to differing fire disturbance regime for several decades prior to the present study. The overstorey vegetation of the island system is dominated by *Pinus sylvestris*, *Betula pubescens* and *Picea abies*, which achieve their greatest relative biomass on large, medium and small islands, respectively. The understory vegetation is dense and short (< 20 cm) and dominated by the dwarf shrubs *Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Empetrum*

149 *hermaphroditum* (which achieve their greatest relative biomass on large, medium and small
 150 islands, respectively) and the understorey feather mosses *Hylocomium splendens* and *Pleurozium*
 151 *schreberi* (which are abundant for all island size classes). The vascular plant species that dominate
 152 on small islands (notably *P. abies* and *E. hermaphroditum*) have resource-conservative strategies
 153 rather than resource-acquisitive strategies, relative to species that dominate on large islands
 154 (Wardle *et al.* 2012; Kumordzi *et al.* 2015).

155 Here, we used an ongoing long term plant removal experiment, as described in Wardle &
 156 Zackrisson (2005), Gundale *et al.* (2010), Fanin *et al.* (2018, 2019) and Kardol *et al.* (2018).
 157 Removal experiments are a type of biodiversity manipulation recognized as a powerful tool for
 158 investigating the effects of subsets of the resident flora on ecological processes in natural
 159 ecosystems (Díaz *et al.* 2003; McLaren & Turkington 2011; Li *et al.* 2018). In August 1996, 14
 160 plots were established on each of the 30 islands (420 plots in total), each representing a different
 161 removal treatment. The experiment consists of two components: (1) a plant functional group
 162 removal component that involves a full factorial combination of three different plant functional
 163 group removals (eight treatments in total); that is, tree root removal (performed by root
 164 trenching), ericaceous shrub removal (performed manually) and feather moss removal (performed
 165 manually) and (2) a plant species removal component that involves a full factorial combination of
 166 three ericaceous dwarf shrub species removals (eight treatments in total; two in common with the
 167 functional group removal component); that is, removal of *V. myrtillus*, removal of *V. vitis-idaea*
 168 and removal of *E. hermaphroditum*. The three plant functional groups represent > 99% of all plant
 169 biomass present on the islands, while the three shrub species represent > 97% of all dwarf shrub
 170 biomass present (Wardle & Zackrisson 2005). There are few other types of plants in the study
 171 system, so when all shrubs and mosses are removed the plots remain largely free of higher plants.
 172 This design allows assessment of the contributions of all possible interactions among functional
 173 groups and among major species within a functional group to ecosystem processes at local spatial
 174 scales across the island size gradient. Removal treatments are implemented on each plot each year

(in its nineteenth year on the sampling date), and we have shown that the legacy effects of removal disturbances had mostly ceased by 2003, i.e., by the seventh year (Wardle & Zackrisson 2005). All plots are 55 cm × 55 cm, but only the inner 45 cm × 45 cm area is measured. Because these soils are nutrient-limited, concentrations of mineral N and P are low and mineral nutrients are unlikely to travel across buffer zones between adjacent plots; this is borne out through an ongoing fertilizer experiment that is near to these plots (Wardle *et al.* 2016), where we did not find large amounts of mineral N and P in control (unfertilized plots) even when high fertilizer additions caused very large increases in mineral N and P in adjacent treatment plots. 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 (Wardle *et al.* 2012).

FIELD SAMPLING AND VEGETATION MEASUREMENTS

Field sampling of soil for the present study is as described by Fanin *et al.* (2018). From 3 August to 15 August 2015, five soil humus cores (3 cm diameter, 10 cm depth) were sampled per plot. One of these cores was taken in the centre of the plot for determining bulk density so that soil nutrient measures could be expressed on a per area basis. The remaining four cores (each taken in one corner of the plot 20 cm from the plot centre) were collected for measurements of soil C and total and mineral N and P. Further, from each plot we collected a sample of at least 20 leaves produced in the current year's growing season from each of the three dwarf shrub species (*V. myrtillus*, *V. vitis-idaea* and *E. hermaphroditum*) whenever present in the plot, for nutrient analyses.

Over 3-15 August 2015, we also quantified each of the dwarf shrub species in each plot using the point intercept method, as described by Wardle & Zackrisson (2005) and Kardol *et al.* (2018). For each plot, we recorded the total number of times each species present was intercepted

by 100 downwardly projected points equally distributed throughout each plot. This provides a measure of cover density of each species, and within species is strongly correlated with standing biomass (Wardle *et al.* 2003). We therefore converted cover data for each species to standing biomass using previously established calibration equations (Wardle *et al.* 2003).

NUTRIENT MEASUREMENTS

For each soil sample measurements were made of total C, N, P, $\delta^{15}\text{N}$, nitrate, ammonium and phosphate. Total C, N and $\delta^{15}\text{N}$ were measured by combustion and thermal conductivity detection using a Flash 1112 Elemental Analyzer (Thermo Scientific, Waltham, MA, USA) linked to an Isotope Ratio Mass Spectrometer. Total P was measured by combustion and dissolution of ash in 1 M H_2SO_4 , followed by phosphate detection using automated molybdate colorimetry on a Lachat Quikchem 8500 analyzer (Milwaukee, WI, USA). Ammonium, nitrate and phosphate were measured on a subsample of soil extracted by KCl by colorimetry on an Auto-Analyzer III (Omni Process, Solna, Sweden). These data were used to calculate the ratios of C to N, C to P, N to P, mineral N (ammonium + nitrate) to total N, phosphate P to total P, and mineral N to mineral N + mineral P. For each plot total and mineral nutrient concentrations were converted to a per unit area basis to 10 cm soil depth by using the measurement from that plot of humus bulk density.

For all dwarf shrub leaf samples, N and P concentrations were determined colorimetrically from micro-Kjeldahl digests, and the N to P ratio was also determined. In addition, for each plot we determined community-weighted values of N, P, and N to P ratio by using the following equation according to Garnier *et al.* (2007):

$$\text{nutrient}_{agg} = \sum_{i=1}^n p_i \times \text{nutrient}_i$$

where nutrient_{agg} is the aggregated (or weighted average) value of the nutrient variable (N, P or N to P ratio) for all species collected in that plot, p_i is the biomass of species i as a

proportion of the total biomass for all vascular plant species collected in that plot, and
 nutrient_i is the value of the nutrient variable for species i .

One mixed bed ionic resin capsule (PST1 capsule, Unibest, Bozeman, USA) was buried at
 5 cm depth in the centre of each plot during August 3-18 2014. These capsules were left for one
 year and harvested over August 3-15 2015 for measurement of ammonium, nitrate and phosphate;
 these measurements were used as an integrated measure of the supply rate of these nutrients from
 the soil solution (De Luca *et al.* 2002). Ionic capsules were extracted using three consecutive
 rinsings of 10 mL 1 M KCl (30 mL total) and nutrient concentrations were reported on a per
 capsule basis (Gundale *et al.* 2014). For each plot this data was also used to calculate the ratio of
 mineral N to mineral N + mineral P in the capsule.

DATA ANALYSIS

For all response variables we analyzed the data separately for the functional group removal and
 species removal components of the experiment, using linear mixed models, as described in Fanin
et al. (2018, 2019). For the functional group component we used 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, with island identity included as a random effect to account for the natural
 variation among ecosystems. For the species removal component the analyses were performed the
 same but with species removals (i.e., *V. myrtillus* removal, *V. vitis-idaea* removal and *E.*
hermaphroditum removal) as subplot factors. Post-hoc testing using Tukey's test at $\alpha = 0.05$ was
 then used to help assess differences among island size classes or among removal treatment
 combinations. All statistical analyses were done using R version 3.1.1 (R Development Core
 Team 2009).

Results

FUNCTIONAL GROUP REMOVAL EFFECTS ON SOIL N AND P

Total C and N per unit area were both significantly enhanced overall by shrub removal (Table S2), by 8.3% and 7.9% respectively. Meanwhile, P per unit area was significantly higher overall when mosses were removed (mean \pm SE = 6.07 ± 0.16 g/m²) than when they were present (5.76 ± 0.14 g/m²). There were also interactive effects of tree root removal, shrub removal and island size class on both N and P per unit area (Table S2, Fig. S1). For N this was because tree root removal effects were most positive on large islands where shrubs were removed, and for P this was because tree root removal effects were most negative on medium islands where shrubs were removed (Fig. S1). Soil C to N ratios were affected by island size (i.e., 21.3% greater on large than small islands), and by tree root removal, the interaction of shrub removal and island size class, and the interaction of all three factors (Table S2) because positive effects of shrub removal were greatest on large islands and when tree roots were not removed (Fig. 1). Soil C to P ratios were stimulated overall by 11.1% by shrub removal but were otherwise unresponsive to treatments (Table S2, Fig. 1). Soil N to P ratios were 28.5% greater on small than large islands, and were promoted by 6.0% by tree root removal and by 7.9% by shrub removal; there was also a significant interactive effect between island size and shrub removal because shrub removal effects were greater on small islands (Table S2, Fig. 1). Meanwhile, soil $\delta^{15}\text{N}$ was enhanced by 17.2% overall by shrub removal and 7.6% by moss removal (Table S2, Fig. 2). Further, there was an interactive effect of island size and tree root removal on soil $\delta^{15}\text{N}$ because removals had their strongest negative effects on medium islands (Table S2, Fig. 2).

Mineral N per unit area and the mineral N to total N ratio were both substantially higher on medium than on large or small islands, and both measures were stimulated overall by ca. 20% by shrub removal (Table S2, Fig. 3). There were also significant interactive effects of island size and tree root removal on both variables because the removals had their largest negative effects on

medium islands. Meanwhile, mineral P per unit area and the mineral P to total P ratio were both least on the small islands, and both measures were reduced overall by ca. 37% when shrubs were removed, and by ca. 32% when tree roots were removed (Table S2, Fig. 3). There were also significant interactive effects of island size and tree root removal on both variables because the removal had their weakest effect on small islands. The ratio of mineral N to mineral N + P ratio was significantly stimulated overall by 11.2% by tree root removal, and by 21.5% by shrub removal (Table S2, Fig. 3).

Nutrients retrieved by resin capsules were strongly affected only by shrub removal (Table S2). As such, resin N and P were reduced overall by 13.0% and 38.8% by shrub removals respectively, while the ratio of resin N to resin N+P was promoted by 11.2% (data not presented).

SHRUB SPECIES REMOVAL EFFECTS ON SOIL N AND P

Total C per unit area was significantly greater when *V. vitis-idaea* was removed (mean \pm SE = 4368 ± 399 g/m²) than when it was present (4156 ± 379 g/m²), but no other treatment effects were significant (Table S3). Total N per area was affected only by island size through being highest on small islands, while P per unit area was unaffected by any factor (Table S3). Soil C to N ratio was greatest on large islands, and reduced overall by 3.6% by the removal of *V. myrtillus* (Table S3, Fig. 4). Soil C to P ratio and N to P ratios were both enhanced overall by *V. vitis-idaea* removals, by 10.6% and 8.0% respectively (Table S3, Fig. 4). Soil N to P ratios were also greatest on small islands, and there was an interactive effect between island size and *E. hermaphroditum* removal because removal promoted N to P ratios on small islands (by 5.6%) but not on medium or large islands (data not presented). Meanwhile, soil $\delta^{15}\text{N}$ was enhanced overall by 8.8% by *V. vitis-idaea* removal, and was marginally non-significantly greater with *V. myrtillus* removal and on medium islands (Table S3, Fig. S2).

Mineral N and the mineral N to total N ratio were both impacted only by island size (Table S3), through being greatest on medium islands (data not presented). Meanwhile, mineral P and the mineral P to total P ratio were both least on the small islands, and both were reduced overall by ca. 17% by removal of *V. myrtillus* and ca. 18% by removal of *V. vitis-idaea* (Table S3, Fig. 5). For the mineral P to total P ratio there was also a significant interactive effect between *V. myrtillus* removal and island size, because removal effects were greatest on large and medium islands. The ratio of mineral N to mineral N+P was enhanced by 6.5% and 7.7% by removal of *V. myrtillus* and *V. vitis-idaea* respectively (Table S3, Fig. 5).

There were no significant effects of removals on data obtained from the resin capsules, except for a very weak three-way interactive effect of island size, *V. myrtillus* removal and *E. hermaphroditum* removal on resin P (Table S3, data not presented). However, there were two-way interactive effects of all possible species pairs on the ratio of resin N to resin N+P (Table S3, Fig. S3), with the highest value occurring when all three species were removed together and the lowest values when particular pairs of species were removed.

REMOVAL EFFECTS ON PLANT BIOMASS AND FOLIAR N AND P

Biomass of *V. myrtillus*, *V. vitis idaea* and *E. hermaphroditum* were overall greatest on large, medium and small islands, respectively (Tables S4, S5, Fig. S4). As such, biomass for *V. myrtillus* (mean \pm SE) overall was 65.5 ± 8.8 g/m², 22.2 ± 5.0 g/m², and 22.5 ± 4.7 g/m² for large, medium and small islands, respectively. Meanwhile, *V. vitis-idaea* biomass was 27.6 ± 4.6 g/m², 67.6 ± 9.3 g/m², and 39.2 ± 6.2 g/m² for large, medium and small islands, respectively, and *E. hermaphroditum* biomass was 6.2 ± 0.7 g/m², 2.7 ± 0.3 g/m², and 64.5 ± 4.8 g/m² for large, medium and small islands, respectively. Total shrub biomass and biomass of each species were unaffected by tree root and moss removals (Table S4). Total shrub biomass was however reduced by the removal of each of the three species (Table S5, Fig. S4), and there were significant

interactive effects between the removal of each species and island size class because loss of each species had its strongest effects on the island size class that it dominated (Fig. S4).

For the functional group removal component of the experiment (Table S4), island size class affected *V. myrtillus* foliar N to P ratio (mean \pm SE = 7.8 ± 0.1 on both large and medium islands; 9.3 ± 0.2 on small islands), *E. hermaphroditum* foliar P (0.206 ± 0.002 %, 0.190 ± 0.004 % and 0.183 ± 0.003 % on large, medium and small islands, respectively) and community-level foliar N (1.25 ± 0.03 %, 1.01 ± 0.02 % and 1.16 ± 0.02 % on large, medium and small islands, respectively). Functional group removals generally had no effects on shrub N or P variables (Table S4), however, tree root removal significantly reduced *E. hermaphroditum* foliar N overall (1.17 ± 0.11 % and 1.26 ± 0.12 % with and without removal, respectively), and moss removal reduced the community-level foliar N to P ratio (7.47 ± 0.11 and 7.18 ± 0.12 with and without removal, respectively).

For the species removal component of the experiment (Table S5), the foliar N to P ratio of *V. vitis-idaea* was reduced by removal of both *V. myrtillus* (7.01 ± 0.14 and 6.55 ± 0.11 with and without removal, respectively) and *E. hermaphroditum* (6.98 ± 0.12 and 6.59 ± 0.13 with and without removal, respectively). Further, *V. vitis-idaea* removal reduced both *E. hermaphroditum* foliar N (1.20 ± 0.02 % and 1.24 ± 0.02 % with and without removal respectively) and foliar P (0.185 ± 0.003 % and 0.192 ± 0.003 % with and without removal respectively).

Most main effects of the removal of all three species on community-level foliar N and P, as well as several of their interactive effects with island size, were highly statistically significant (Table S5, Fig. 6). As such, *V. myrtillus* removal reduced community foliar N and P with this effect being greatest on large islands, *V. vitis-idaea* removal enhanced community foliar N and P with this effect being greatest on medium and small islands, and *E. hermaphroditum* removal enhanced community foliar N but only on medium and small islands. Further, *V. myrtillus* removal reduced community foliar N to P ratios on all island size classes, *V. vitis-idaea* removal

promoted community N to P ratios but most strongly on medium islands, and *E. hermaphroditum* removal promoted community N to P ratios but most strongly on small islands (Table S5, Fig. 7).

Discussion

EFFECTS OF FUNCTIONAL GROUPS ON SOIL N AND P

Of the three functional groups, dwarf shrubs consistently had the strongest effects on both soil N and P variables, which is in line with their high productivity and turnover in our study system; despite comprising about 5% of total plant biomass they account for nearly half of total net primary productivity (Wardle *et al.* 2012). As such, throughout the chronosequence they reduced total and mineral soil N while promoting mineral soil P. For the resin capsules, which quantify uptake of nutrients at a specific position in the manner that might be encountered by a plant root (Pampolino & Hatano 2000; Qian & Schoneau 2002), we found shrubs to promote both N and P uptake, though with much greater promotion of P. Two likely mechanisms for this are that the dwarf shrubs actively promote microbial biomass and activity (Wardle & Zackrisson 2005), and that these shrubs support ericoid mycorrhizal fungi that are highly capable of extracting recalcitrant compounds in the humus layer (Read *et al.* 2004), which in turn would lead to greater mineralization of N and P from the soil humus. In the case of N, this promotion of N mineralization would enhance N uptake by ericaceous shrubs, which may cause the reduction of humus $\delta^{15}\text{N}$ that we observed. It is well known that ericaceous shrubs are depleted in ^{15}N relative to other plant functional types (Craine *et al.* 2009). Thus inputs of new plant derived organic matter associated with ericaceous shrub production should be ^{15}N depleted relative to N losses that occur during N mineralization processes, which in turn would lead to the long term decline in soil organic matter $\delta^{15}\text{N}$ through time, as we observed (Högberg 1997, Houlton *et al.* 2006). The differential effects of shrubs on soil N and P led to them reducing ratios of total N to P, mineral N

to mineral N + P and resin N to resin N + P, providing consistent evidence that shrubs change soil stoichiometry by depleting soil N relative to P.

Tree roots also had some effects on soil N and P measures, though generally lesser than the shrubs, and when they did occur they generally worked in the same direction. The strongest effect was for the promotion of soil mineral P by tree roots, which was likely because of greater mineralization due to ectomycorrhizal fungi associated with the roots, and turnover of labile necromass produced by the fungi (Clemmensen *et al.* 2015). In contrast to shrubs, tree roots only promoted $\delta^{15}\text{N}$ values. This can be explained by tree roots and associated ectomycorrhizal fungi redistributing ^{15}N enriched N from deeper soil layers to the surface (Hobbie and Högberg 2012), or alternatively as a result of recalcitrant ectomycorrhizal necromass accumulation in surface soils which would be ^{15}N enriched relative to soil N losses (Craine *et al.* 2009, Hobbie *et al.* 1999). Feather mosses reduced total soil P but had little effect on measures of soil total or mineral N. This is despite their role in governing decomposition processes and in serving as an important source of ecosystem N input through N_2 -fixation by associated symbiotic cyanobacteria (Lindo *et al.* 2013, Jonsson *et al.* 2015). However, mosses did reduce $\delta^{15}\text{N}$, which could be due either to increased inputs of fixed N_2 that would have a lower $\delta^{15}\text{N}$ signature, or reduced N mineralization and turnover and thus decreased fractionation of N (Hyodo & Wardle 2009).

Despite relatively strong effects of plant functional group removals on soil N and P variables, effects of removals varied with island size class (as revealed by significant interactions between removal factors and size class) in relatively few cases. Further, when these effects did occur then they were usually strongest on the most productive medium or large islands, and this included effects of tree root and/or shrub removals on soil total N and P, C to N ratio, $\delta^{15}\text{N}$, and various measures of mineral N and P. These results align with previous work pointing to removals of subsets of the vegetation from these plots having the strongest effect on the remaining vegetation on medium or large islands (Kardol *et al.* 2018). This suggests that ecological effects of plant identity may be greatest in ecosystems that are more productive and where plant nutrient

uptake and litter inputs are therefore highest. These findings are generally contradictory to our first hypothesis predicting greater positive effects of removals on nutrients on small islands that had undergone retrogression. However, our finding that positive effects of tree roots on soil mineral N and P were generally strongest on medium and large islands suggests that retrogression on the small islands could be associated with the effects of tree roots on available nutrients being less positive. This is in line with previous work in this study system showing that tree root-associated fungal mycorrhizal communities on larger islands are likely to produce more labile and less defended necromass that is more readily mineralized (Clemmensen *et al.* 2015). Meanwhile, the only effect of removals that was actually largest on the small islands was that of shrub removal on soil N to P ratios, and this involved the shrubs suppressing the ratio. Since the soil N to P ratio increases during retrogression, this means that the shrubs have an effect on this ratio that works in the opposing direction to the changes that occur in soil during retrogression. The increase in the soil N to P ratio that often characterizes retrogression (Wardle *et al.* 2004; Peltzer *et al.* 2010), including in our study system, therefore cannot be driven by effects of plants during the 19-year time scale of our study, but is instead likely determined by pedogenic changes that operate over a much longer period (Walker & Syers 1976; Vitousek 2004).

We recognize that while changes in mineral N and P caused by manipulation of plant groups may be rapid, changes in total soil N and P (and their ratio) will be considerably slower. Indeed, after 7 years of removals in this experiment there was no detectable change in total soil N and P caused by the removals (Wardle and Zackrisson 2005), while changes were clearly detectable after 19 years (this study). Although no other comparable study of this duration exists, it is conceivable that as the experiment continues to run for longer, stronger effects of removals on total soil N and P may arise. However, there is no reason to expect that these stronger effects would work in a different direction to that which we are already detecting after 19 years.

EFFECTS OF SHRUB SPECIES ON SOIL N AND P

The shrubs had the strongest effect of all three functional groups, and in our experiment they are represented by three species, *V. myrtillus*, *V. vitis-idaea* and *E. hermaphroditum*. Of these, the two *Vaccinium* species, which are more resource-acquisitive than *E. hermaphroditum*, also had the strongest effects. These effects worked in a similar way as for the effects of all shrubs: *V. myrtillus* enhanced soil C to N ratios, *V. vitis idaea* reduced C to P and N to P ratios, and both species promoted mineral P and reduced mineral N to mineral N + P ratios.

There were few interactive effects between removals of any of the three species and island size, although we did find that positive effects of *V. myrtillus* on soil mineral P were greatest on the medium and large islands where it is most abundant, suggesting that retrogression on small islands could be linked in part to less-positive effects of *V. myrtillus*. In that light, *V. myrtillus* has a high relative turnover of biomass, and produces high quality litter that stimulates decomposition processes relative to other plant species in the study system (Wardle *et al.* 2003); it also has its lowest abundance on small islands. Further, we found little support for our second hypothesis predicting the greatest positive effects of species removals when *E. hermaphroditum* is lost from the small islands that it dominates. We did however find a greater negative effect of *E. hermaphroditum* on soil N to P ratios on small islands, and this aligns with what we found for the effects of total shrub removals. This means that despite producing poor quality and well-defended litter that suppresses soil processes (Nilsson *et al.* 1993; Tybirk *et al.* 2000), an increasing abundance of this highly resource-conservative species during retrogression does not contribute to the increase over time in humus N to P ratios and may actually serve to counteract it.

FUNCTIONAL GROUP AND SPECIES EFFECTS ON FOLIAR N AND P

Despite removal treatments sometimes exerting strong effects on belowground N and P variables, there were few effects of removals on foliar nutrients for each of the three shrub species. When

these did occur, they always revealed weakly positive responses of foliar N, P or N to P ratios to the presence of other shrub species or functional groups. This only partially aligns with what we observed belowground, where plant functional groups or species sometimes promoted the availability of N and P, but consistently only reduced ratios of N availability to P availability. Further, there was virtually no evidence for these effects varying among island size classes, meaning that in contrast to what we sometimes observed belowground, any effects of removals on foliar nutrients are constant across highly contrasting ecosystems. The relatively weak response of foliar nutrient levels of these species, both to removal treatments and to the variety of environmental conditions that occur across island size classes, suggests that their nutrient status has low plasticity relative to most other plant species (see Siefert *et al.* 2015) and that they respond to variation in soil nutrients mostly by altering their biomass rather than their nutrient levels. This result also points to contrasting responses of the aboveground and belowground subsystems, in line with what has sometimes been found for other boreal and subarctic ecosystems (Marshall *et al.* 2011; Wardle *et al.* 2013), and is inconsistent with our third hypothesis predicting that responses of foliar N and P to plant removals should mirror belowground responses of N and P availability.

We did however find clear effects of shrub species removal on community-level measures of foliar nutrients for the remaining shrubs. This was driven by differences in nutrients between and not within species, and by the differences among species being large (averaged across all plots in the experiment, foliar N was 1.53%, 0.90% and 1.21%, foliar P was 0.187%, 0.038% and 0.189%, and N to P ratio was 8.27, 6.93 and 6.44, for *V. myrtillus*, *V. vitis-idaea* and *E. hermaphroditum* respectively). This means that loss of *V. myrtillus* will inevitably depress community-level measures of foliar N and P and promote N to P ratios, while loss of *V. vitis-idaea* will elevate community-level foliar N and P. Further, there were strong interactive effects of species removals with island size on community-level foliar nutrient measures, but these were driven by species turnover across the gradient, with the loss of each species having its strongest

effect on the island size class for which it made up the greatest proportion of total biomass. As such, although within- and across-species trait variation often both drive community-level trait variation (Albert *et al.*, 2010; Violle *et al.* 2012), the low intraspecific plasticity of foliar nutrients in our study means that only across-species variation has an important role. Our finding that effects of removals on community-level foliar N and P are determined by the relative abundance of the species that is removed and the magnitude of its difference from the remaining community, and not by changes in belowground N and P, also contradicts our third hypothesis.

CONCLUSIONS

We found by exploring the effects of plant removals on a number of measures of ecosystem N and P availability that although plant removal effects sometimes varied across the island size gradient, this seldom happened in a way that explains the decline in nutrient availability and increase in soil N to P ratios that characterizes ecosystem retrogression. The only exceptions were the less positive effects of tree roots on soil mineral N and P, and of *V. myrtillus* on soil mineral P, on small islands. As such, we propose that retrogression is driven mostly by longer-term pedogenic processes as opposed to direct effects of plant communities on these properties.

Our results have several implications. First, they contribute more generally to understanding how effects of biodiversity loss may vary across natural ecosystems. Despite some claims to the contrary (e.g., Duffy 2009), there is growing recognition that the effects of biodiversity loss can indeed vary greatly among ecosystems (Ratcliffe *et al.* 2017; Baert *et al.* 2018). The findings of this work, in combination with other recent results from this experiment (Fanin *et al.* 2018, 2019, Kardol *et al.* 2018), reveal how large scale, long term experiments repeated across contrasting systems, offer robust insights about the mechanisms by which biodiversity loss impacts on ecosystems is mediated by environmental context. Second, they show that effects of plant biodiversity loss depend both on which floristic components are lost and the

ecosystems from which they are lost, and that this is not a simple function of the biomass of the components that are lost. For example, the loss of *Vaccinium* species which occupy only a few percent of total plant biomass had a particularly strong impact on our response variables. This contrasts with the predictions of the ‘mass ratio hypothesis’ of Grime (1988), but is in line with recent suggestions that minor components of the community often exert disproportionate effects on ecosystems (Isbell *et al.* 2017). We recognize however that our experiment only removed tree root inputs and not leaf litter; long term productivity measurements reveal that tree litter inputs are about the same as the sum of shrub and moss litter inputs on large islands and half the sum of shrub and moss inputs on small islands (Wardle *et al.* 2012). Finally, they help understand how biotic controls of ecosystem properties change as ecosystems change over time, for example during succession or as a result of disturbances or global change. In the boreal zone, human-induced changes in climate, land use and fire regime are all likely to greatly modify forest ecosystems; our results suggest that impacts of plant functional groups and species on ecosystem properties, including on N and P availability, may show considerable shifts as these ecosystems change.

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Data accessibility

Data are available from the Dryad Digital Repository

<http://datadryad.org/resource/doi:10.5061/dryad.mr36sp2> (Wardle *et al.* 2019)

Author contributions

D.A.W. acquired the necessary funding and designed, set up and maintained the experiment. All authors participated in the field sampling, and in planning the sampling and analyses. N.F. performed all statistical analyses and prepared the figures. D.A.W. wrote the first draft of the manuscript with subsequent input and improvements from all authors.

References

Albert, C.H., Thuiller, W., Yoccoz, N.G., Soudant, A., Boucher, F., Saccone, P. & Lavorel, S. (2010). Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology*, **98**, 604–613.

Baert, J.M., Eisenhauer, N., Janssen, J.R. & De Laender, F. (2018) Biodiversity effects on ecosystem functioning respond unimodally to environmental stress. *Ecology Letters*, **21**, 1191-1199.

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H.,... Lindahl, B. D. (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, **339**, 1615-1618.

- 555 Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D. A. & Lindahl, B. D. (2015)
 556 Carbon sequestration is related to mycorrhizal fungal community shifts during long term
 557 succession in boreal forests. *New Phytologist*, **205**, 1525-1536.
- 558 Craine, J.M., Elmore, A.J., Aidar, M.P.M., Bustamante, M., Dawson, T.E., Hobbie, E. A.,
 559 Wright, I.J. (2009). Global patterns of foliar nitrogen isotopes and their relationships with
 560 climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New*
 561 *Phytologist*, **183**, 980-992.
- 562 Crews, T.E., Kitayama, K, Fownes, J.H., Riley, R.H., Herbert, D.A., Mueller- Dombois, D. &
 563 Vitousek, P.M. 1995. Changes in soil phosphorous fractions and ecosystem dynamics
 564 across a long chronosequence in Hawaii. *Ecology*, **76**, 1407–1424.
- 565 De Luca, T., Nilsson, M.-C. & Zackrisson, O. (2002) Nitrogen mineralization and phenol
 566 accumulation along a fire chronosequence in northern Sweden. *Oecologia*, **133**, 206-214.
- 567 Díaz, S., Chapin, F.S. III, Symstad, A., Wardle, D.A. & Huenneke, L. (2003) Functional diversity
 568 revealed through removal experiments. *Trends in Ecology and Evolution*, **18**, 140-146.
- 569 Duffy, J.E. (2009) Why biodiversity is important to functioning of real-world ecosystems.
 570 *Frontiers in Ecology and the Environment*, **7**, 437–444.
- 571 Fanin, N., Gundale, M.J., Farrell, M., Ciobanu, M., Baldock, J.A., Nilsson, M.-C., Kardol, P. &
 572 Wardle, D.A. (2018) Consistent effects of biodiversity loss on multifunctionality across
 573 contrasting ecosystems. *Nature Ecology and Evolution*, **2**, 269-278.
- 574 Fanin, N., Kardol, P., Farrell, M., Kempel, A., Ciobanu, M., Nilsson, M.-C., Gundale, M. J. &
 575 Wardle, D. A. (2019) Effects of plant functional group removal on structure and function
 576 of soil communities across contrasting ecosystems. *Ecology Letters* (in press, online early;
 577 DOI: 10.1111/ele.13266).
- 578 Garnier, E., Lavorel, S., Ansquer, P., Castro, H., Cruz, P., Dolezal, J. *et al.* (2007) Assessing the
 579 effects of land-use change on plant traits, communities and ecosystem functioning in

- 580 grasslands: a standardized methodology and lessons from an application to 11 European
581 sites. *Annals of Botany*, **99**, 967–985.
- 582 Griffiths, B.S., Welschen, R., van Arendonk, J.J.C.M. & Lambers, H. (1992) The effect of nitrate-
583 nitrogen on bacteria and bacterial-feeding fauna in the rhizosphere of different grass
584 species. *Oecologia*, **91**, 253–259.
- 585 Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects.
586 *Journal of Ecology*, **86**, 902–910.
- 587 Gundale, M. J., From, F., Bach, L. H. & Nordin, A. (2014) Anthropogenic nitrogen deposition in
588 boreal forests has a minor impact on the global carbon cycle. *Global Change Biology*, **20**,
589 276–286.
- 590 Gundale, M.J., Wardle, D.A. & Nilsson, M.-C. (2010) Vascular plant removal effects on
591 biological N-fixation vary across a boreal forest island gradient. *Ecology*, **91**, 1704–1714.
- 592 Hayes, P., Turner, B.L., Lambers, H. & Laliberté, E. (2014). Foliar nutrient concentrations and
593 resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-
594 million-year dune chronosequence. *Journal of Ecology*, **102**, 396–410.
- 595 Hobbie, E.A. & Hogberg, P. (2012) Nitrogen isotopes link mycorrhizal fungi and plants to
596 nitrogen dynamics. *New Phytologist*, **196**, 367–382.
- 597 Hobbie, E.A., Macko, S.A. & Shugart, H.H. (1999) Insights into nitrogen and carbon dynamics of
598 ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia*, **118**, 353–360.
- 599 Hobbie, S.E. (1992) Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution*,
600 **7**, 336–339.
- 601 Houlton, B.Z., Sigman, D. M. & Hedin, L.O. (2006) Isotopic evidence for large gaseous nitrogen
602 losses from tropical rainforests. *Proceedings of the National Academy of Sciences of the*
603 *USA*, **103**, 8745–8750.

- 604 Hyodo, F. & Wardle, D.A. (2009). Effect of ecosystem retrogression on stable nitrogen and
 605 carbon isotopes of plants, soils and consumer organisms in boreal forest islands. *Rapid*
 606 *Communications in Mass Spectrometry*, **23**, 1892-1898.
- 607 Högberg, P. (1997) ^{15}N natural abundance in soil-plant systems. *New Phytologist*, **137**, 179-203.
- 608 Huston, M.A. & De Angelis, D.L. (1994) Competition and coexistence: the effects of resource
 609 transport and supply rates. *American Naturalist*, **144**, 854-877.
- 610 Isbell, F., Gonzalez, A., Loreau, M., Cowles, J., Díaz, S., Hector, A. Larigauderie, A. (2017)
 611 Linking the influence and dependence of people on biodiversity across scales. *Nature*, **546**,
 612 65-72.
- 613 Jonsson, M., Kardol, P., Gundale, M.J., Bansal, S., Nilsson, M.-C., Metcalfe, D. and Wardle, D.A.
 614 (2015) Direct and indirect drivers of moss community structure, function and associated
 615 microfauna across a successional gradient. *Ecosystems*, **18**, 154-169.
- 616 Kardol, P., Fanin, N. & Wardle, D.A. (2018) Long-term impacts of species loss on community
 617 properties across contrasting ecosystems. *Nature*, **557**, 710-713.
- 618 Kumordzi, B.B, Wardle, D.A. & Freschet, G.T. (2015) Plant assemblages do not respond
 619 homogenously to local variation in environmental conditions: functional responses differ
 620 with species identity and abundance. *Journal of Vegetation Science*, **25**, 32-45.
- 621 Lagerström, A., Nilsson., M.-C. & Wardle, D.A. (2013) Decoupled responses of tree and shrub
 622 leaf and litter trait values to ecosystem retrogression across an island area gradient. *Plant*
 623 *and Soil*, **367**, 183-197.
- 624 Laliberté, E., Kardol, P., Didham, R.K., Teste, F.P., Turner, B.L. & Wardle, D.A. (2017) Soil
 625 fertility shapes belowground food webs across a regional climate gradient. *Ecology*
 626 *Letters*, **20**, 1273-1284.
- 627 Laliberté, E., Turner, B.L., Costes, T., Pearse, S.J., Wyrwoll, K.-H., Zemunik, G. & Lambers, H.
 628 (2012). Experimental assessment of nutrient limitation along a 2-million year dune

- 629 chronosequence in the southwestern Australia biodiversity hotspot. *Journal of Ecology*,
 630 **100**, 631–642.
- 631 Li, W., Zhang, R., Liu, S., li, W., Li, J., Zhou, H. & Knops, J. M. H. (2018) Effect of loss of plant
 632 functional group and simulated nitrogen deposition on subalpine ecosystem properties on
 633 the Tibetan Plateau. *Science of the Total Environment*, **631**, 289–297.
- 634 Lindo, Z., Nilsson, M.-C. & Gundale, M.J. (2013) Bryophyte-cyanobacteria associations as
 635 regulators of the northern latitude carbon balance in response to global change. *Global*
 636 *Change Biology*, **19**, 2022-2035.
- 637 Marshall, C. B., McLaren, J. R. & Turkington, R. (2011) Soil microbial communities resistant to
 638 changes in plant functional group composition. *Soil Biology and Biochemistry*, **43**, 78–85.
- 639 McLaren, J.R. & Turkington, R. (2011) Biomass compensation and plant responses to 7-years of
 640 plant functional group removals. *Journal of Vegetation Science*, **22**, 503-515.
- 641 Nilsson, M.-C., Högborg, P., Zackrisson, O. & Fengyou, W. (1993) Allelopathic effects by
 642 *Empetrum hermaphroditum* on development and nitrogen uptake by roots and mycorrhizae
 643 of *Pinus silvestris*. *Canadian Journal of Botany*, **71**, 620-628
- 644 Northup, R.R., Zeng, S. Y., Dahlgren, R. A. & Vogt, K. (1995) Polyphenols control of nitrogen
 645 release from pine litter. *Nature*, **377**, 227–229.
- 646 Odum, E.P. (1969) The strategy of ecosystem development. *Science*, **164**, 262– 270.
- 647 Pampolino, M.F. & Hatano, R. (2000) Comparison between conventional soil tests and the use of
 648 resin capsules for measuring P, K and N in two soils under two moisture conditions. *Soil*
 649 *Science and Plant Nutrition*, 46, 461- 471.
- 650 Qian, P. & Schoneau, J.J. (2002) Practical applications of ion exchange resins in agricultural and
 651 environmental soil research. *Canadian Journal of Soil Science*, **82**, 9 – 21.
- 652 Peltzer, D.A., Wardle, D.A., Allison, V.J., Baisden, W.T., Bardgett, R.D., Chadwick, O.A., ...
 653 Walker, L.R. (2010) Understanding ecosystem retrogression. *Ecological Monographs*, **80**,
 654 509-529.

- 655 Ratcliffe, S., Wirth, C., Jucker, T., der Plas, F., Scherer-Lorenzen, M., Verheyen, K. Baeten,
 656 L.. (2017). Biodiversity and ecosystem functioning relations in European forests depend
 657 on environmental context. *Ecology Letters*, **20**, 1414-1426.
- 658 Read, D.J., Leake, J.R. & Perez-Moreno, J. (2004) Mycorrhizal fungi as drivers of ecosystem
 659 processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, **82**, 1243-
 660 1263.
- 661 Richardson, S.J., Peltzer, D.A., Allen, R.B., McGlone, M.S. & Parfitt, R.L. (2004) Rapid
 662 development of phosphorus limitation in temperate rainforest along the Franz Josef soil
 663 chronosequence. *Oecologia*, **139**, 267–276.
- 664 Selmants, P.C. & Hart, S.C. (2010) Phosphorus and soil development: does the Walker and Syers
 665 model apply to semiarid ecosystems? *Ecology*, **91**, 474–484.
- 666 Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., Fajardo, A., , Wardle,
 667 D.A. (2015) A global meta-analysis of the relative extent of intraspecific trait variation in
 668 plant communities. *Ecology Letters*, **18**, 1406-1419.
- 669 Turner, B.L. & Laliberté, E. (2015). Soil development and nutrient availability along a 2 million-
 670 year coastal dune chronosequence under species-rich mediterranean shrubland in
 671 southwestern Australia. *Ecosystems*, **18**, 287–309.
- 672 Turner, B.L., Condron, L.M., Richardson, D.J., Peltzer, D.A. & Allison, V.J. (2007) Soil organic
 673 phosphorus transformations during pedogenesis. *Ecosystems*, **10**, 1166–1181.
- 674 Tybirk, K., Nilsson, M.-C., Michelsen, A., Kristensen, H.L., Shevtsova, A., Tune, S.M.,
 675 Johnsen, I. (2000). Nordic *Empetrum* dominated ecosystems: function and susceptibility to
 676 environmental changes. *Ambio*, **29**, 90–97.
- 677 Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C.,, Messier, J. (2012)
 678 The return of the variance: intraspecific variability in community ecology. *Trends in*
 679 *Ecology and Evolution*, **27**, 244–252.

- 680 Vitousek, P.M. (2004) *Nutrient Cycling and Limitation. Hawaii as a Model System*. Princeton
 681 University Press, Princeton, USA.
- 682 Walker, L.R. & del Moral, R. (2003) *Primary Succession and Ecosystem Rehabilitation*.
 683 Cambridge University Press, Cambridge, UK.
- 684 Walker, T.W. & Syers, J.K. (1976) The fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1–
 685 19.
- 686 Wardle, D.A. & Zackrisson, O. (2005) Effects of species and functional group loss on island
 687 ecosystem properties. *Nature*, **435**, 806-810.
- 688 Wardle, D.A., Bardgett, R.D., Walker, L.R. & Bonner, K.I. (2009) Among- and within-species
 689 variation in plant litter decomposition in contrasting long term chronosequences.
 690 *Functional Ecology*, **23**, 442-453.
- 691 Wardle, D.A., Bellingham, P.J., Kardol, P., Giesler, R. and Tanner, E.V.J. (2015). Coordination
 692 of aboveground and belowground responses to local-scale soil fertility differences between
 693 two contrasting Jamaican rainforest types. *Oikos*, **124**, 285-297.
- 694 Wardle, D.A., Gundale, M. J., Jäderlund, A. and Nilsson, M.-C. (2013) Decoupled long term
 695 effects of nutrient enrichment on aboveground and belowground properties in subalpine
 696 tundra. *Ecology*, **94**, 904-919.
- 697 Wardle, D.A., Gundale, M. J., Kardol, P., Nilsson M.-C. and Fanin, N. (2019) Impact of plant
 698 functional group and species removals on soil and plant nitrogen and phosphorus across a
 699 retrogressive chronosequence. *Journal of Ecology*, doi:10.5061/dryad.mr36sp2.
- 700 Wardle, D.A., Hörnberg, G., Zackrisson, O., Kalela-Brundin, M. & Coomes, D.A. (2003) Long term
 701 effects of wildfire on ecosystem properties across an island area gradient. *Science*, **300**, 972-
 702 975.
- 703 Wardle, D.A., Jonsson, M., Bansal, S., Bardgett, R.D., Gundale, M.J. and Metcalfe, D.B. (2012)
 704 Linking vegetation change, carbon sequestration and biodiversity: insights from island
 705 ecosystems in a long term natural experiment. *Journal of Ecology*, **100**, 16-30.

- 706 Wardle, D. A., Jonsson, M., Mayor, J. R. and Metcalfe, D. B. (2016) Aboveground and
707 belowground responses to long-term nutrient addition across a retrogressive
708 chronosequence. *Journal of Ecology* 104: 545-560.
- 709 Wardle, D.A., Walker, L.R. & Bardgett, R.D. (2004) Ecosystem properties and forest decline in
710 contrasting long-term chronosequences. *Science*, **305**, 509-513.
- 711 Wardle, D.A., Zackrisson, O., Hörnberg, G. & Gallet, C. (1997) Influence of island area on
712 ecosystem properties. *Science*, **277**, 1296-1299.

Figure legends

Fig. 1. Soil humus C:N, C:P and N:P ratios in response to island size class (Large, L; Medium, M; Small, S) and removal versus non-removal of tree roots (-T, +T) and shrubs (-S, +S) after 19 years. Error bars are standard errors. Linear model results reporting statistical analyses of this data are in Table S2. Island size classes topped by the same capital letter do not differ, and within island size classes removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Fig. 2. Stable isotope N concentrations ($\delta^{15}\text{N}$) of humus in relation to island size and removal versus non-removal of tree roots (-T, +T), shrubs (-S, +S) and mosses (-M, +M) after 19 years. Error bars are standard errors. Linear model results reporting statistical analyses of this data are in Table S2. Within each island size class, removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Fig. 3. Humus mineral N (ammonium + nitrate), mineral P (phosphate), and ratios of mineral N to total N, mineral P to total P, and mineral N to (mineral N + mineral P), in response to island size class (Large, L; Medium, M; Small, S) and removal versus non-removal of tree roots (-T, +T) and shrubs (-S, +S) after 19 years. Error bars are standard errors. Linear model results reporting statistical analyses of this data are in Table S2. Island size classes topped by the same capital letter do not differ, and within island size classes removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Fig. 4. Soil humus C:N, C:P and N:P ratios in response to island size class (Large, L; Medium, M; Small, S) and removal versus non-removal of *Vaccinium myrtillus* (-Vm, +Vm) and *Vaccinium vitis-idaea* (-Vv, +Vv) after 19 years. Error bars are standard errors. Linear model results

reporting statistical analyses of this data are in Table S3. Island size classes topped by the same capital letter do not differ, and within island size classes removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Fig. 5. Humus mineral P (phosphate) and ratios of mineral P to total P, and mineral N to (mineral N + mineral P), in response to island size class (Large, L; Medium, M; Small, S) and removal versus non-removal of *Vaccinium myrtillus* (-Vm, +Vm) and *Vaccinium vitis-idaea* (-Vv, +Vv) after 19 years. Error bars are standard errors. Linear model results reporting statistical analyses of this data are in Table S3. Island size classes topped by the same capital letter do not differ, and within island size classes removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Fig. 6. Community level shrub foliar N and P concentrations in response to island size class (Large, L; Medium, M; Small, S) and the removal versus non-removal of *Vaccinium myrtillus* (-Vm, +Vm), *Vaccinium vitis-idaea* (-Vv, +Vv) and *Empetrum hermaphroditum* (-Eh, +Eh) after 19 years. Error bars are standard errors. Linear model results reporting statistical analyses of this data are in Table S5. Island size classes topped by the same capital letter do not differ, and within island size classes removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Fig. 7. Community level shrub foliar N to P ratios in response to island size class and the removal versus non-removal of *Vaccinium myrtillus* (-Vm, +Vm), *Vaccinium vitis-idaea* (-Vv, +Vv) and *Empetrum hermaphroditum* (-Eh, +Eh) after 19 years. Error bars are standard errors. Linear model results reporting statistical analyses of this data are in Table S5. Within each island size class, removal treatments topped with the same lower case letter do not differ, according to Tukey's post-hoc tests at $\alpha = 0.05$.

Figure 1

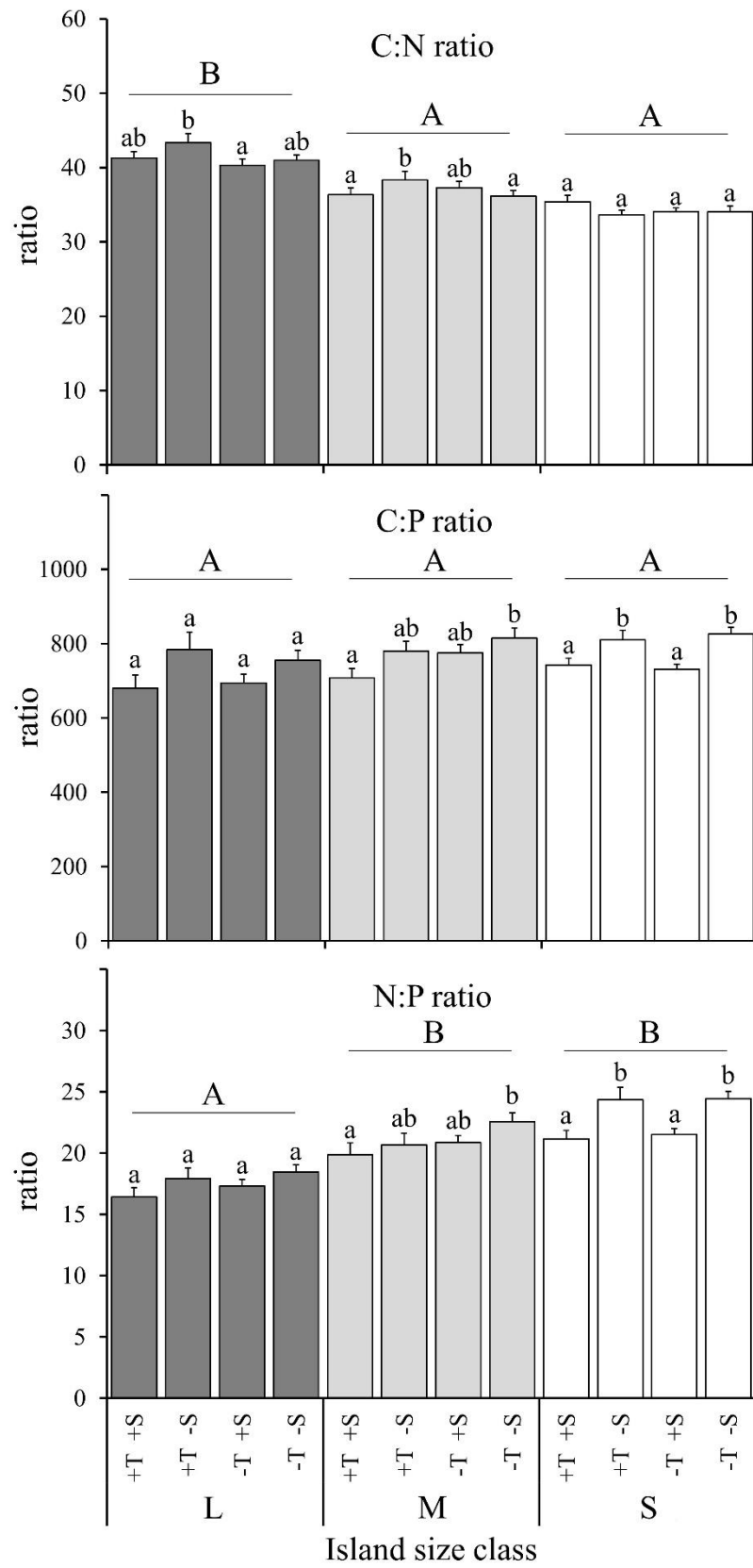


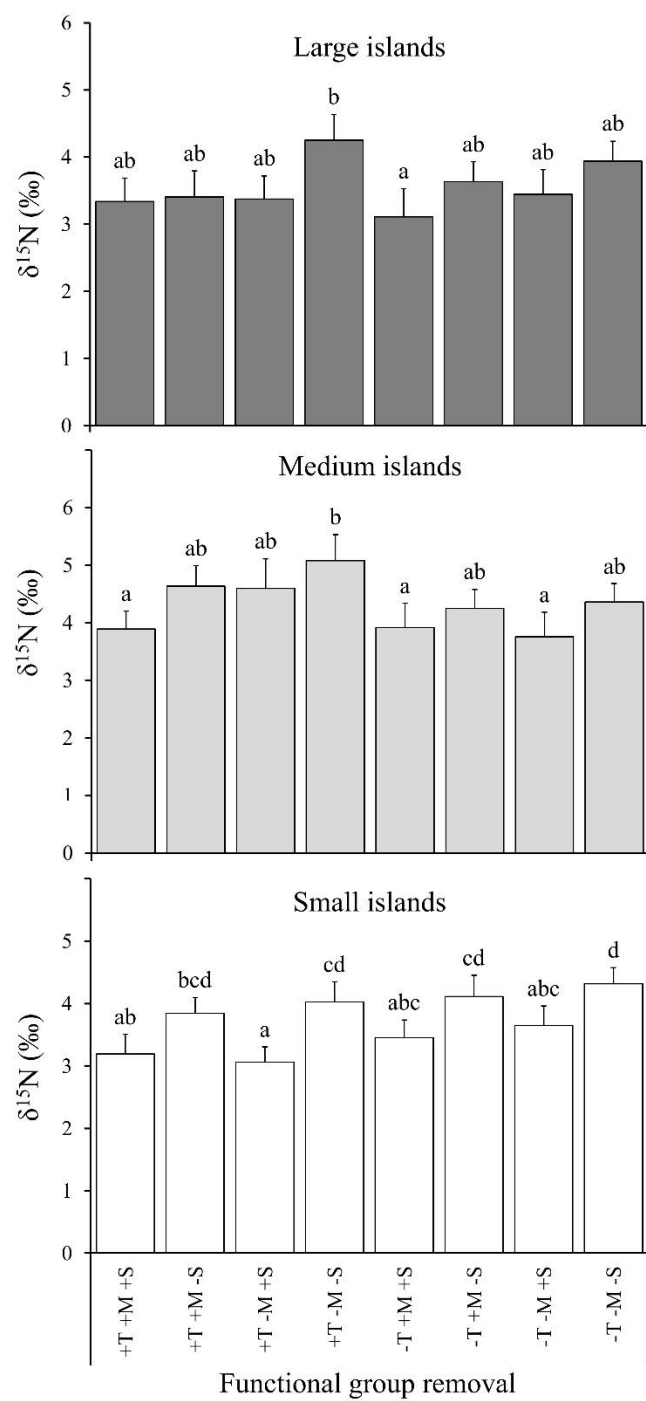
Figure 2

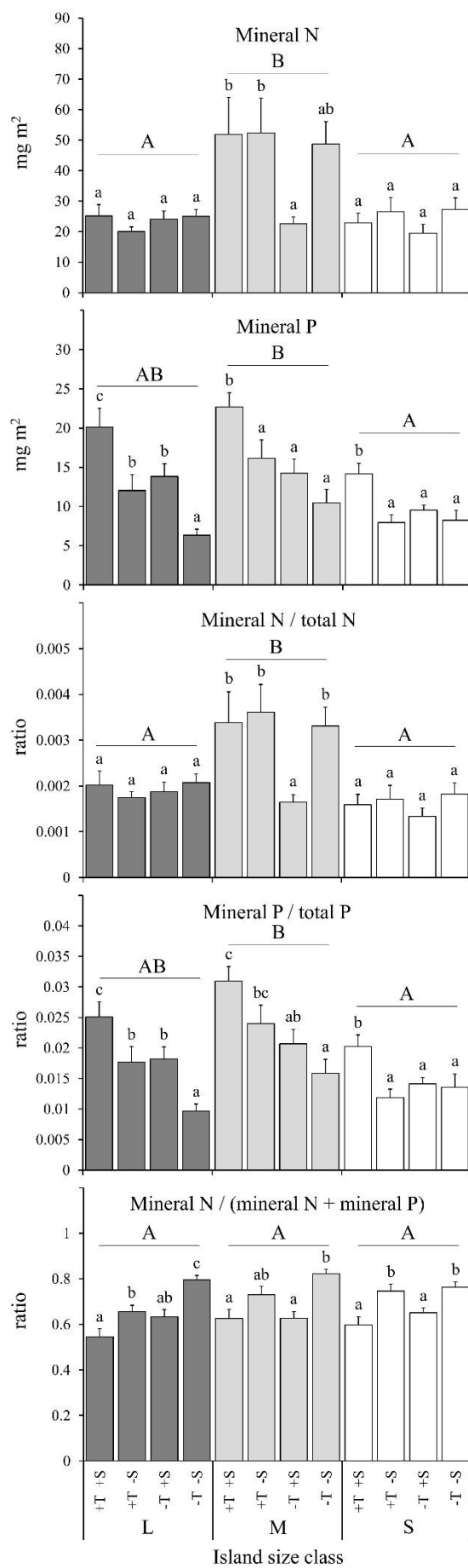
Figure 3

Figure 4

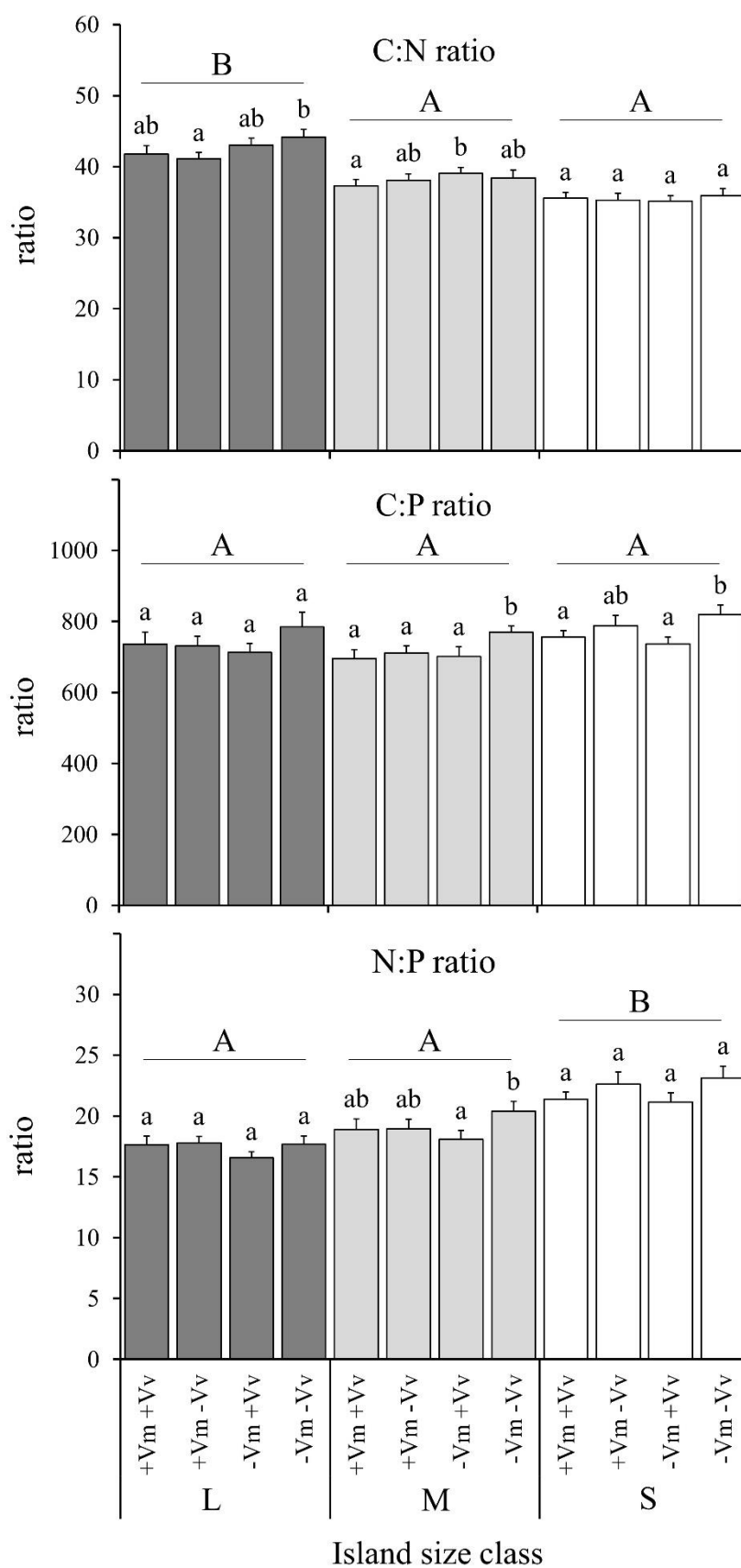


Figure 5

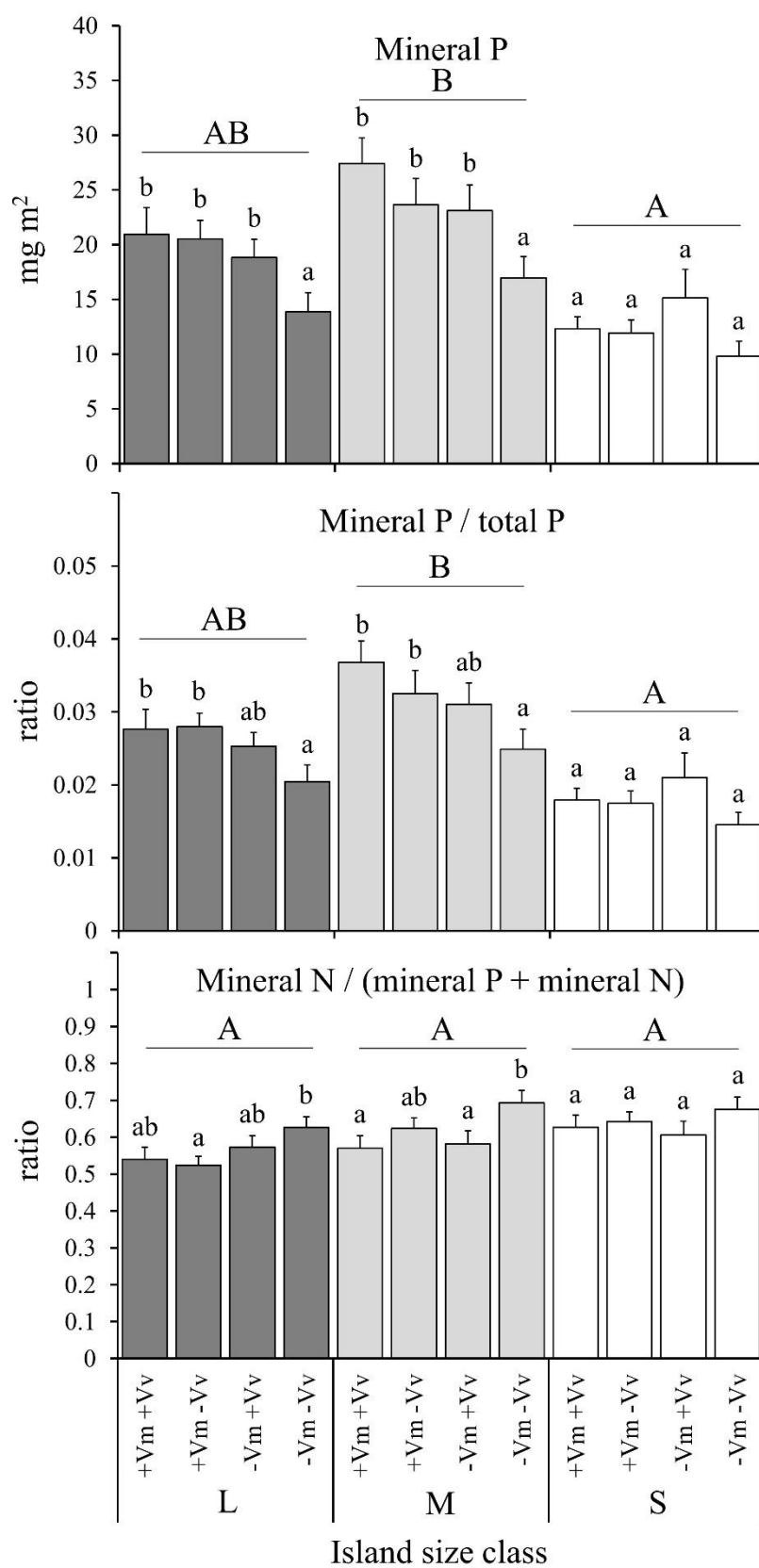


Figure 6

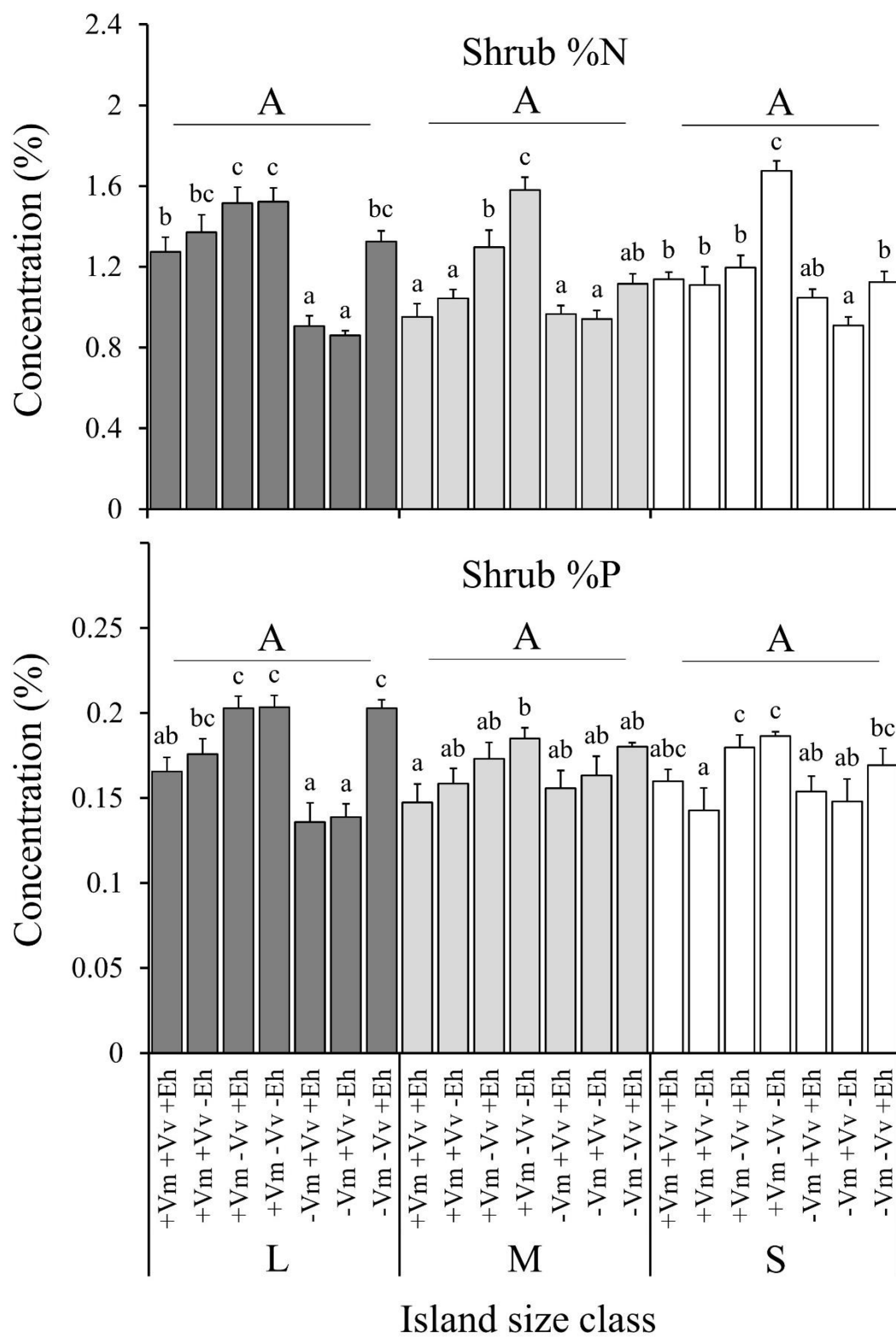


Figure 7

