Belowground effects of deer in a temperate forest are time-dependent

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Abstract

The past century witnessed a dramatic increase in deer abundance in North America, Western Europe, and Japan, that triggered profound changes in the vegetation structure of temperate forests. Considering the effects large herbivores can have on soil properties and organisms, it is likely that such increased deer abundance will have consequences belowground. Current studies in temperate forests, however, found inconsistent results regarding the effect of deer on soils within, and across, ecosystems. These inconsistencies may be the result of a time-dependent response of the soil to deer presence. Short-term belowground modifications may reflect the direct interactions of deer on soil (i.e. trampling and waste deposition), while long-term belowground modifications may reflect both direct and indirect effects of deer on soil (e.g. through vegetation shifts). To test these ideas, we measured the effects of deer on soil properties and prokaryotic communities in the temperate forests of Haida Gwaii, Canada. We compared three complementary systems varying in duration of deer presence or exclusion, so as to be able to assess the short- (before and after a deer cull), intermediate- (inside vs. outside deer exclosures) and long- (comparing islands with and without deer) term effects of deer, respectively. We found no change in soil physical and chemical properties and in prokaryotic community structure after one year of deer removal. Twenty years of deer exclusion significantly reduced soil compaction but had no effect on soil prokaryotic community structure. Over 70 years of deer presence significantly correlated with: increased soil

compaction, reduced total soil phosphorus content and soil prokaryotic diversity, and modified soil prokaryotic community structure and composition. Such effects of deer on the soil may have consequences for nutrient cycling. Revealing the belowground effects of deer in temperate forests, therefore, requires long-term studies, longer than most of those currently available in the literature.

Keywords: above-belowground interactions, soil properties, prokaryotic communities, trampling, aboveground herbivores, vegetation shift.

1. Introduction

Large herbivores can influence belowground soil properties and communities directly through trampling and waste deposition, and indirectly through plant removal (Bardgett and Wardle, 2003; Schrama et al., 2013). To date, interactions between large herbivores and soil have been highlighted for a broad range of ecosystems and herbivores, from sheep-grazed pastures to moose-browsed old-growth boreal forests (Andriuzzi and Wall, 2017; Bardgett et al., 1997; Pastor et al., 1993). The effects of large herbivores on soils depend on ecosystem characteristics such as ecosystem type, climate, herbivore size, and soil properties (Andriuzzi and Wall, 2017; Bardgett and Wardle, 2003; Schrama et al., 2013). Soil properties and organisms are central to carbon and nutrient recycling (Wardle et al. 2004). As a result, belowground modifications caused by large herbivores can have major feedbacks on ecosystem functioning and on aboveground organisms through the acceleration or the deceleration of these biogeochemical cycles (Bardgett and Wardle, 2003; Wardle et al., 2004).

The past century witnessed a dramatic increase in deer abundance at continental scales in temperate forests of North America, Western Europe, and Japan (Côté et al., 2004; Fuller and Gill, 2001; Takatsuki, 2009). This massive increase has triggered major changes in the structure of temperate forests including the prevention of tree regeneration, a reduction in understory biomass, the modification of understory plant composition, and negative reverberating effects on other trophic layers such as birds and insects [see among others (Cardinal et al., 2012; Côté et al., 2004; Martin et al., 2010; Nuttle et al., 2011; Ramirez et al., 2018; Takada et al., 2008)]. Considering the interactions between large herbivores and soil described above, increased deer

54 abundance in temperate forests may have significant consequences belowground. In forest
55 ecosystems, the effects of large herbivores on soil have been predicted to be driven mainly by
56 the reduction of litter quantity and quality. Such reduction is a consequence of the promotion
57 of less palatable plant species due to selective browsing, that surmounts the effects of nutrient
58 input from dung and urine deposition (Bardgett and Wardle, 2003; Chollet et al., 2020). As a
59 result, a negative effect of deer on nutrient availability and biological activity is expected in
60 forest ecosystems (Bardgett and Wardle, 2003). Current studies on the belowground effects of
61 deer in temperate forests, however, found inconsistent results within, and across, systems
62 (Bardgett et al., 1998; Bardgett and Wardle, 2003; Harrison and Bardgett, 2008). For example,
63 the effects of deer on soil properties were found to be significant (e.g. Bressette et al., 2012;
64 Gass and Binkley, 2011; Niwa et al., 2011), neutral (Relva et al. 2014), or idiosyncratic (Wardle
65 et al., 2001; Harrison and Bardgett, 2004). In light of the profound aboveground modification of
66 forest ecosystems by persistent abundant deer populations, understanding the interactions
67 between deer and soil, and being able to predict their effects on edaphic properties and
68 processes, is a forest management and conservation necessity. It is also essential for a
69 comprehensive understanding of ecological processes in temperate forests.

70 We hypothesised that some of the discrepancies currently observed across and within
71 belowground studies in temperate forests may result from the approaches and methodologies
72 used. Particularly, the length of the study could act as a key confounding factor. To date, the
73 method of choice to study deer effects on ecosystems has been by excluding deer from fenced
74 areas known as exclosures. The comparison of ecosystem characteristics inside and outside of

exclosures over time provides information on the ecosystem's resilience following deer exclusion and, therefore, on the effects deer have exerted on the ecosystem. The duration of exclusion varies widely across studies. Exclusion usually lasts in the range of a decade (Andriuzzi and Wall, 2017); however, the mechanisms through which deer interact with soil are not all operating at the same temporal and spatial scale. Changes in the plant community could take decades and operate at the ecosystem scale, while the deposition of dung or urine, or its cessation through deer exclusion or severe cull, are local and near instantaneous processes. Time since deer exclusion must, therefore, play a key role in the patterns revealed by exclosure studies.

To test the hypothesis of the importance of study length, we compared the effect of different deer browsing histories on soil ecosystem properties and soil prokaryotic communities in a temperate forest. We used three complementary systems varying in length of deer presence or exclusion to assess the short-, intermediate- and long-term effects of deer. We conducted our study on the Canadian archipelago of Haida Gwaii (B.C., Canada), where Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), introduced over 100 years ago, inhabit all but a few islands. We first followed the short-term effects of deer in response to a recent deer cull on Ramsay Island (Haida Gwaii). In this system, we assessed the rapid (one month after the cull) to short-term (one year after the cull) responses of the vegetation and soil to deer removal. We then studied a set of 20-year old deer exclosures distributed on Graham Island, the largest of the archipelago's islands, where deer have been present since the late 1800s to early 1900s. This deer exclosure system enabled us to compare the medium-term (20 years) effects of total deer

exclusion to the effects of a century-long presence of an abundant deer population. Finally, we took advantage of a unique situation on Haida Gwaii where deer colonisation of the archipelago resulted in the presence, in close proximity, of a small number of islands that had never been colonised by deer, and islands that had been colonised for more than 70 years at the time of this study (Vila et al., 2004). In this third system, the comparison of the deer-colonised islands to the un-colonised islands allowed us to study the long-term effects of deer colonisation on the soil.

We predicted that the short-term modifications of the belowground subsystem, investigated using our recent deer cull study system, would be driven by the direct interaction of deer with edaphic properties through trampling and/or waste deposition. The local-scale nature of waste deposition by deer and the soil-type specific response to compaction may, therefore, explain part of the idiosyncrasies observed within and among short studies (Murray et al., 2013; Schrama et al., 2013). Conversely, the indirect effects of large herbivores via changes in the vegetation composition and structure should be longer-term processes acting at the ecosystem scale. Revealing their consequences belowground will, therefore, require lengthier studies (Bardgett et al., 2005). In this respect, we predicted that such indirect effects of deer would drive the differences belowground in our deer exclusion study system and in our deer colonisation study system.

2. Materials and Methods

2.1. Study sites and sampling

116 Haida Gwaii is an archipelago located off the west coast of British Columbia, Canada (latitude
117 53.255, longitude -132.087). The climate is cool, temperate and oceanic. Mean annual
118 temperature and precipitation are 7.6°C and 1349 mm, respectively (Meidenger and Pojar,
119 1991). At low altitude, Haida Gwaii is covered with a coastal temperate rainforest that is
120 dominated by western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and
121 Sitka spruce (*Picea sitchensis*). Soil bedrock geology is volcanic and sedimentary, together with
122 intrusions of sedimentary rocks with basalt (Sutherland Brown, 1968). Soil types range from
123 organic soils that are classified as Folisols, to podzols, brunisols and gleysols (The Canadian
124 System of Soil Classification, 3rd ed.).

125 Sitka black-tailed deer, native to the adjacent mainland, were first introduced to these islands
126 in 1878 by Europeans for hunting. In the absence of natural predators, deer populations
127 increased rapidly, modifying the aboveground forest ecosystem (Allombert et al., 2005a, 2005b;
128 Martin et al., 2010; Stockton et al., 2005). The presence of islands varying in browsing histories
129 offered a remarkable context for the long-term accumulation of empirical and experimental
130 data on these aboveground consequences. The 30 year-long accumulation of data provided an
131 ideal situation to study the impact of deer belowground.

132 A challenge in using island comparisons is that they may not be subject to the same climate and
133 natural disturbances, which will influence plant community composition and succession, or
134 parent geology which will influence soil type. For this study on the impact of deer belowground
135 we selected islands and study sites in ways to ensure their comparability in the other aspects
136 than deer histories. Within each of our study systems we selected islands in close physical

proximity along the east coast of Haida Gwaii with similar annual rainfall (1250 mm) and sites with identical or, at least similar underlying geology (see Table1). Lost, Low, Louise, and Graham Island are formed from rocks from the Yakoun formation (andesite, lapilli tuff, sandstone, shale, coal); Ramsey, Lyell and Tar Island are composed solely of rocks from the Masset formation (basalt flows and breccias, rhyolite ash flows and dacite). All sampling sites within each system were similar in altitudinal ranges (50 - 300 m)(coastal and forest interior conditions at similar distance from the shoreline on the island systems, and, for the exclosure system, sites all situated on the Skidegate plateau on Graham Island). We only selected sites situated in mature old growth forests that had not been affected by industrial forestry or other recent human land-use. Within each study system these mature primary forest sites belonged to the submontane wet hypermaritime subzone of the Coastal Western Hemlock biogeoclimatic zone (CWHwh1) (Meidenger and Pojar, 1991). Soils on all islands had characteristic organic forest floors, with a carbon content greater than 40% and a F-layer deeper than 10cm.

Recent deer cull system: In response to the recognized negative effects of deer on plants, invertebrates, and songbird communities (Martin et al., 2010), and the documented evidence of a potential for recovery (Chollet et al. 2016), Parks Canada launched “The Llgaay gwii sdiihlda: Restoring Balance project” in 2017. The aim of this project was to remove deer completely from several islands in order to restore the ecosystems of this protected area. We took advantage of this initiative to study the short-term response of the ecosystem after the very severe deer cull, estimated to have removed more than 80% of the initial deer population. We sampled the vegetation and soil prior to (summer 2016), a month after (summer 2017), and

158 one year after (summer 2018) the cull on Ramsay Island (Table 1). As controls, we used Tar
159 Island that had never been colonised by deer, and Lyell Island that had been colonised for over
160 70 years. We established plots randomly on each island with a minimum distance of 100 m
161 from the shoreline and between plots (Table 1). Each plot was 20 m x 20 m in size. We surveyed
162 the vegetation cover in each plot as described below. We sampled the forest floor layer of soil
163 using a 2.5 cm diameter x 30 cm long soil core. We sampled approximately 100 cores within
164 each plot and composited them to cover plot heterogeneity.

165 **Deer exclosure system:** In 1997, 20 years prior to this study, the Research Group on Introduced
166 Species (RGIS) built twenty deer exclosures distributed in pairs at 10 sites across Graham Island
167 (Table 1), in the northern half of the archipelago. Deer densities on Graham Island have been
168 estimated at 13 deer/km² (Engelstoft, 2001). Each exclosure was 5 m x 5 m in size and consisted
169 of a 2.4 m high, large-mesh wire fence that prevented deer access. We used this experimental
170 set-up to study the resilience of the vegetation and soil after 20 years of deer exclusion. For this
171 system, we sampled vegetation and soil during the summer of 2017. We defined two plots per
172 exclosure – one placed inside and one outside – to compare the vegetation and soil
173 characteristics with and without deer exclusion. We set the size of the plots to 4 m x 4 m to
174 account for edge effects in the exclosure. We surveyed the vegetation cover in each plot as
175 described below. We sampled and composited into one sample the soil from five small pits
176 randomly dug inside the plot. One exclosure had been destroyed by a fallen tree a few weeks
177 before this study, leaving 19 exclosures to be sampled.

178 **Deer colonisation system***:* We selected five islands all covered by mature forests – Low, Lost,
179 Tar, Louise and Lyell Islands – that differed in deer presence (Table 1). Low, Lost and Tar Islands
180 had never been colonized by deer due to their distance from the coast and difficulty of access.
181 Louise and Lyell Islands have a long colonisation history, with deer being present for more than
182 70 years (Vila et al., 2004) at the time of study. Deer density on these islands was estimated to
183 range between 21 and 37 deer/km² (Stockton et al., 2005). We compared these two sets of
184 islands to study the long-term response of the ecosystem to deer presence. For this study
185 system, we sampled the vegetation and soil during the summer of 2017. We established 20 m x
186 20 m plots, surveyed their vegetation (see below) and sampled the soil following the same
187 protocol as for the recent deer cull system.
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190 **Table 1 –** Sampling locations and details for the three study systems.

System	Island	Island size (ha)	Deer presence	# plots	Parent material
Deer colonisation	*Low*	9.6	Never colonised	3	Yakoun formation, porphyritic andesite
	Lost	7.3	Never colonised	5	Yakoun formation, porphyritic andesite
	Tar	6	Never colonised	6	Masset formation, basalt, rhyolite
	Louise	35,000	Colonisation > 70 yrs	4	Yakoun formation, porphyritic andesite
	Lyell	> 17,300	Colonisation > 70 yrs	6	Masset formation basalt, rhyolite
Deer exclosures	*Graham*	636,100	Inside/Outside.	19 exclos.	Yakoun formation, porphyritic andesite (1 exclosures) and Quartenary sediments (18 exclosures)
Recent deer cull	*Tar*	6	No Deer	6	Masset formation, basalt, rhyolite
	Lyell	> 17,300	Deer > 70 yrs	6	Masset formation, basalt, rhyolite
	Ramsay	1,622.8	Culled	13	Masset formation, basalt, rhyolite

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194 ### 2.2. Vegetation survey

195 For each of the three study systems, we surveyed vascular plant cover in every plot using a
196 modified Braun-Blanket scale (Braun-Blanquet, 1932) (Table A1). We surveyed bryophyte plant
197 cover by randomly placing a quadrat on the forest floor twenty times in each plot and recording
198 bryophyte species presence in each iteration. We used a 5 x 5 cm quadrat for the bryophyte
199 survey in the deer exclosure system and a 20 x 20 cm quadrat in the other two study systems

200 (i.e. recent deer cull and deer colonisation system). We estimated the percent cover of each
201 bryophyte species as the number of occurrences of the species divided by 20 and multiplied by
202 the total bryophyte cover on the plot. We assigned a percent cover value of 0.01 % to the
203 bryophytes that were present in the plot but absent from the quadrat survey.

204 **2.3. Soil physical and chemical properties**

205 We sampled all the soil samples exclusively from the F layer of the forest floor according to the
206 Canadian system of soil classification (The Canadian System of Soil Classification, 3rd ed.), which
207 is biologically the most active soil horizon. Soil samples were kept cool at 4°C for transport back
208 to the laboratory within one month. Soil samples were then sieved to ensure homogenization
209 and kept frozen at -20°C prior to chemical analyses.
210 We measured soil penetration resistance, as a proxy for soil compaction, using a hand-held
211 penetrometer (Gilson HM-500 pocket penetrometer, Lewis, OH, US). We recorded 50
212 penetration resistance measurements per plot to account for soil heterogeneity. A logistical
213 mishap prevented us from assessing soil penetration resistance the first year of the recent deer
214 cull study system (i.e. 2016, one year before the cull). Soil water content was measured by
215 drying the fresh soil at 105° until constant weight was achieved (~48 hours) and subtracting the
216 dry weight from the fresh weight. We measured soil pH in duplicate on air-dried soil in a 0.01M
217 $CaCl_2$ solution using a 1:10 (air dry soil : solution) ratio. We measured total soil carbon and
218 nitrogen content (g / g dry soil) on 3.5 mg of freeze-dried soil using an Elementar Vario El Cube
219 Analyzer (Elementar, Langenselbold, Germany). We measured total soil phosphorus content (µg
220 / g dry soil of P) in 0.1 g of freeze-dried soil using the sodium hypobromite alkaline oxidation

method (Dick and Tabatabai, 1977) followed by the colorimetric method developed by Murphy and Riley (1962) and modified by Watanabe and Olsen (1965). We extracted soil ammonium (NH_4) and nitrate (NO_3) (µg / g dry soil of N) in a 2M KCl solution using a 1:10 ratio (fresh soil : solution). We shook the solution for one hour and filtered through a fiberglass G6 microfilter. We further analysed the extracts by colorimetry with the phenol-hypochlorite reaction method for NH_4 quantification (Weatherburn, 1967) and the VCl_3 reduction method for NO_3 quantification (Hood-Nowotny et al., 2010).

2.4. Molecular analyses

We extracted soil DNA from 0.05 g of freeze-dried soil using the DNeasy PowerSoil Kit from Qiagen (Qiagen, Venlo, Netherlands). We controlled DNA purity and quantity using both a quantus fluorometer (Promega corporation, Madison, WI, USA) and a nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). We measured soil bacterial abundance by qPCR using a set of general bacterial primers targeting the 16S RNA gene. We used the forward primer U16SRT-F (ACTCCTACGGGAGGCAGCAGT) and the reverse primer U16SRT-R (TATTACCGCGGCTGCTGGC) designed by Clifford et al. (2012). Reactions were 10 µL with 500 nM of primers, 0.5 µL of DNA template, 3 µL of H_2O and 5 µL of PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific Inc). The conditions of the reactions were 2 min at 50 °C and 2 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. We produced the standard curves using *E. coli* DNA extracted from DH5 alpha cells (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Standard curves were made with seven dilutions starting from 3.025×10^8 copy numbers and with a 1:4 dilution factor. Mean R^2 and efficiency of the

242 reactions were 0.998 and 91.12 % respectively. All the measurements were made in triplicate.
243 Illumina sequencing of the 16S RNA gene took place at the Integrated Microbiome Resource
244 platform in Halifax (NS, Canada) using the primer pair 515F (Parada) – 806R (Apprill) (Apprill et
245 al., 2015; Parada et al., 2016). We used the pipeline DADA2 with the package dada2 and the
246 software R to analyse these sequences (Callahan et al., 2016; R Core Team, 2018). We filtered
247 and trimmed reads using the function *filterAndTrim*. We used the standard filtering parameters
248 of the function and trimmed the reads after the 250 and the 200 nucleotides for the forward
249 and reverse reads, respectively. Error rates were calculated for both forward and reverse reads
250 using the function *learnErrors* and, were used to calculate the number of true sequence
251 variants using the sample inference algorithm of DADA2. The denoised forward and reverse
252 reads were then merged using the function *mergePairs*. Chimeras were removed using the
253 function *removeBimeraDenovo* with the method "consensus". At the end of the reads cleaning,
254 we retained a total of 16603, 5939, 8628 Operational Taxonomic Units (OTUs) for the recent
255 deer cull, deer exclosure, and deer colonisation systems, respectively. Rarefaction curves are
256 given on Figure A1. One sample (“OB1OUT”) from the deer exclosure system had a low
257 sequencing depth; we therefore removed this exclosure from the analysis (Figure A1). We
258 rarefied samples to the minimum read count in each system using the function
259 *rarefy_even_depth* from the package phyloseq in R. Rarefaction did not change the results of
260 the analysis. We assigned taxonomy with the Ribosomal Database Project (RDP) database to
261 genus level (Maidak et al., 1996).

262 **2.5. Data analyses**

263 We calculated vascular plant, bryophyte, and prokaryotic alpha diversities using the Shannon
264 index. We used Principal Component Analysis (PCA) to visualise the effect of deer on the
265 environmental factors measured (plant and soil characteristics) for the three systems. We
266 performed PCA on normalised data using the function *prcomp* from the package stats on R (R
267 Core Team, 2018). We assessed differences in aboveground properties, belowground
268 properties and prokaryotic abundance and diversities between treatments with the *nparLD*
269 function with a F1-LD-F1 design for the recent deer cull system (Noguchi et al., 2012), a paired
270 Wilcoxon test for the deer exclosures system, and a Wilcoxon test for the deer colonisation
271 system. The *nparLD* method applied with a F1-LD-F1 design is suitable for nonparametric
272 analysis of paired data in factorial experiments with one whole-plot factor and one sub-plot
273 factor design (Brunner et al., 2001).
274 We Hellinger-transformed OTUs prior to any further analyses of the microbial community
275 structure. We assessed the significance of the difference in microbial community structure
276 among treatments with a PERMANOVA using the function *adonis* from the package vegan in R
277 (Oksanen et al., 2019). We calculated the β diversity of the prokaryotic community in each
278 treatment with the function *betadisper* of the package vegan, using the group centroid analysis
279 (Oksanen et al., 2019) and the Bray Curtis distance. We used a Redundancy Analysis (RDA) to
280 investigate the correlation between the plant and the soil data and the soil prokaryotic
281 community. We performed the RDA using the function *rda* from the package vegan in R. We
282 first realised the RDA using the first axes of the PCA realised on the plant and soil data as
283 explanatory variables. For the deer colonisation system, we also realised a RDA using the
284 vegetation and soil variables, that we selected by forward selection. Prior to the variable

285 selection, we verified the significance of the model resulting from the RDA on all explanatory
286 variables using an ANOVA with 999 permutations, as recommended by Blanchet et al. (2008).
287 We ran the forward selection on all the explanatory variables using the function *forward.sel*
288 from the package adespatial on R (Dray et al., 2019). We corrected p-values for multiple testing
289 using the function *p.adjust* from the package stats, and with the method 'holm'. We calculated
290 the percent variation of the soil prokaryotic community explained by the explanatory variables
291 with the function *varpart* from the package vegan.

292 ## 3. Results

293 ### 3.1. Deer affected understory vegetation in a consistent way across the three study
294 systems

295 In the recent deer cull system, the first axis of the PCA discriminated vegetation from the plots
296 on the islands that have or had deer present ('present' and 'culled' treatments) from plots on
297 islands without deer ('absent' treatment) (Figure 1A). The second PCA axis discriminated
298 between years of sampling (Figure 1A). Interaction between treatments and year of sampling
299 was significant for the vascular plant and bryophyte diversities, and the bryophyte and forb
300 cover (Table A2 and Figure A2). Among these variables, bryophyte diversity decreased the year
301 after the cull, while vascular plant diversity and forb cover increased the year after the cull
302 (Table A2 and Figure A2). Concerning bryophyte cover we found a change through time on both
303 culled and control islands, indicating that these change cannot be attributed to the deer cull
304 treatment (Figure A2).

In the deer exclosure system, the first axis of the PCA fully discriminated the vegetation data according to deer presence or absence (Figure 1B). Vascular plant diversity, shrub cover and forb cover were significantly higher inside the 20-year-old deer exclosures (Table A2 and Figure A3). Conversely, bryophyte cover was significantly lower with deer exclusion (Table A2 and Figure A3).

In the deer colonisation system, we found a pattern of deer effect on the plant community structure similar to the one we observed in the deer exclosure system (Figure 1B). Vascular plant diversity, shrub cover, and pteridophyte cover were lower on the islands colonised by deer for over 70 years when compared to the islands without deer (Table A2 and Figure A4). Conversely, bryophyte diversity and cover, graminoid cover, and conifer cover were higher on islands colonised by deer for over 70 years when compared to islands without deer (Figure 1B, Table A2, and Figure A4). On the first axis of the PCA, vegetation plots from the exclosure system had coordinates intermediate between those from islands without deer and those from long-term colonised islands (Figure 1B).

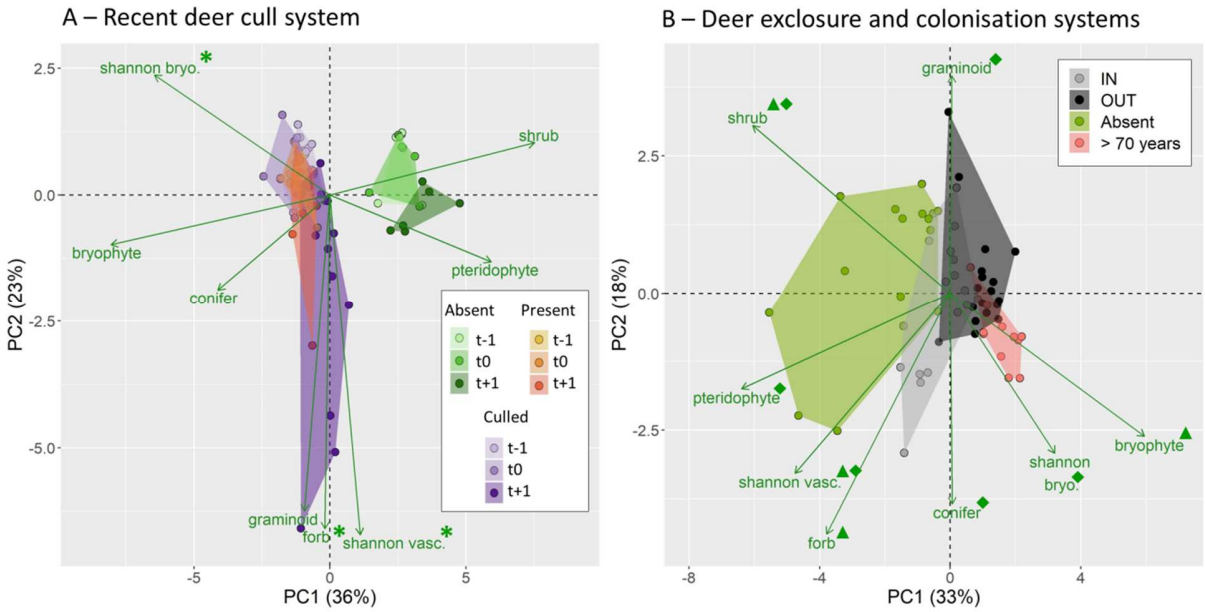


Figure 1 – PCA showing discrimination of the plant community structure in **A)** the recent deer cull system and **B)** the deer exclosures and the deer colonisation systems. Plant community structure includes the percent cover of the different guilds and the vascular and bryophyte diversity. The symbols *, Δ and ◊ indicate the variable significantly different between treatments in the recent deer cull, the deer exclosure, and the deer colonisation system respectively. t_{-1}, t_0 and t_{+1} correspond to the year before, the month after and the year after the cull respectively. IN = plots inside deer exclosure, OUT = plots outside deer exclosure.

3.2. Soil physical and chemical properties responded differently to deer presence in the three study systems

In the recent deer cull system, soil properties from the island without deer and those from the island with deer discriminated along the first axis of the PCA (Figure 2A). The second axis of the PCA discriminated soils between years of sampling, with lower scores observed for the sampling done the year before and the month after the cull, and higher scores observed for the sampling done the year after the cull. The interaction between year of sampling and treatment was significant for soil pH and total phosphorus (Table A2), but was not correlated to the cull

(Figure A2). The interaction was marginally significant for soil ammonium, and corresponded to a decrease in soil ammonium the month following the cull (Figure A2, W = 70, p-value = 0.08). In the deer exclosure system, soils taken from inside and outside exclosures were segregated by the PCA axes (Figure 2B) as a result of a significantly higher soil penetration resistance outside of the exclosures (Table A2 and Figure A3, W = 0, p-value < 0.001). The other soil properties did not differ significantly between the inside and outside of the exclosures (Table A2).

In the deer colonisation system, soil properties discriminated plots across treatments on the second axis of the PCA (Figure 2B). Samples from islands with long-term deer presence had a significantly higher water content, lower pH and lower total phosphorus (Table A2 and Figure A4, W = 5, p-value < 0.001; W = 152, p-value < 0.001and W = 118, p-value = 0.02, respectively). Soil penetration resistance was three times higher on islands with long-term deer presence (Table A2 and Figure A4, W = 0, p-value < 0.001).

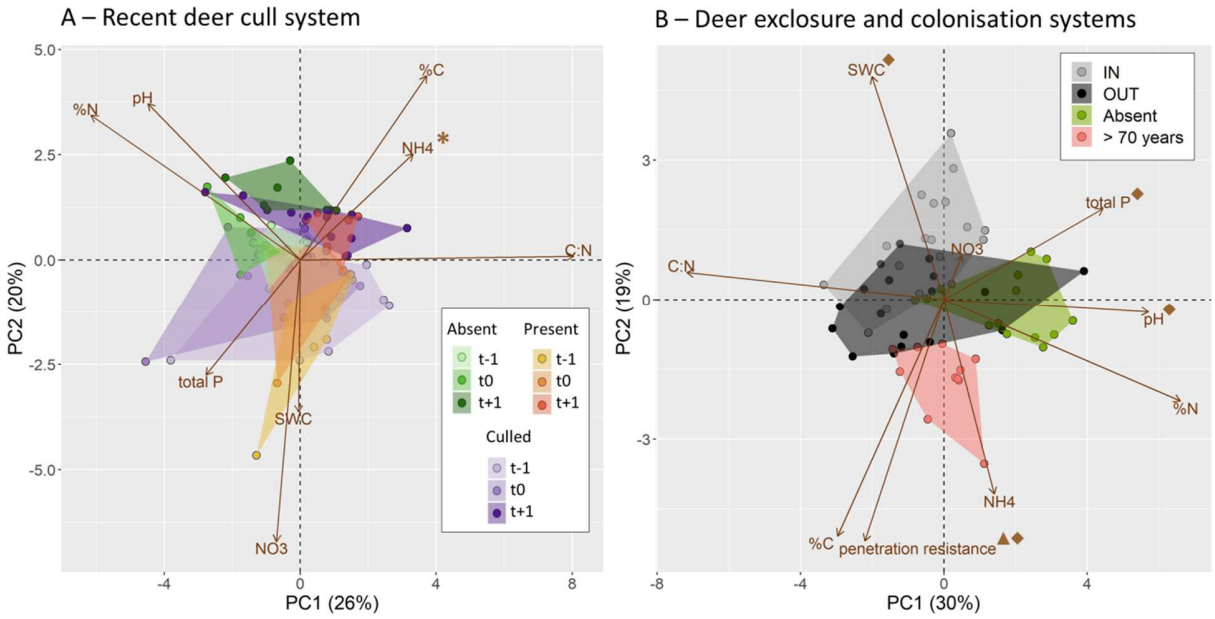


Figure 2 – PCA showing discrimination of the soil physical and chemical properties in **A)** the recent deer cull system and **B)** the deer exclosures and the deer colonisation systems. Soil properties include the following variables: SWC = Soil Water Content, P = total phosphorus content, N = percent nitrogen content, C = percent carbon content, C:N = ratio carbon to nitrogen, NH4 = ammonium, NO3 = nitrate, and soil penetration resistance. The symbols *****, **Δ** and **◊** indicate the variable significantly different between treatments in the recent deer cull, the deer exclosure, and the deer colonisation system respectively. t_{-1}, t_0 and t_{+1} correspond to the year before, the month after and the year after the cull respectively. IN = plots inside deer exclosures, OUT = plots outside deer exclosure.

3.3. Soil prokaryotic community structure was significantly modified by deer, but only in the deer colonisation system

We retained a total of 18,542 unique Operational Taxonomic Units (OTUs) after filtering and rarefaction across the three systems, with 82.8 % of the total OTUs shared among the three study systems. On average, 99.5 % of the OTUS belonged to the Bacterial kingdom. They were classified into 608 genera from 229 families, 71 classes and 32 phyla. 0.5 % of the OTUs

belonged to the Archaeal kingdom. The archaeal family *Nitrososphaera* from the Thaumarchaeota phylum largely dominated the archaeal population with an average representation of 86.7 % across treatments and systems. The ten most important prokaryotic genera across treatments and systems were *Mycobacterium*, *Conexibacter*, *Aquisphaera*, *Bradyrhizobium*, *Actinoallomurus*, *Roseiarcus*, *Singulisphaera*, *Burkholderia*, *Povalibacter* and *Gaiella*.

In the recent deer cull system, although soil prokaryotic abundance and α diversity increased significantly with the year of sampling (Figure 3A, $F = 59.1$, p-value < 0.001; and Figure 3D, $F = 8.81$, p-value < 0.001 respectively), the interaction between treatments and the year of sampling was not significant, indicating that the cull did not drive soil prokaryotic abundance and diversity (Figure 3A, $F = 0.37$, p-value = 0.74; and Figure 3D, $F = 1.83$, p-value = 0.15 respectively). Similarly, the interaction between year and treatment was not significant for the β diversity of the soil prokaryotic community (Figure 3G, $F = 1.70$, p-value = 0.16). The PERMANOVA showed significant differences in the soil prokaryotic community composition both between treatments and years of sampling ($F = 10.45$, p-value = 0.001 and $F = 4.24$, p-value = 0.001 respectively). Differences in soil prokaryotic community composition between treatments were correlated with the first axes of the PCA realised on the vegetation and soil variables, which accounted for 4.0 % and 9.2 % of the variation, respectively (Figure 4A). However, the interaction between treatment and year of sampling was not significant ($F = 0.84$, p-value = 0.831), indicating that the change over time was the same for the three treatments and could not be attributed to the deer cull.

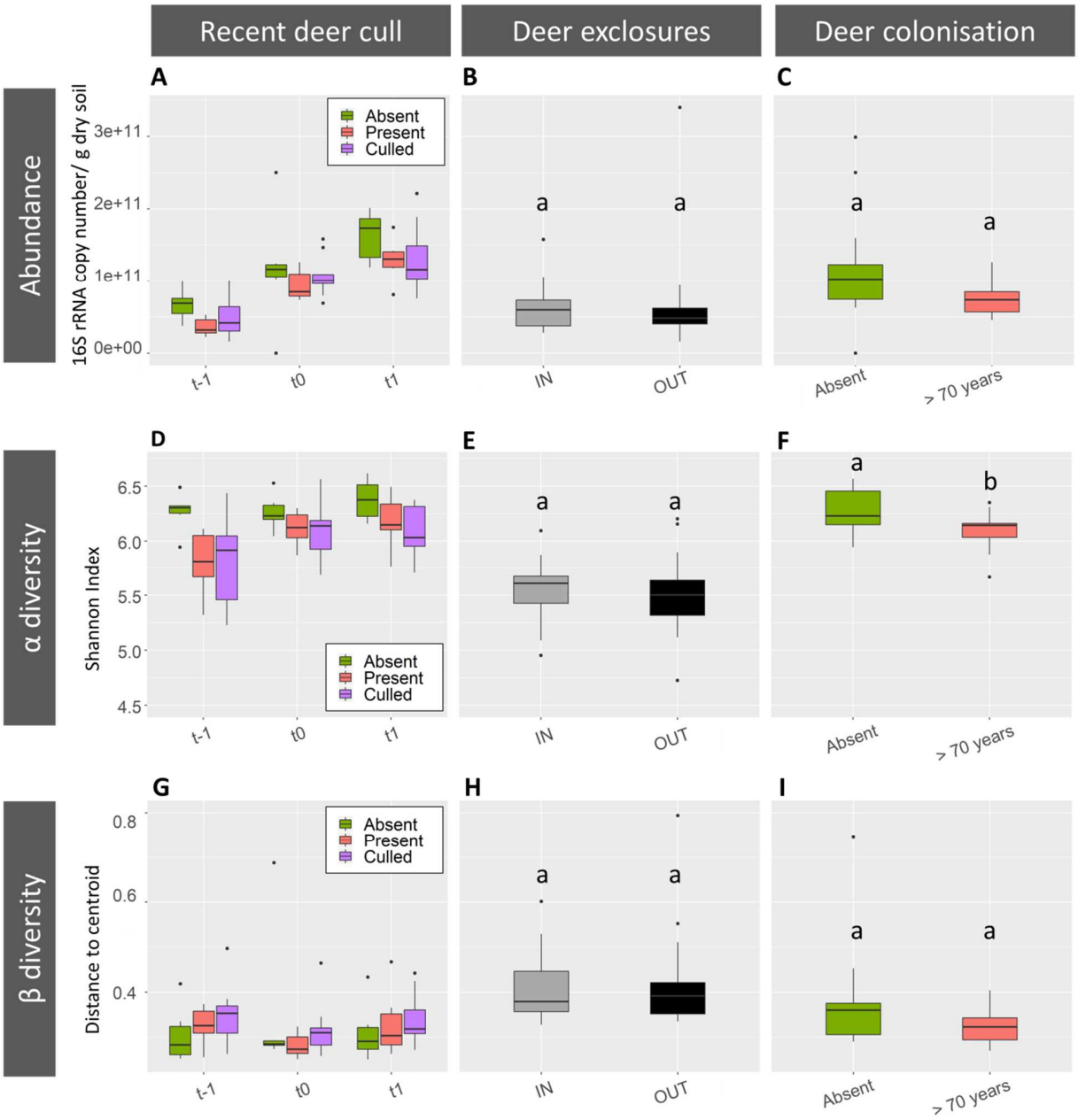
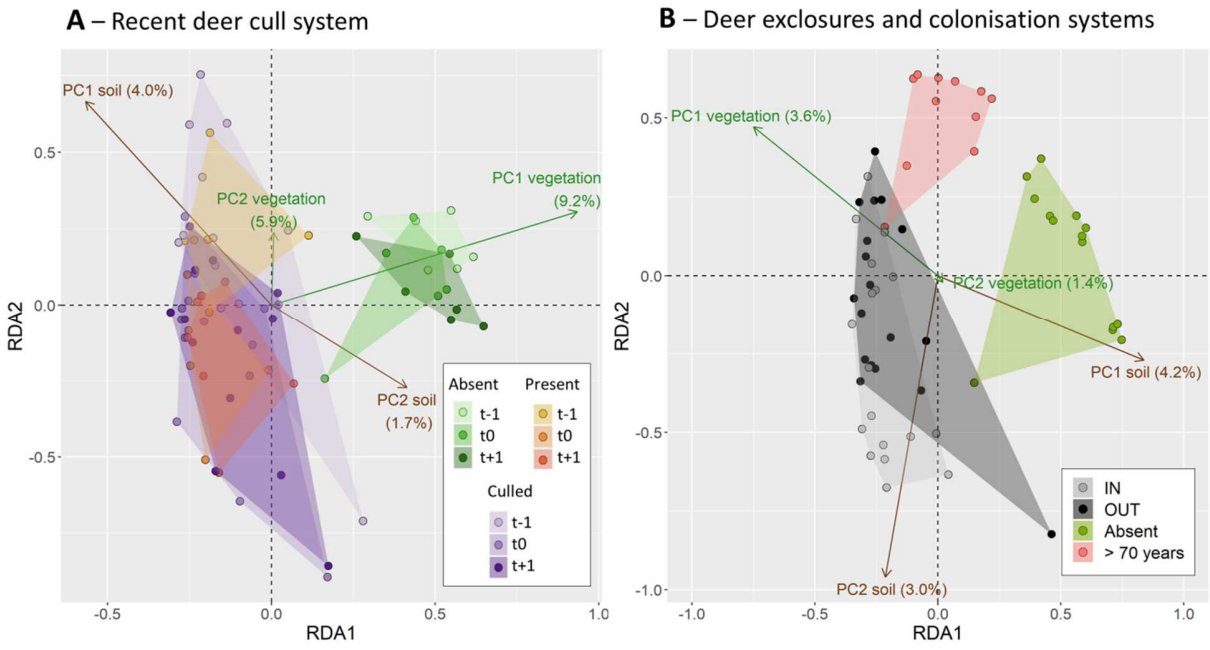


Figure 3 – Soil prokaryotic community structure. Soil microbial abundance in **A)** the recent deer cull system, **B)** the deer exclosures system and **C)** the deer colonisation system. Soil prokaryotic α diversity in **D)** the recent deer cull system, **E)** the deer exclosures system and **F)** the deer colonisation system. Soil prokaryotic β diversity within each treatment in **G)** the recent deer cull system, **H)** the deer exclosures system and **I)** the deer colonisation system. t_{-1}, t_0 and t_{+1} correspond to the year before, the month after and the year after the cull respectively. IN = plots inside deer exclosures, OUT = plots outside deer exclosure.

In the deer exclosure system, we found no significant differences in soil prokaryotic abundance, α diversity and β diversity after 20 years of deer exclusion (Figure 3B, W = 103, p-value = 0.768; Figure 3E, W = 89, p-value = 0.899; and Figure 3H, W = 92, p-value = 0.80 respectively). Similarly, we found no significant difference in the soil prokaryotic community composition after 20 years of deer exclusion, as evidenced by the overlap of communities in the plots from inside and outside deer exclosures in Figure 4B (F = 0.781, p-value = 0.297).

In the deer colonisation system, the difference in soil prokaryotic abundance was marginally significant between islands without deer and islands with deer for over 70 years (Figure 3C, W = 44, p-value = 0.08), with a higher bacterial abundance in soil samples from the islands without deer. Prokaryotic α diversity was significantly higher in soils from islands without deer than on the islands with deer present for over 70 years (Figure 3F, W = 41, p-value = 0.05). The β diversity of the soil prokaryotic community was not significantly different between the islands without deer and the islands colonised for more than 70 years (Figure 3I, W = 104, p-value = 0.15). Soil prokaryotic community composition was significantly different between the islands colonised for more than 70 years and the islands without deer (Figure 4B, F = 7.21, p-value = 0.001). The difference in soil prokaryotic community composition was correlated with the first and second axes of the PCA based on the soil variables, and the first axis of the PCA based on the plant variables (Figure 4B). Mainly, the RDA analysis revealed that differences in soil prokaryotic community structure between islands colonised and un-colonised by deer were mainly correlated with soil pH and soil penetration resistance (Figure A5). Soil penetration

418 resistance accounted for 7 % of the variation in soil prokaryotic community structure, with high
419 scores being associated with soil samples from islands colonised by deer for more than 70 years
420 (Figure A5). Soil pH accounted for 5 % of the variation in soil prokaryotic community structure,
421 with high scores being associated with soil from islands that have never been colonised by deer
422 (Figure A5).



423
424 **Figure 4 –** Redundancy Analysis (RDA) on the OTUs and the axes of the PCA realised on the
425 plant and the soil data for **A)** the recent deer cull system and **B)** the deer exclosures and the
426 deer colonisation systems. Percent values correspond to the variation in soil prokaryotic
427 community explained by the PCA axes, and calculated by variation partitioning.
428

429

430 ## Discussion

431 Current studies investigating the belowground effects of deer in temperate forests have found
432 inconsistent results within, and across, systems (Bardgett et al., 1998; Bardgett and Wardle,

2003; Harrison and Bardgett, 2008). In this study, we compared three different approaches varying in length of deer presence and exclusion to investigate the effects of deer belowground. While the effects of deer on the vegetation were consistent among the three study systems, we found that the response of the soil properties and organisms to deer pressure depended on the approach used (see Figure 5 for a synthesis).

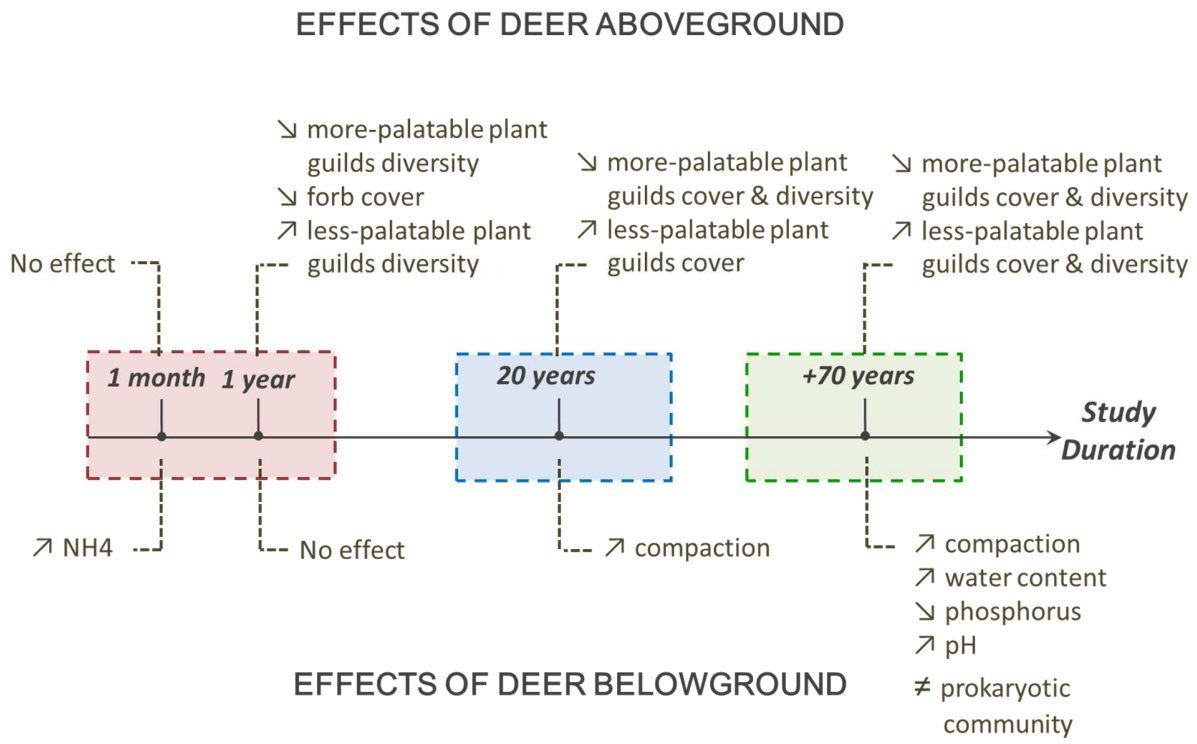


Figure 5 – Effects of deer above- and belowground as concluded from the three different study methods. Boxes in red, blue and green represent the recent deer cull, the deer exclosure and the deer colonisation systems respectively. Plant guilds included in the more-palatable plants are: shrubs, forbs and pteridophytes (Stockton et al., 2005). Plant guilds included in the less-palatable plants are: bryophytes, conifers and graminoids (Chollet et al., 2013b; Stockton et al., 2005).

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451 ### 3.4. Different study methods lead to different conclusions on the effects of deer
452 ### belowground

453 Aboveground, we found across the three study systems that deer presence significantly
454 reduced vascular plant abundance and diversity, and significantly promoted the dominance of
455 less-palatable conifers and unpalatable bryophytes. Such modification of the plant community
456 composition is in agreement with previous studies on the same islands (Chollet et al., 2016,
457 2013b, 2013a; Stockton et al., 2005) and in other temperate forests of the world (e.g. Horsley et
458 al. 2003, Côté et al. 2004, Boulanger et al. 2018). The longer deer were present, the stronger
459 the modifications, and the more plant guilds involved (Figure 1, Table A2). The differences in
460 vegetation structure we documented across the three systems, therefore, reflect different
461 stages of a consistent response of the vegetation to deer presence (Figure 5).

462 Belowground, however, the response of soil physical and chemical properties to deer presence
463 and removal differed among our three study systems. In the recent deer cull system, soil
464 ammonium was the only edaphic variable that changed following deer removal. The marginal
465 decrease in soil ammonium concentration in the month following the deer cull could be
466 explained by the sudden cessation of urine input, which constitutes a source of ammonium to
467 the soil. However, the moderate decrease in ammonium did not persist to the year following
468 the cull, nor was there a change in nitrate or total N, indicating a transitory process. Soil

469 penetration resistance, a proxy for soil compaction, was found to be higher with deer presence
470 in both the deer exclosures and the deer colonisation system. The high foot pressure of
471 ungulates can indeed induce physical compaction of the soil (Duncan and Holdaway, 1989). Soil
472 compaction values inside the deer exclosures were similar to those observed on islands never
473 colonised by deer, indicating that twenty years of deer exclusion were sufficient to restore
474 initial soil bulk density (Figures S3 and S4). This reversion of soil compaction following deer
475 exclusion was not correlated with other changes in edaphic properties in the deer exclosure
476 system. Higher soil water retention has been documented previously as a direct consequence
477 of soil compaction (Cambi et al., 2015). We did not observe such differences in soil water
478 content between the less-compacted soils sampled inside, and the more-compacted soils
479 sampled outside deer exclosures. However, in the deer colonisation system, soil water content
480 was significantly higher after 70 years of deer presence (Table A2, Figure A4). An explanation
481 for this discrepancy between the two systems could come from the heavy rains that occurred
482 during the soil sampling in the exclosures, which might have brought the soil samples close to
483 their water holding capacity (average soil water content was 601 % ± 183 % and 311 % ± 85 %
484 for the deer exclosures and the deer colonisation system respectively). We further found that
485 total phosphorus and soil pH were significantly altered after 70 years of deer presence
486 (Supplementary material Appendix, Table A2, Figure A4). The lower levels of soil phosphorus
487 observed on islands colonised by deer may be the consequence of the higher cover of
488 bryophytes. Mosses have been shown to sequester large quantities of phosphorus in coniferous
489 forests (Chapin et al., 1987). The acidification of the soil after long-term deer presence may be
490 explained by the higher relative abundance of both conifers and moss, whose litters have been

491 shown to be acidic (Cornelissen et al., 2006; Finzi et al., 1998). Long-term urine deposition by
492 deer might also explain this acidification, as ammonia input to soil may stimulate nitrification
493 with consequent production of H^+ ions (Ball et al., 1979; Black, 1992).
494 Contrary to what we might have expected in response to the replacement of palatable plants
495 (nutrient-rich) by unpalatable plants (nutrient-poor) (Pastor et al., 1993), we did not observe
496 changes in the soil C:N in any of the three study systems. This result is consistent with the fact
497 that litter C:N was not modified by deer despite the drastic modification of the plant
498 community composition on these islands (Chollet et al., 2020). This result is also consistent with
499 the results of the exclosure study by Binkley et al. (2003), who found no change in soil C:N after
500 35 years of elk exclusion in the Rocky Mountain National Park (United States). Similarly, deer
501 presence or absence for more than one month did not affect the concentration of soil inorganic
502 nor total nitrogen in our study, which suggests a resilience of the soil to the local addition or
503 removal of dung and urine inputs. Indeed, dung deposition did not influence carbon or nitrogen
504 decomposition at the ecosystem level on these same islands (Chollet et al., 2020).

505 We found that the soil prokaryotic community structure was significantly affected by deer, but
506 only in the deer colonisation study system. In both the recent deer cull and the deer exclosure
507 system, soil prokaryotic abundance, α and β diversities and composition were indeed not
508 affected after one month, one year, nor twenty years of deer removal or exclusion. This lack of
509 influence of deer on the soil prokaryotic community structure in the short-term or medium-
510 term is consistent with previous results found in western North American, Patagonian, and New
511 Zealand temperate forests (Gass and Binkley, 2011; Relva et al., 2014; Wardle et al., 2001).

In the deer colonisation system, we found that soil microbial biomass tended to decrease after 70 years of deer colonisation, which is consistent with the results found in boreal and Japanese temperate forests (Niwa et al., 2011; Pastor et al., 1988). In Australian woodlands, Eldridge et al. (2017) found that grazing by domestic and wild herbivores increased bacterial diversity through the exclusion of Actinobacteria, the competitive microbial phylum, due to a reduction in soil carbon content. In contrast, we observed a reduction in prokaryotic diversity, which was driven by a shift in composition rather than a modification of taxa abundance, in response to 70 years of deer presence. The lower prokaryotic α diversity in soils from islands colonised by deer could result from the simplification of the vegetation observed aboveground. Low belowground diversity may, indeed, be linked to low aboveground diversity as a consequence of reduced litter and root exudate diversity (Haichar et al., 2008; Wardle et al., 2004). Conversely, β diversity of the soil prokaryotic communities was not modified by deer, suggesting that the simplification of the vegetation by deer does not lead to a homogenisation of the soil prokaryotic community in our system. Deer colonisation was an important factor structuring the soil prokaryotic community (Figure 4B and S5). The differences in soil prokaryotic community structure were partly due to the significantly lower soil pH on islands colonised by deer. This result is not surprising considering that soil pH has been shown to be one of the major edaphic properties structuring soil microbial communities (Fierer and Jackson, 2006). Soil penetration resistance, which was significantly higher on islands with deer, also explained part of the variation in soil prokaryote communities between islands colonised and un-colonised by deer. Soil compaction has been linked to a reduction in microbial abundance and the modification of microbial composition towards microbes adapted to low oxygen availability

534 (Hartmann et al., 2014). Similarly, simulated trampling has been shown to decrease soil
535 microbial biomass in sub-arctic grasslands (Sørensen et al., 2009). The lack of difference in the
536 soil prokaryotic community structure observed in the deer exclosure system, where soil
537 penetration resistance was strongly alleviated by deer exclusion, is therefore surprising (Figure
538 4 and Figure A3). This result suggests that it is not only the level of compaction, but also the
539 duration, that plays a role in restructuring the soil microbial community. The absence of
540 variation in the soil microbial communities of elk grazed and un-grazed temperate forests after
541 15 years of elk exclusion, despite significant reduction in soil compaction by elk exclusion,
542 supports this hypothesis (Gass and Binkley, 2011).
543 Previous studies found a top-down regulation of the microbial community structure by wild
544 ungulates in a sagebrush steppe (Cline et al., 2017; Peschel et al., 2015), in an alpine grassland
545 (Yang 2013) and in Australian woodlands (Eldridge et al., 2017). Our results show that such top-
546 down regulation also operates in temperate forests. However, this modification was only
547 observed after more than 70 years of deer presence, suggesting that regulation of the soil
548 prokaryotic communities by deer is a slow process in such ecosystems.

549 **3.5. Effects of deer belowground: the importance of study duration**

550 Our three study approaches led to diverse results when investigating the effects of deer
551 belowground. The comparison of the results found among these approaches suggests that the
552 modifications of the ecosystem components by deer are time dependant (Figure 5).
553 Aboveground, changes in the plant community in response to deer presence or removal were
554 relatively fast and consistent, because they are primarily the result of direct negative impacts of

555 browsing and trampling. Belowground, changes in edaphic properties varied according to the
556 length of deer presence or exclusion. Consistent with our prediction, short- and intermediate-
557 term effects of deer belowground were probably the result of the direct interactions of deer on
558 the soil (i.e. dung and urine deposition and trampling). Long-term effects of deer belowground
559 appeared to be the result of both direct interaction, due to trampling, and indirect interaction
560 due to a vegetation shift.
561 Deer density has previously been shown to play a significant role in the extent of the
562 belowground response to deer in temperate forests (Ramirez et al., 2018). Our results highlight
563 that difference in study duration among studies can be another confounding factor when
564 comparing findings on the effects of deer belowground. Currently, the method of choice to
565 study the impact of large herbivores, exclosures, generally last in the range of a decade
566 (Andriuzzi and Wall, 2017). The longest period of deer exclusion in temperate forests has been
567 investigated by Wardle et al. (2001) in New Zealand. The authors found idiosyncratic effects of
568 20 to 50 years of deer exclusion on soil properties and communities, with responses to deer
569 exclusions varying from site to site without apparent consistency among sites. In our study, the
570 effects of deer on soil chemistry (pH and total phosphorus) and soil prokaryotes were
571 detectable after 70 years of deer colonisation. This suggests that several decades are necessary
572 to observe non-idiosyncratic effects of deer belowground.
573 The effect of deer on soil compaction particularly illustrates the time-dependence of different
574 soil responses observed among studies. Indeed, previous studies in temperate forests did not
575 find an impact of deer on soil compaction for deer exclusion that lasted less than 15 years
576 (Burke et al., 2019; Furusawa et al., 2016; Relva et al., 2014; Suzuki and Ito, 2014). However,

consistent positive effects of deer on soil compaction were observed for studies lasting over 15 years (Gass and Binkley, 2011; Iida et al., 2018; Kumbasli et al., 2010; Sabo et al., 2017). This is consistent with our study, where one year of deer exclusion after a deer cull did not change soil penetration resistance, whereas, twenty years of deer exclusion and 70 years of deer presence significantly decreased or increased compaction, respectively (Table A2).

In their meta-analysis on the effect of the exclusion of wild herbivores on the soil, Andriuzzi and Wall (2017) found that time since herbivore exclusion, which was ranging from less than 5 years to more than 50 years, was the weakest predictor of soil microbial community structure. However, their analysis combined results of exclosure studies from various biomes and herbivore sizes, both of which have been shown to strongly influence herbivore effects belowground (Andriuzzi and Wall, 2017). It is likely that the time-dependence of the soil response to herbivores depends on both the biome and the herbivore size, which could explain the absence of a general pattern in their study. For example, effects of deer on the soil via vegetation replacement may be expected to be faster in grassland ecosystems, where plant tolerance to herbivores is higher, than in forest ecosystems where tree and shrub tolerance to herbivores is lower and plant regrowth slower (Augustine and McNaughton, 1998).

4. Conclusions

We found that aboveground effects of deer were consistent among the three study systems, reflecting a temporal shift in the vegetation in response to deer presence that was consistent with plant growth patterns and requirements. The effects of deer on soil properties and organisms were time-dependent. The belowground response to deer was driven by waste

598 deposition and trampling in the short-term and by trampling and vegetation shift in the long-
599 term. Long-term changes in soil compaction and pH by deer contributed to a modification of
600 soil prokaryotic community structure and composition. Detection of changes in soil chemical
601 and biological properties by deer in temperate forests, therefore, requires long-term studies
602 which are currently scarce in the literature.

603

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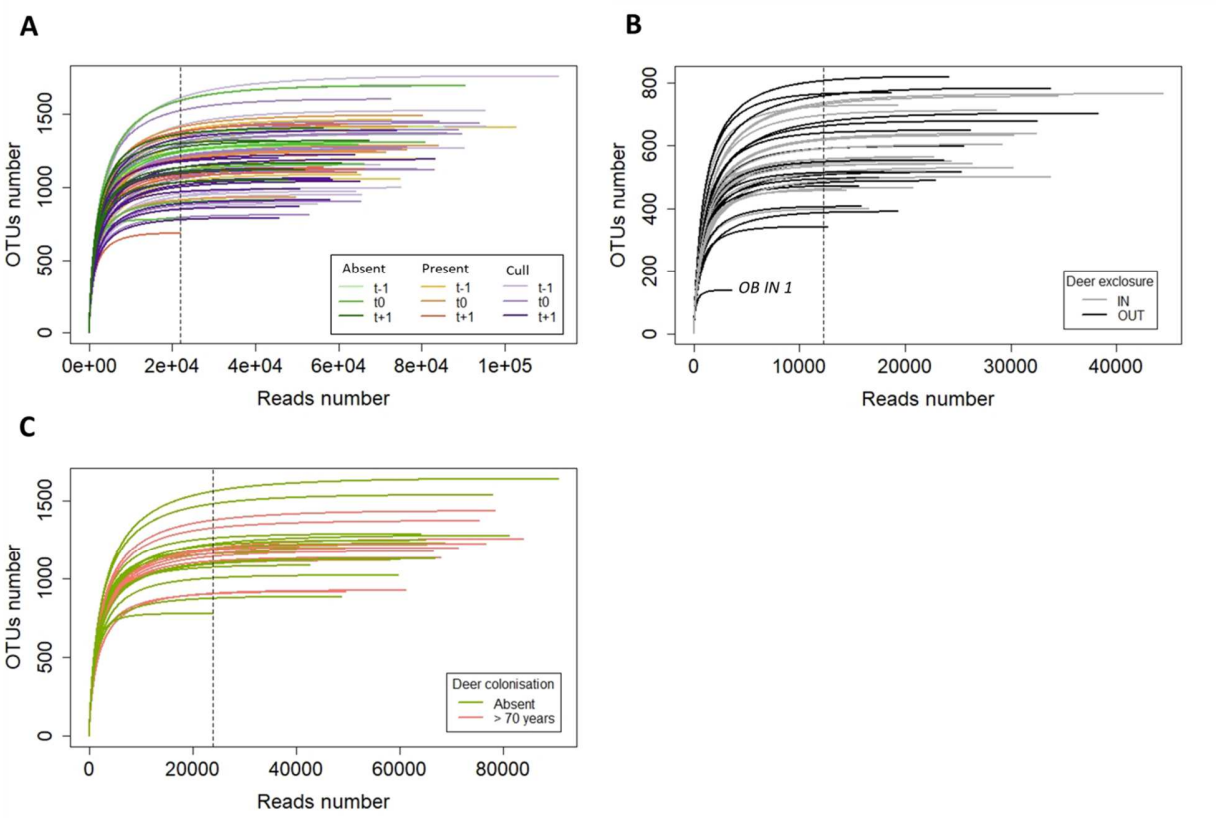
7. Appendices

Table A1 – Modified Braun-Blanket scale used for estimating plant species cover in the vegetation surveys.

Cover class	A	B	C	D	E	F	G	H	I	J
% cover range	<0.25	0.25-0.5	0.5-1	1-5	5-15	15-25	25-50	50-75	75-95	95-100
Midpoint (%)	0.125	0.375	0.75	3	10	20	37.5	62.5	85	97.5



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828 **Figure A1 –** Rarefaction curves in **A)** the recent deer cull system, **B)** the deer exclosure system
829 and **C)** the deer colonisation system. The dashed line indicates the reads number value at which
830 data were rarefied in each system.

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Table A2 – Results of the statistical tests in each system and for each variable. Cull = recent deer cull system, Exc. = deer exclosure system and Col. = deer colonisation system. F1-LD-F1 nparLD test, paired Wilcoxon test and Wilcoxon test were used for the three systems respectively. Statistical values given for the recent deer cull system correspond to the interaction between treatments and year of sampling. Values in bold and underlined correspond to significant p-value < 0.05 that were attributed to a deer effect. Values in bold correspond to significant p-value < 0.05, but that were not attributed to any deer effect. Values in bold and italic correspond to marginally significant p-value < 0.1 that were attributed to a deer effect.

	Statistic			**p-value**		
Variables	1/Cull	2/Exc.	3/Col.	1/Cull	2/Exc.	3/Col.
Vegetation						
Shannon vasc.	3.78	183	114	**0.0097**	**7e^{-05}**	**0.04**
Conifer	-	117.5	29.5	-	0.38	**0.01**
Forb	6.30	145.5	78	**0.0043**	**0.001**	0.98
Graminoid	1.32	11.5	17.5	0.27	0.74	**5.5e^{-04}**
Pteridophyte	1.34	62	147	0.26	0.08	**1.3e^{-04}**
Shrub	2.26	179	154	0.11	**2.1e^{-04}**	**3e^{-05}**
Shanon bryo.	4.42	114	30	**0.0035**	0.47	**0.01**
Bryophyte	5.20	13.5	9.5	**0.0049**	**0.002**	**2.3e^{-04}**
Soil						
Penetrometer	0.45	0	0	0.59	**1.4e^{-04}**	**3e^{-05}**
SWC	0.15	127	5	0.91	0.21	**1e^{-05}**
pH	4.39	107	152	**0.0067**	0.64	**5e^{-05}**
%C	1.02	51	50	0.39	***0.08***	0.15
%N	1.87	73	83	0.12	0.4	0.77
C:N	0.91	95	56	0.44	1	0.27
Total P	2.46	127	118	**0.057**	0.21	**0.02**
NH4	2.32	70	70	***0.077***	0.33	0.73
NO3	1.65	37	61.5	0.19	0.58	0.30

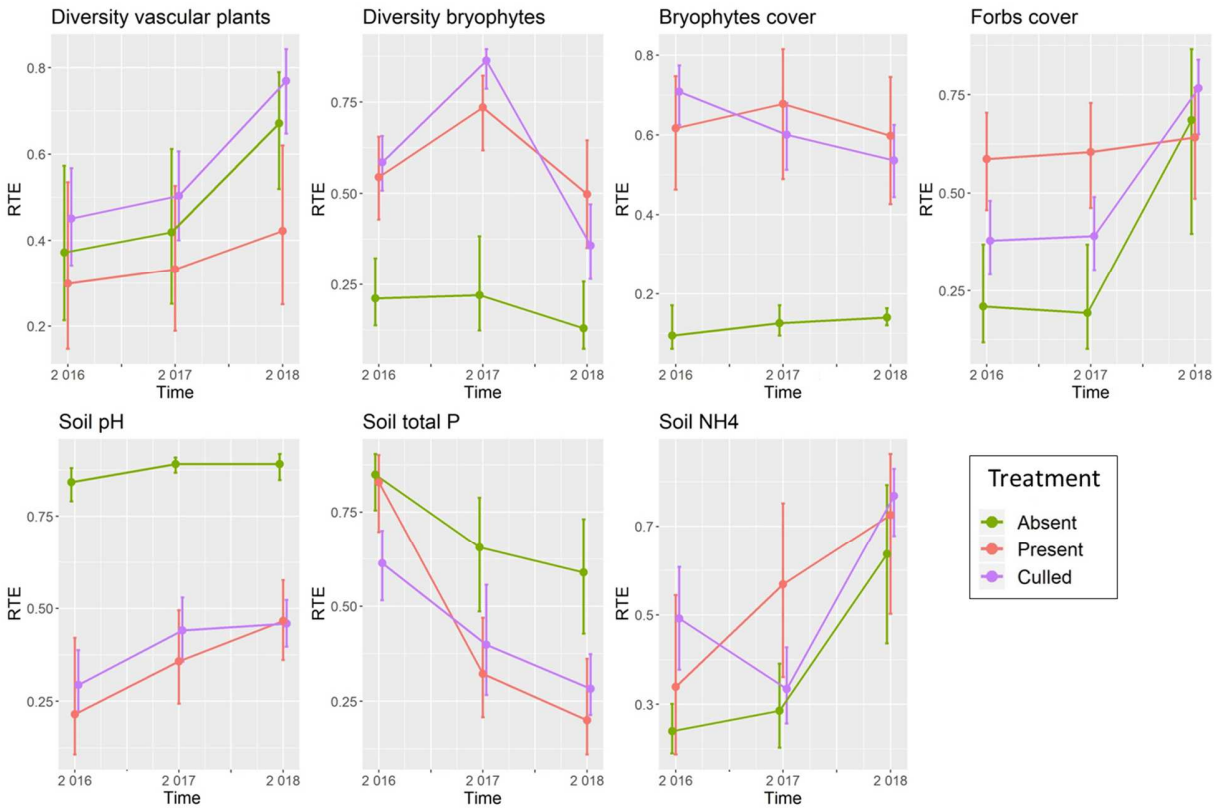
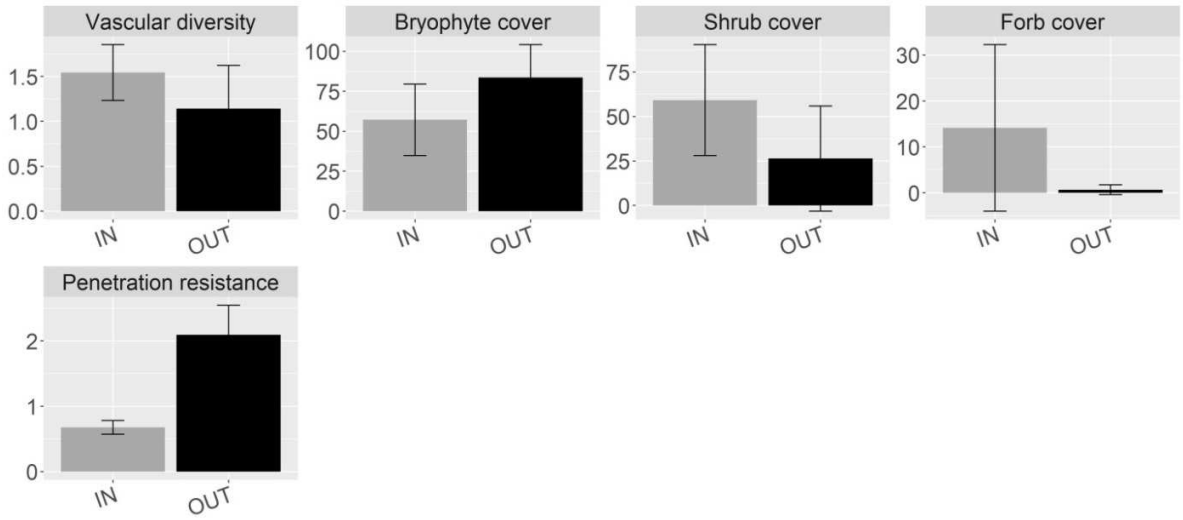


Figure A2 – Relative Treatment Effect (RTE) in the recent deer cull system for plant and soil variables showing a significant interaction between the treatment and the year of the cull. The RTE is the probability that a value randomly sampled in the entire dataset is lower than the value randomly sampled in a sub-dataset (Noguchi et al., 2012). It represents the interaction between two factors, here 'Time and 'Treatment'. Bars correspond to the 95% confidence intervals.



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879 **Figure A3 –** Variables found to be significantly different between inside (IN) and outside (OUT)
880 exclosures in the deer exclosure system. Vascular plant diversity is represented with the
881 Shannon index. Plant covers are expressed in %. Penetration resistance is expressed in kg/cm².

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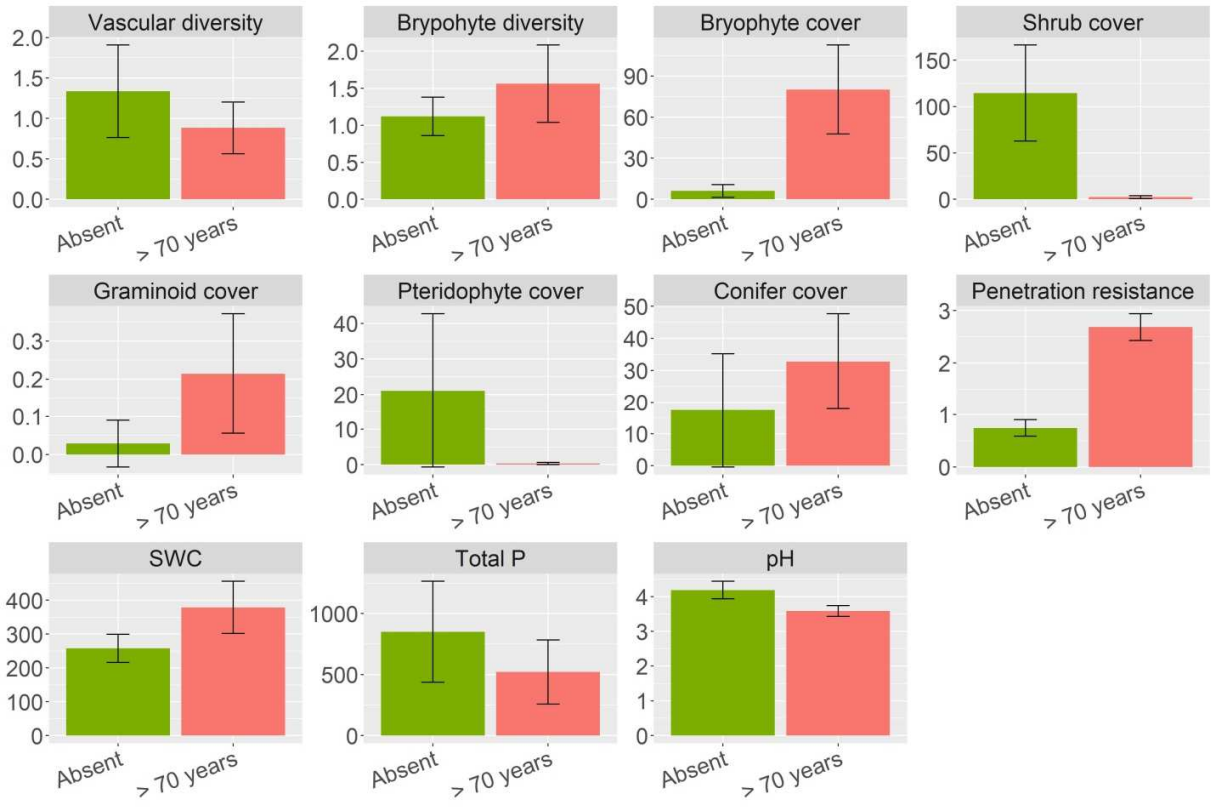

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885 **Figure A4 –** Plant and soil variables that differed significantly between un-colonised islands and
886 islands colonised by deer for more than 70 years in the deer colonisation system. Plant
887 diversities are represented with the Shannon index. Plant covers are expressed in %.
888 Penetration resistance is expressed in kg/cm². Soil Water Content (SWC) is expressed in
889 percent. Total phosphorus (P) is expressed in µg P/g dry soil.

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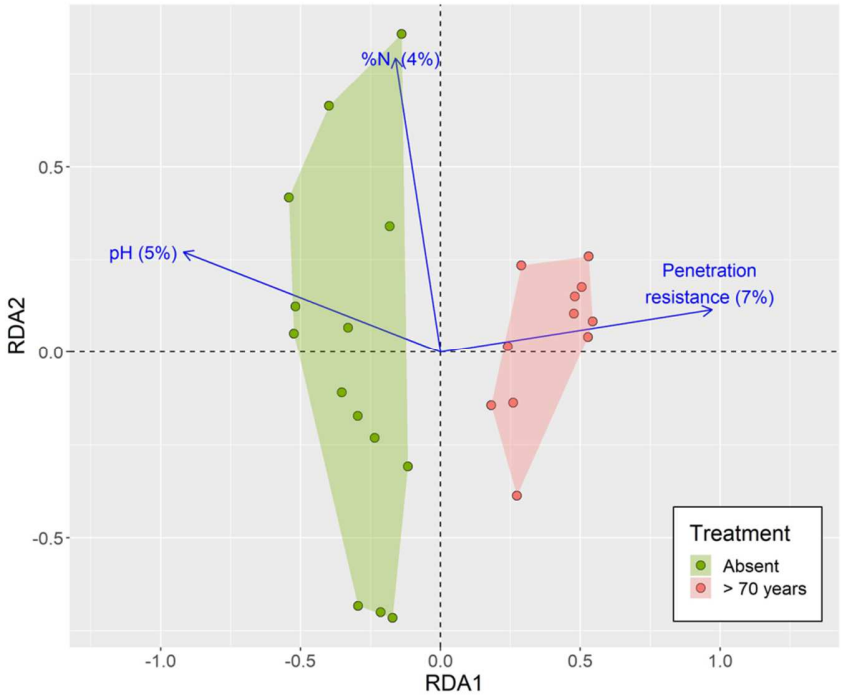

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893 **Figure A5 –** Redundancy Analysis (RDA) on the OTUs and the environmental variables selected
894 by forward selection for the deer colonisation systems. Percent values correspond to the
895 variation in soil prokaryotic community explained by the PCA axes, and calculated by variation
896 partitioning.

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