



De l'abondance des cerfs aux propriétés du sol : Une étude de cas dans les forêts d'Haïda Gwaii

Morgane Maillard

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From deer abundance to soil properties: A case study in the forests of Haida Gwaii

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Le 19 décembre 2019

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Abstract

The past century witnessed a dramatic increase in deer abundance in North America and Western Europe. Deer overabundance prevented temperate forest regeneration, dramatically reduced their understory vegetation cover and composition, with negative consequences for other trophic layers such as birds and insects. While impacts of abundant deer aboveground have been well documented, effects on the soil of temperate forests remain unclear. Deer interact with the soil through waste deposition, trampling, and reduction of litter quantity and quality through selective foraging. The multiplicity of these pathways makes it difficult to predict the net effect deer will have on soil communities and processes. As a result, current studies in temperate forests have found inconsistent results within, and across, systems. In an attempt to resolve these inconsistencies, we used the unique configuration of the Canadian archipelago of Haida Gwaii which offers a quasi-experimental situation with the presence of islands without and with deer, the latter varying in deer colonisation history. This unique context is complemented by the knowledge gathered in the course of 30 years of studies on the effect of abundant deer aboveground. We measured the effect of deer presence on litter decomposition, soil properties, soil prokaryotic communities and nitrogen cycling rates. We compared three complementary study systems varying in time of deer presence and exclusion. We found that the response of the soil to deer presence was time dependant. Short-term and intermediate effects of deer belowground were the results of the direct interactions deer have with the soil, i.e. waste deposition and trampling. Long-term effects of deer belowground appeared to be the results of both direct interactions, due to trampling, and indirect interactions, due to vegetation shifts. Through the reduction in litter quality by selective browsing, long term deer presence significantly reduced the rate of carbon and nitrogen lost by litter during decomposition. Under long-term deer presence, soil prokaryotic community diversity decreased, and composition was shifted, by trampling. In the absence of deer it had a better ability in decomposing carbon. A preliminary analyse on the nitrogen cycle suggest no effect of deer on the kinetics of nitrogen rates in the forest floor.

Résumé

L'augmentation récente et spectaculaire de l'abondance des cerfs en Amérique du Nord et en Europe occidentale a entraîné de profonds changements dans la structure des forêts tempérées. Si ces changements sur la partie aérienne de la forêt sont aujourd'hui bien caractérisés, les interactions avec le compartiment 'sol' restent encore largement méconnues. Les cerfs peuvent interagir avec le sol par le rejet de fèces et d'urine, par leur piétinement, et par un abrouissement sélectif qui favorise la dominance de plantes ayant une litière de pauvre qualité. Ces interactions multiples rendent difficile la prédiction de l'effet net des cerfs sur les organismes et les processus du sol. En conséquent, les études actuelles dans les forêts tempérées ont révélé des résultats idiosyncratiques. Pour résoudre cette problématique, nous avons utilisé la configuration unique de l'archipel canadien d'Haida Gwaii, qui offre une situation quasi expérimentale avec la présence d'îles présentant un gradient de colonisation. S'y ajoute l'accumulation de 30 ans de connaissances sur l'effet des cerfs sur la structure des forêts de ces îles. Nous avons quantifié l'effet des cerfs sur la décomposition de la litière, les propriétés du sol, l'abondance et la structure des communautés procaryotes du sol, ainsi que sur les taux de transformation de l'azote. Nous l'avons fait en comparant trois systèmes d'études complémentaires différant en temps de présence et d'exclusion des cerfs. Nous avons trouvé que la réponse du sol aux pressions exercées par les cerfs dépendait de la durée de présence ou d'exclusion de ces herbivores. Pour des durées inférieures à 35 ans, cette réponse dépend des interactions directes que les cerfs ont avec le sol, à savoir le rejet de fèces et d'urine ainsi que du piétinement. Pour des durées supérieures à 70 ans, cette réponse dépend à la fois d'interactions directes dues au piétinement et d'interactions indirectes dues à la modification de la végétation. En réduisant la qualité de la litière, la présence prolongée des cerfs a considérablement ralenti la décomposition du carbone et de l'azote. 70 ans de compaction du sol par le piétinement des cerfs a modifié de manière significative la structure de la communauté procaryote du sol, réduisant également leur habilité à décomposer le carbone. Cependant, nos analyses préliminaires sur le cycle de l'azote suggèrent une absence d'effet des cerfs sur la cinétique des taux d'azote dans la première couche du sol.

Lay Summary

The past century witnessed a dramatic increase in deer abundance in North America and Western Europe that triggered profound changes in the structure of temperate forests. If these changes are today well characterised, the effects of abundant deer belowground in these forests remain unclear. Deer can interfere with the soil through waste deposition, trampling, and reduction of litter quantity and quality by preferential browsing of palatable plants. What are the consequences of these interactions for the soil? To answer this question, we studied the soil response to the colonisation and removal of Sitka black-tailed deer in the forests of Haida Gwaii. We found that deer slowed-down litter decomposition by reducing litter quality. They also modified microbial community structure and ability in decomposing carbon via soil trampling. Most of these effects became only apparent in the long term, hence questioning the results obtained through short term studies.

Résumé court

L'augmentation récente et spectaculaire de l'abondance des cerfs en Amérique du Nord et en Europe occidentale a entraîné de profonds changements dans la structure des forêts tempérées. Si ces changements sont aujourd'hui bien caractérisés, les effets de cette forte abondance sur le sol restent cependant mal compris. Les cerfs peuvent interagir avec le sol par le rejet de fèces et d'urine, le piétinement et la réduction de la quantité et de la qualité de la litière par le broutage préférentiel des plantes appétantes. Quelles sont les conséquences de ces interactions pour le sol ? Pour répondre à cette question, nous avons étudié la réponse des sols à la colonisation et à l'élimination du cerf de Sitka dans les forêts d'Haida Gwaii. Nous avons constaté que les cerfs ralentissaient la décomposition en réduisant la qualité de la litière. La structure de la communauté microbienne et sa capacité à décomposer le carbone était impactée par la compaction du sol dû au piétinement. Nous avons également constaté que les effets des cerfs à court et moyen termes n'avaient que peu ou pas d'effet sur le sol, remettant en question les conclusions des études actuelles basées sur de plus court terme.

Preface

Outlines of this project were thought and designed by Sue Grayston and Jean-Louis Martin. This thesis was realised under a joint PhD between the University of Montpellier (UM) in France, and the University of British Columbia (UBC) in Vancouver, Canada. Data analysis and redaction of the thesis were conducted within the team Dynamic of Landscape and Biodiversity (DPB) at the Center of Functional and Evolutionary Ecology (CEFE). All the laboratory analyses were conducted within the laboratories of the Belowground Ecosystem Group (BEG) at UBC in Vancouver. All the field work was conducted on Haida Gwaii, with the support of the Research Group on Introduced Species (RGIS).

Simon Chollet conceived the ideas and designed the methodology of the litter bag experiment in close interaction with Morgane Maillard. Maria Continentino, Yonadav Anbar and Morgane Maillard built the litter bags. Simon Chollet, Juliane Schörghuber, Jean-Louis Martin and Morgane Maillard collected the data. Morgane Maillard realised the chemical measurements on litter and litter bags content. Simon Chollet and Morgane Maillard analyzed the data and led the writing on the manuscript. A version of this chapter has been published in Peer Community In Ecology (PCI).

Morgane Maillard designed the methodology of the third chapter. Vegetation survey was designed by Catch Catomeris and Dylan Mendenhall. Sampling was realised by Sue Grayston, Jean-Louis Martin, Catch Catomeris, Simon Chollet, Juliane Schörghuber, Maria Continentino, Yonadav Anbar, Dylan Mendenhall and Morgane Maillard. Experimental analyses of the samples were realised by Morgane Maillard. Léna Simon contributed to the soil DNA extraction, the qPCR and the total phosphorus measurements.

Morgane Maillard designed the methodology of the ^{15}N tracing experiment. Sampling was realised by Morgane Maillard with the help of Sue Grayston, Jean-Louis Martin, Catch Catomeris, Simon Chollet, Juliane Schörghuber, Maria Continentino, Yonadav Anbar and Dylan Mendenhall. Experimental analyses of the samples were realised by Morgane Maillard. Development of the ^{15}N tracing model was realised Christoph Müller. Simulations of the model were realised by Anne Jansen-Willems.

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MultiDimensional Scaling. LD = Linear Discriminant. Absent = deer absent; < 35 = deer present for less than 35 years; > 70 = deer present for more than 70 years; Reduced = deer culled on Reef Island between 1997 and 2010; IN = inside deer exclosures; OUT outside deer exclosures; Culled = recent severe cull on Ramsay Island; t_{-1} , t_0 and t_{+1} correspond to the year before, month after and year after the cull respectively. 83

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Chapter 1: What role are deer playing in temperate forests?

1.1– Interactions or the definition of an ecosystem

“Organisms interact with one another and with their environment”.

This simple statement is one of the few universal laws in ecology (Lawton, 1999). Interactions indeed drive the concept of ecosystem. Within an ecosystem, species are organised in food webs that reflect the trophic interactions from top predators to primary producers. Modification of these network components can lead to a profound restructuring of the ecosystem, which in turn will affect its resilience to perturbations (Mills et al., 1993). For example, the removal of top predators may initiate a trophic cascade resulting in the proliferation of some species and the decline of others (Estes and Palmisano, 1974; Paine, 1966). Today, ecosystem perturbations stem increasingly, directly and indirectly, from human activities. Understanding the dynamics of these complex shifts in the networks of interactions surrounding us, and in particular understanding the consequences of dramatic changes in the abundance of key species, is increasingly necessary if we want to anticipate changes in ecosystems in response to such perturbations. It is also essential to our coexistence with nature.

1.2 – Deer overabundance in temperate forests

When can we speak of “overabundance”, and what are the consequences of species overabundance for ecosystems? The notion of overabundance is difficult to define without subjectivity. Caughley (1981) defined four classes of overpopulation. According to his definition, a species is overabundant if it (1) *“threatens human life or livelihood”*, (2) *“depresses the densities of favored species”*, (3) *“is too numerous for their own good”* or (4) *knocks “the system of plants and animals off their equilibrium”*. If human activities are responsible for the extinction of numerous species (Ceballos et al., 2015), they have also largely contributed to the overabundance of others. Such overabundances are often the

result of exotic species introduction or of the removal of competitors or predators (Coblentz, 1990; Estes and Palmisano, 1974). They can also result from the proliferation of segments of the local fauna that are able to take advantage of ecosystem perturbation by humans (Côté et al., 2004). The resulting modification of food webs can lead to profound changes in ecosystem functioning and biodiversity (Augustine and Decalesta, 2003; Estes and Palmisano, 1974; Paine, 1966). The dramatic increase in deer abundance in temperate forests of North America and Western Europe during the past century is a textbook case, and falls into the four classes of overpopulation defined by Caughley (1981) (Côté et al., 2004; Fuller and Gill, 2001). Studying the ecological consequences of deer overabundance is not only a management necessity, but also provides a unique opportunity to obtain a more comprehensive understanding of ecological processes in temperate forests.

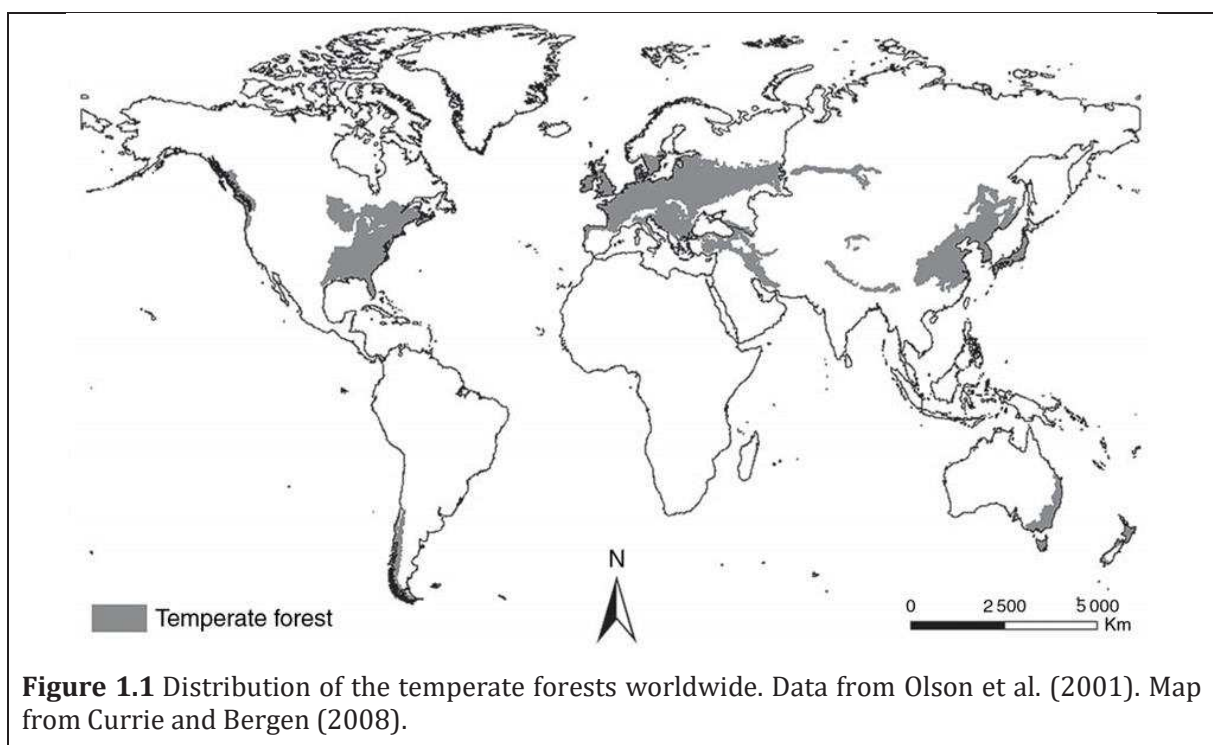
1.2.1 – Temperate forests

Forests have always played an important function in society. Source of food and traditional medicines for gatherers and hunters, they also provide wood, an essential material used for fuel, habitat and construction materials (Landsberg and Waring, 2014). Forests are also important carbon sinks, and therefore of outmost importance for our warming planet (Pan et al., 2011). Yet, forests have been significantly affected by human activities.

Temperate forests represent 25 % of the forests worldwide, and are located in both the Northern and the Southern hemisphere. They are an important component of the temperate biome and are found in five regions of the world, in North America, South America, Europe, Asia and Oceania (Figure 1.1). Agriculture and the timber industry have been responsible for either the deforestation of forested land globally or for the transformation of the remaining area of natural forests into managed forests (Sedjo et al., 1998). In contrast to tropical forests, temperate forests are characterised by a seasonality that drives their physiological processes (Currie and Bergen, 2008). They differ from boreal forests due to their higher mean annual temperatures that range from 5°C to 20°C, and by a frost-free season lasting at least four months (Currie and Bergen, 2008). Temperate forests are diverse. They can be classified into three main biogeographic units based on their

vegetation characteristics. Evergreen coniferous temperate forests are found in mountainous regions of North and South America, Europe and Asia. They are dominated by conifers such as pine (*Pinus* spp.), fir (*Abies* spp.), spruce, (*Picea* spp.), hemlock (*Tsuga* spp.) and cedar (*Thuja* spp.) (Landsberg and Waring, 2014). Evergreen broad-leaved temperate forests are dominated by trees that conserve their leaves throughout the year. They mainly contain the eucalyptus (*Eucalyptus* spp.) forests of Australia, and the southern beech (*Nothofagus* spp.) forests of Australia, South America and New Zealand. Mixed temperate forests are dominated by both evergreen coniferous species and deciduous broad-leaved trees, such as oaks (*Quercus* spp.), maples (*Acer* spp.), beeches (*Fagus* spp.) or alders (*Alnus* spp.) (Landsberg and Waring, 2014). These forests are mainly located in North America, Asia and Europe.

The past century has witnessed a dramatic increase in deer abundance at continental scales in North America, Western Europe and Japan (Côté et al., 2004; Fuller and Gill, 2001; Takatsuki, 2009). This recent overabundance has important implications for the structure and functioning of temperate forests (Côté et al., 2004).



1.2.2 – Overabundant deer: what consequences for the forest?

The increase in deer populations this past century is believed to be the result of several mechanisms. Following a massive decline in deer populations that occurred between the 15th and the 20th centuries, measures have been taken by wildlife managers to restore deer populations. The will to restore deer populations was driven by the wish to restore hunting opportunities for subsistence, recreation and trophies. Strict hunting regulations were applied, with hunting restricted to adult male deer. Several re-introductions occurred through Europe and the United-State (McShea et al., 1997). Indirectly, re-forestation and the abandonment of agricultural land, or, in cropland, the shift to winter cereals that provided abundant winter forage to deer, have all contributed to a rehabilitation of deer habitat and populations. All these factors have paved the way to current high deer abundance in North America, Europe and Japan (Côté et al., 2004; Fuller and Gill, 2001; Takatsuki, 2009). Last, but not least, the extirpation, or severe reduction, of the deer predators has also played a role in deer overabundance. Ripple and Beschta (2012) indeed found that deer density was six times higher in areas from where wolves were absent, based on data from 42 published studies on North American and Eurasian forests. Similarly, the re-introduction of wolves in 1995 in Yellowstone National Park, United States, allowed to control elk population and reduce their high abundance (Ripple and Beschta, 2012b). Reduction in cougar density due to increasing human presence also resulted in significantly larger deer populations in two national parks of Western United States (Ripple and Beschta, 2008, 2006).

The massive increase in deer abundance across continents has triggered important changes to the structure of forests (Côté et al., 2004; Ramirez et al., 2019). High deer abundance has been shown to prevent forest regeneration in north temperate forests due to over-browsing and trampling of tree seedlings (Gill, 1992). Deer over-abundance can also lead to a dramatic reduction in vegetation cover and composition in forest understories (Horsley et al., 2003; Stockton et al., 2005). Selective browsing towards more palatable (i.e. nutrient-rich) plant species promotes the dominance in the forest of less palatable (i.e. nutrient-poor) plant species (Pastor et al., 1993; Tamura, 2016). These modifications in the

forest plant community composition and structure have negative consequences for population abundances and diversities of other trophic layers such as forest birds and insects that depend on the understory vegetation for food and habitat (Cardinal et al., 2012; Chollet and Martin, 2013; Martin et al., 2010; Nuttle et al., 2011; Takada et al., 2008). Reduction in forest plant and animal biodiversity by overabundant deer therefore simplifies and homogenises forest ecosystem properties (Martin et al., 2010). Deer over-abundance also has important economic implications. Damage caused by deer to the timber industry is estimated to exceed \$750 million per year in the United States through both the reduction in volume of harvestable timber and the cost associated with seedling protection from herbivory with cones or chemical repellents (Conover, 1997). In addition, collisions between deer and vehicles are not negligible, representing more than a million accidents each year in both Europe and the United States, causing death, injuries and material damage (Bruinderink and Hazebroek, 1996; Romin and Bissonette, 1996).

Extensive studies in North America and Europe on the effects of over-abundant deer on vegetation have led to good characterisation of the interactions between deer and plant communities in temperate forests. These studies have further highlighted the top-down control that deer exert on other trophic layers, through the modification of vegetation structure in the absence of control by predators and/or hunters. However, this is only half of the story. Beneath our feet, deer may modify the hidden part of the forest: the soil.

1.3 - What are the consequences of deer overabundance beneath our feet?

Soil occupies a central role in forest functioning. Complex and dynamic, soils are not only necessary to provide a physical support for aboveground organisms. They also allow the recycling of the nutrients sustaining the life of the forest.

1.3.1 – The soil: a complex ecosystem

The importance of soil for forest functioning comes from the activity of billions of interacting organisms. Soil supports a biodiversity largely exceeding the one found aboveground

(Thomas and Packham, 2007). Soil organisms have a broad range of body sizes, and can thus interact at a broad range of spatial scales (Wardle, 2002). Larger than 2 mm, soil macrofauna are represented by 9 orders, including the *Araneae*, the *Diplopoda* and the *Lumbricidae*. Some of these organisms live at the surface of the soil, such as millipedes; others, like centipedes and worms, spend their life digging through the soil matrix. Smaller than macrofauna organisms but larger than 0.1 mm, the mesofauna includes many arthropods such as the *Acari*, the *Collembola* and the *Tardigrada*. The small, but abundant, microbiota includes the fraction of organisms smaller than 0.1 mm. Among them protists, rotifers and nematodes constitute the micro-fauna. Bacteria and archaea represent the prokaryotic fraction of the soil microbial community. Prokaryotes are extremely diverse, soil bacteria for example are estimated to exceed 500 000 species (Thomas and Packham, 2007). They are also extremely abundant, and it is estimated that 200 million bacteria can be found in a single gram of soil (Thomas and Packham, 2007). Finally, fungi are eukaryotic microorganisms, whose abundance can represent up to 70 % of the soil biomass (Thomas and Packham, 2007). Like bacteria, the diversity of soil fungi can be enormous, exceeding thousands of species (Bridge and Spooner, 2001). Similarly to the organisation found aboveground, all these organisms interact with each other and with the soil matrix. In these belowground food webs, predators feed on other soil organisms and herbivores feed on plant roots and root exudates. The vast majority however are detritivores and decomposers and drive the fundamental process of litter decomposition.

In forests, the fuel of soil food webs is the litter produced by the vegetation aboveground. When a dead leaf – or litter – falls on the ground, it is transformed through a series of processes into biomass and humus. This process, called decomposition, is essential for the recycling of carbon and nutrients and is driven by many players belowground. Detritivores, macro- and meso- faunal decomposers feed on this food input. By chewing the dead leaf, they participate in the physical breakdown and mixing of litter, favouring the activity of the soil microbiome. The soil microbiome comprises bacteria, archaea and fungi. Functional guilds of microbes chemically degrade litter, using and transforming organic molecules in order to produce energy and sustain their growth. Fungi are particularly

efficient at degrading complex structural molecules, such as cellulose and lignin, through the production of cellulolytic and oxidative enzymes, respectively. Some species of bacteria, such as *Pedobacter* and *Mucilaginibacter*, have also been shown to produce such enzymes (López-Mondéjar et al., 2016). Through specific metabolisms, soil microbes contribute to the mineralisation of organic nutrients into a form available for plants. Nitrogen, in particular is transformed through a range of reactions conducted by the interactions of specific micro-organisms. As a constituent of proteins, nitrogen is an essential nutrient for any living organism. In temperate forest soils, where nitrogen is limiting for plant growth, nitrogen mineralisation by soil microbes is of utmost importance (Vitousek and Howarth, 1991). Proteins contained in litter are first depolymerised by soil microbial proteolysis. The resulting organic nitrogen molecules are then mineralised into ammonium, a process called ammonification that is conducted by a broad range of soil microbes. Ammonium is further transformed into nitrite by the ammonia-oxidizing bacteria and archaea, such as the *Nitrosomonas*, *Nitrosococcus* and members of the Crenarchaeota, during the first step of the nitrification process (Levy-Booth et al., 2014). In the second and last step of nitrification, nitrite is oxidized to nitrate by nitrite-oxidizing bacteria such as the *Nitrobacter* or the *Nitrospira* (Levy-Booth et al., 2014). Mineral nutrients, such as ammonium or nitrate, will then be assimilated by the roots, and used by plants to sustain their growth. A new leaf will be created. Eventually, this new leaf will die and fall to the ground, and the cycle will start again.

Soil is constantly interacting with the above-ground sub-system. Litter fall provides organic nutrients to the soil food web, and soil organisms decompose and recycle organic nutrients into a mineral form that sustains plant homeostasis and growth. As a result, any modification of these biological processes may influence the plant community, which might reverberate on other trophic layers aboveground (Wardle, 2002). Similarly to plants and other living beings, soil microbes depend on nutrients to maintain their homeostasis and growth. When nutrient content of litter is sufficient to fill microbial requirements, microbes release the surplus into mineral form in the soil through mineralisation processes described above. However, when the nutrients content of litter is insufficient, nutrients are retained by

microbes, in a process called immobilisation. In this last scenario, plants and soil micro-organisms are, therefore, in competition for nutrients. Modification of nutrient availability in soil can influence tree growth and chemical defenses by plants, and change understory species composition and abundance due to increased plant competition and stimulation of fungal pathogens (Mateirć, 2016; Nohrstedt, 2001; Nordin et al., 2006; Strengbom and Nordin, 2008).

Soil is therefore an essential and indivisible part of the forest ecosystem. Any modification of soil properties and organisms can interfere with functioning, which are likely to have implications for the aboveground forest compartment (Wardle, 2002). Fully understanding the role of deer over-abundance in the ecological functioning of forests is, therefore, not possible without considering the effect on the soil.

1.3.2 – Overabundant deer: which consequences for the soil?

Deer can interact directly and indirectly with the soil ecosystem through three main pathways. **1) Deposition of waste.** By excreting urine, deer release ingested organic nitrogen directly into a mineral ammonium form. This by-passes nitrogen mineralisation steps in soil. This mechanism may speed up decomposition and nitrogen cycling processes (Bardgett et al., 1998; Molvar et al., 1993). By eliminating feces, deer release a digested form of plant litter to the soil. Ungulate feces are a significant source of nutrients that are more easily processed by micro-organisms than plant litter (Ruess and McNaughton, 1987). Through waste deposition, deer, therefore, provide pulsed inputs of nutrients to the soil which may increase soil microbial biomass and activity (Bardgett et al., 1998). **2) Trampling.** The high foot pressure of ungulates can cause an important physical compaction of the soil (Duncan and Holdaway, 1989). Soil compaction, through trampling, can modify soil habitat by reducing pore size, increasing water retention and soil temperature, and decreasing oxygen levels (Cambi et al., 2015). Depending on the initial soil water and mineral content, such modifications can have positive, negative, or no effects on soil microbial activity, with extreme soil moisture content (very wet or very dry) and high soil clay content favouring negative effect of deer on nitrogen mineralization and decomposition, and intermediate soil

moisture and sandy soils favouring positive and neutral effect, respectively (Schrama et al., 2013b, 2013a). **3) Modification of vegetation abundance and composition.** In forests, deer can strongly reduce the quantity of plant litter reaching the soil due to over-browsing, which implies a reduction in organic nutrients entering soil to feed faunal and microbial communities (Bardgett and Wardle, 2003). Deer can also contribute to a decreased quality of litter entering soil through preferential consumption of palatable plant species, which are characterised by being nutrient-rich, with low content of structural carbohydrate compounds such as lignin (Bardgett and Wardle, 2003; Pastor et al., 1993). The loss of palatable plant species results in a dominance of poor-quality herbivore-resistant plant species in forests inhabited by deer (Pastor et al., 1993). The resulting reduction in litter quality is further enhanced by the production by these plants of chemical defences against herbivory, such as recalcitrant tannins and terpenes, making litter less easily decomposable for soil microorganisms (Grime et al., 1996). Lower litter quality can, therefore, slow down the activity of microorganisms involved in decomposition and nutrient cycling (Pastor et al., 1993).

A decelerating effect of deer on soil properties and microbial activities has been predicted to dominate in forest ecosystems (Bardgett et al., 1998; Bardgett and Wardle, 2003; Harrison and Bardgett, 2008). This decelerating effect is driven by the vegetation shift toward less palatable plant species resulting from preferential browsing of herbivores (Bardgett et al., 1998; Bardgett and Wardle, 2003; Harrison and Bardgett, 2008). However, current studies in temperate forests have found inconsistent results within and across systems (Figure 1.2 and Table S1.1). Perhaps the most notable study on the effect of deer belowground in temperate forests comes from the analysis of soil properties inside and outside 30 deer exclosures in New Zealand (Wardle et al., 2001). Wardle *et al.* (2001) found that deer exclusion had significant effects on soil chemistry and soil organisms, but that the direction of these changes was idiosyncratic across exclosures. Other studies have documented idiosyncratic effects of deer on soil properties. A comparison of four exclosures in the forests of northern Britain showed that 14 years of native red deer (*Cervus elaphus*) exclusion had an idiosyncratic effect on soil total C:N, soil water content and microbial C biomass (Harrison and Bardgett, 2004). Two years of native red deer exclusion in an oak

forest of central Europe resulted in an increase or decrease in organic C and total N with or without the presence of other ungulates respectively (Mohr et al., 2005; Mohr and Topp, 2005). Some have studies found a consistent effect of deer, but these effects were not consistent across studies. Harrison and Bardgett (2004) for example, found that deer significantly increased soil pH and decreased N availability in a regenerating native forest of Northern Britain, whereas Kumbasli *et al.* (2010) and (Mohr et al., 2005) found that soil pH decreased when deer are present in a Turkish and a German forest, and Stritar et al. (2010) found a positive effect of elk on N availability in Arizonian forests in the United States. Other studies, such as the work of (Relva et al. (2014) on exotic deer in Argentinian forests, showed that deer did not have any impact on soil properties and functioning.

The effect of deer on the soil microbial community is also idiosyncratic among studies. The effect of deer on soil microbial biomass in temperate forest was found to be negative in a Japanese and an Arizonian forest (Niwa et al., 2011; Stritar et al., 2010), idiosyncratic in British and New Zealand forests (Harrison and Bardgett, 2004; Wardle et al., 2001) or neutral in forests in the Colorado (US), Germany and Argentina (Gass and Binkley, 2011; Mohr et al., 2005; Relva et al., 2014).

As a result, the consequences of deer presence on soil functioning or nutrients cycling show similar lack of patterns. The effect of deer on soil microbial respiration, a proxy for microbial activity, was neutral (Harrison and Bardgett, 2004; Relva et al., 2014) or idiosyncratic in New Zealand forests (Wardle et al., 2001). Similarly, no trend in the effect of deer on soil nitrogen cycling was found, with deer effects on N mineralisation being negative (Gass and Binkley, 2011; Harrison and Bardgett, 2004), positive (Furusawa et al., 2016), or neutral (Niwa et al., 2011; Relva et al., 2014).

1.4 – Explaining the idiosyncrasies in deer effects belowground

Deer interact with soil through many pathways. The diversity of the mechanisms involved makes a prediction of the net effect deer on soil properties and functioning difficult. When investigated in temperate forests, the effects of deer belowground indeed show inconsistent results that reflect this difficulty. Several causes could explain the inconsistencies found

within and across studies. Among them, the heterogeneous nature of the soil may play an important role.

1.4.1 – Soil heterogeneity within and among temperate forests

Soil is spatially heterogeneous. Root exudates, animal waste deposition and carcasses can all create nutrient-rich patches with consequent temporary hotspots of microbial activity (Kuzyakov and Blagodatskaya, 2015; Murray et al., 2013). The solid nature of the soil matrix retards or prevents the mobility of these pulsed inputs of nutrients, contributing to their restricted localisation (Kuzyakov and Blagodatskaya, 2015). Deer might strongly increase this spatial and temporal heterogeneity through differential use of the ecosystem and through local waste deposition (Murray et al., 2013). By doing so, deer may create an irregular landscape with patches more or less affected by their presence. This increased heterogeneity of the soil response to deer presence could therefore partly explain the idiosyncrasies found within studies.

Soil is vertically heterogeneous. Several horizons can be distinguished in a soil profile. These horizons show different physical and chemical properties, different communities of organisms and different biological activities (Fang and Moncrieff, 2005; Fierer et al., 2003; Will et al., 2010). In forests, the upper organic layers, or forest floor, receive the fresh litter and contain most of the organic matter. As we go deeper, we reach the mineral horizons where the organic carbon and the microbial biomass decrease with depth, and different microbial communities are found (Fierer et al., 2003). Studies on the effect of deer belowground in temperate forests have focused on the organic horizons (Prietzl and Ammer, 2008; Wardle et al., 2001), specific mineral horizons (*e.g.* Furusawa et al., 2016; Mohr and Topp, 2005; Niwa et al., 2008), or on fixed soil depth without considering horizons (*e.g.* Burke et al., 2019; Harrison and Bardgett, 2004; Relva et al., 2014). Differences in soil sampling techniques and the resulting differences in microbial and biological activity in the collected soil samples might, therefore, explain part of the differences among results.

Soil also varies with forest type and location. In forests dominated by deciduous broad-leaved trees, the forest floor is usually associated with a mull humus (Ponge, 2003). Mull humus forms are characterised by nutrient-rich deciduous litter and a high abundance of soil fauna which incorporate the organic layer into the mineral soil horizons (Petersen and Luxton, 1982; Ponge, 2003). In coniferous-dominated forests, such as the ones found in parts of western North America, soil is usually associated with a mor humus (Ponge, 2003). Mor humus forms are characterised by nutrient-poor litter and matted, fungal-dominated structure with low or absent fauna (Thomas and Packham, 2007). Mull and mor humus therefore contain different soil communities and nutrient dynamic. Parent material also affects soil communities and functioning, and differs among regions (Anderson, 1988). The amount of clay, sand and silt in soil depends on the parent material, and influences soil organisms and their activity (Chau et al., 2011; Hassink, 1994). For example, the effect of physical compaction on nitrogen mineralisation has been shown to be negative in clay soils and neutral in sandy soils (Schrama et al., 2013a). Soil texture further influences nutrient mobility, with clay favouring nutrient binding while sandy soil favours nutrient mobility (Anderson, 1988). Soils from different temperate forests are therefore likely to show important differences in term of organisms, nutrient dynamics and response to perturbations. These differences, due to their vegetation and parent material characteristics, might, therefore, partly explain the different to deer found among belowground studies.

1.4.2 – Importance of the temporal scale

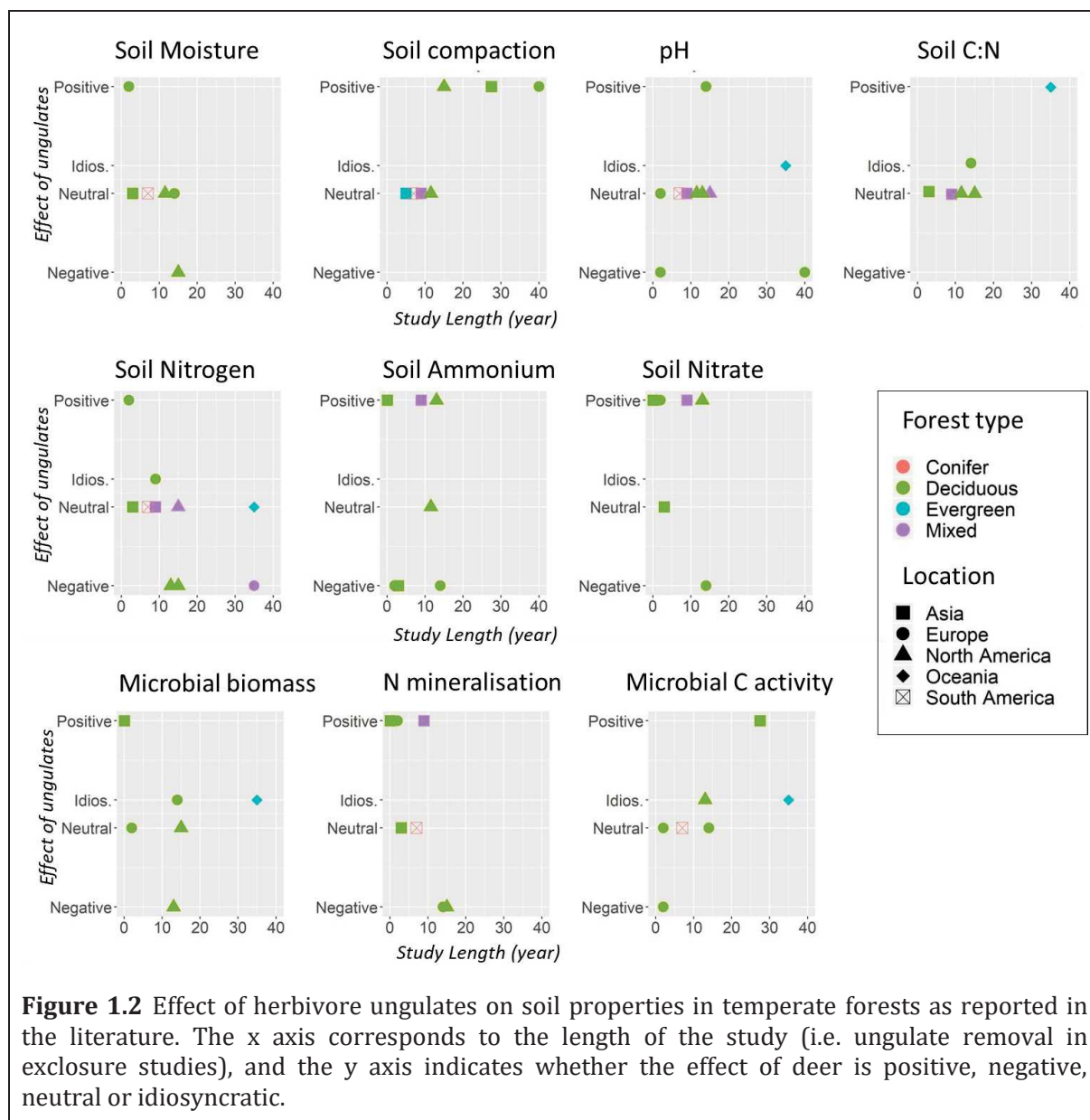
Inconsistencies in belowground responses to deer within and across studies may also result from a time-dependent response of soil to pressure by ungulates. All the mechanisms through which deer interact with soil are not operating at the same temporal and spatial scale. It takes years to decades for the plant community to be restructured by deer, while deposition of dung and urine is an instant process. As a result, short-term modifications of the belowground subsystem may be driven by the direct interaction deer have with edaphic properties through trampling or dung and urine deposition. Conversely, indirect effects of ungulates via effects on vegetation structure are longer processes. The effects of modification of vegetation structure by deer on the belowground system should, therefore,

take longer to appear (Bardgett et al., 2005). The method of choice to study the effect of deer on ecosystems is through their exclusion using fenced areas. Comparison of the ecosystem inside and outside such exclosures provides information on ecosystem resilience following deer exclusion, and, therefore, on the pressure deer exerted on the ecosystem. The time of deer exclusion varied a lot across studies, and generally consisted of relatively short periods of time, in the range of a decade (Andriuzzi and Wall, 2017). The effect of deer in these studies must, therefore, reflect short-term changes following deer exclusion. As these short-term changes might be driven by direct and local effects of deer, i.e. waste deposition, the heterogeneity induced by these local effects might explain the idiosyncrasies in belowground responses to deer found within studies.

Figure 1.2 shows the effect of deer on soil properties according to the time of deer exclusion and the forest type among studies. Soil compaction shows a clear pattern, with soil being consistently more compacted by deer trampling after 15 years of deer exclusion. Other variables, however, did not show such a clear pattern. This might be due to the simultaneous influence of local- and time- dependent responses of the soil to deer influence.

1.5 – Haida Gwaii: an *in situ* laboratory

Despite several studies on the subject, the effects of deer on soils in temperate forests remain unresolved. The idiosyncrasies observed within and among studies may reflect the spatial and temporal specificity of the interactions between deer and the soil. To dig deeper into this question, we proposed to combine different and contrasting approaches to investigate the effect of deer on the soil. Haida Gwaii, Canada's largest and most isolated archipelago, offers a unique opportunity for such a multi-pronged study with the ambition to uncover general patterns behind the effects of deer belowground and the diagnosed idiosyncrasies.



1.5.1 – The archipelago of Haida Gwaii

Haida Gwaii is an archipelago located in north-west British Columbia (Canada), situated 50-130 km off the Canadian mainland (latitude 53.255, longitude -132.087). With a total size of 995 000 ha, the archipelago consists of two main islands – Graham (640 000 ha) and Moresby (260 000 ha) – and an approximate number of 150 smaller islands and islets (Figure 1.3). Its ecological characteristics correspond to the wet and very wet hypermaritime

subzones of the Coastal Western Hemlock, Mountain Hemlock and Mountain Heather Alpine biogeoclimatic zones (Banner, 2014; Meidinger and Pojar, 1991). Climate of this subzone is cool, temperate and oceanic. Mean annual temperature and precipitation are 7.6°C and 1349 mm respectively (Meidinger and Pojar, 1991). Haida Gwaii is covered with coastal temperate rainforests that are dominated at low elevation (< 600m) by western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Sitka spruce (*Picea sitchensis*). The typical understory vegetation includes bryophytes, shrubs such as red huckleberry (*Vaccinium parvifolium*), false azalea (*Menziesia ferruginea*) or salal (*Gaultheria shallon*), and herbs such as several species of ferns (e.g. *Blechnum spicant*, *Dryopteris assimilis*) and forbs (e.g. *Listera caurina* and *cordata*, *Moneses uniflora*, *Cornus canadensis*) (Pojar, 2002). Three physiographic regions can be distinguished on the archipelago (Brown, 1968; Figure 1.3). The Queen Charlotte lowlands, which cover the north eastern part of the archipelago, have a bedrock that mainly consists of sedimentary rocks with basalt and granite. The Skidegate plateau and the Queen Charlotte Ranges include the rest of the islands. Their bedrock consists mainly of volcanic rocks with minor intrusions of sedimentary rocks. Soil types on the archipelago are classified as organic soils in the Folisol, Humo-Ferric/ Ferro-Humic Podzols, gleysols and brunisols orders (Banner, 2014; Pojar and Banner, 1984).

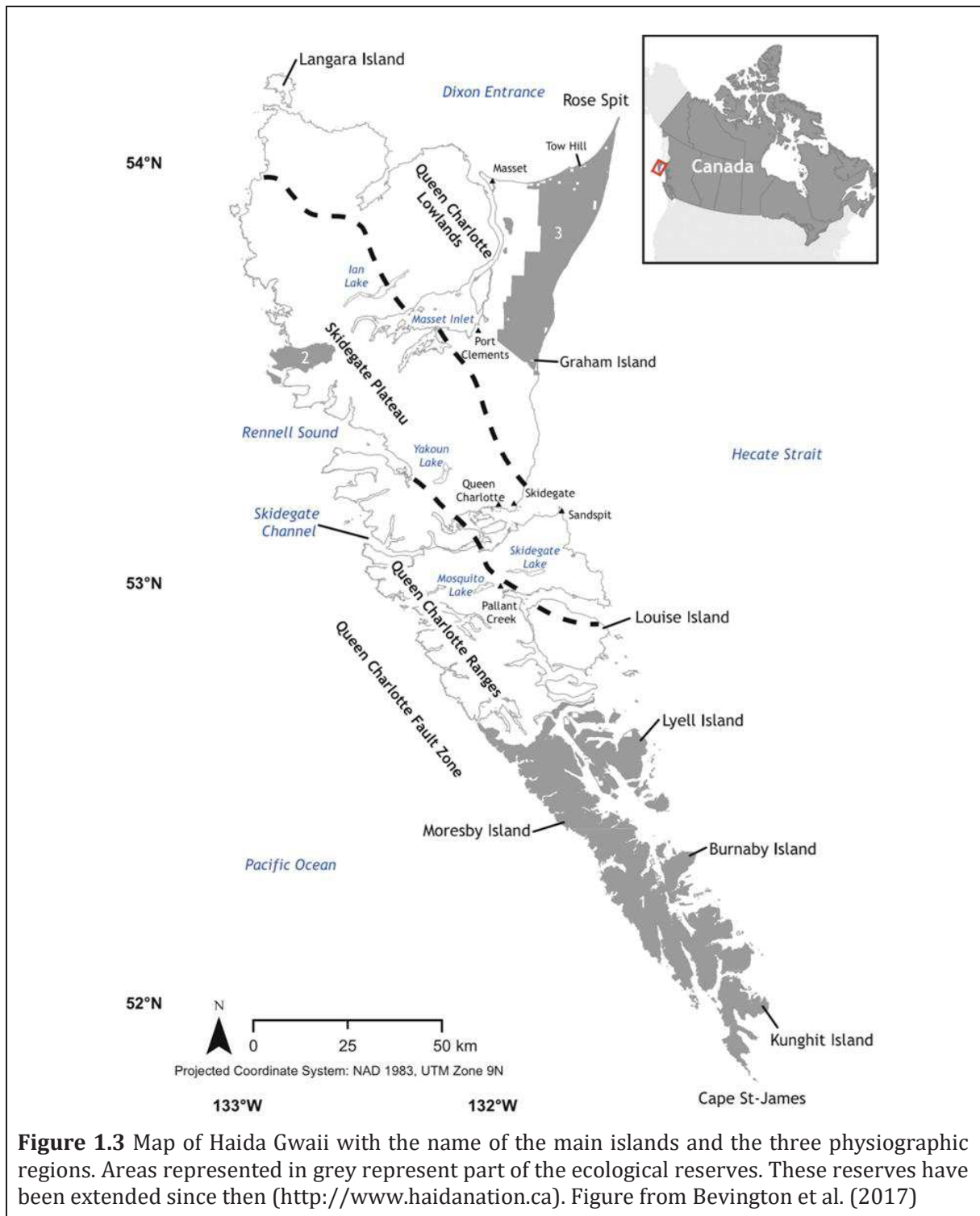
Haida Gwaii is home of the highest number of endemic species and subspecies in Canada, hence its nickname of “The Canadian Galapagos” (Foster, 1982). Because of its island status, food webs on Haida Gwaii are a simplified version of the one found on the nearby mainland (MacArthur and Wilson, 1967). Prior to European settlement, eleven terrestrial mammal species were present on the archipelago, nine times less than on the nearby mainland of British Columbia (Golumbia et al., 2008). Among them, the extinct Dawson caribou (*Rangifer tarandus*), was the only large herbivore on the island. Extinct in 1920s-1930s, this species is believed to have had a low abundance, and a distribution restricted to the muskegs and open woodlands of north-eastern Graham Island (Byun et al., 2002). The Black bear (*Ursus americanus carlottae*), which feeds mainly in the intertidal and on land vegetation, can occasionally feed on young deer. It is the only large predator on the islands. Other large deer predators are only found on the nearby mainland, such as wolves, cougars and occasionally

grizzly bears. There are no recent or historical records of these predators presence on the archipelago (Golumbia et al., 2008).

The first known contact of Europeans with Haida Gwaii in 1774 marked a shift in the archipelago's ecology. Eradication of the sea otter through hunting for fur, the introduction of numerous non-native species and, more recently, extensive industrial-scale logging by the timber industry, have all contributed to the modification of the terrestrial and marine ecosystems on most of the archipelago. Nowadays, strict regulations have been established to protect and restore the islands. As a result, about 50 % of the archipelago is now in protected areas. It is estimated that at least 217 vertebrates and 25 % of the vascular plant species are non-native to the archipelago (Golumbia et al., 2008). Some of them, such as black rat (*Rattus rattus*) were introduced by accident; some others, such as red and Sitka black-tailed deer (*Cervus elaphus elaphus* and *Odocoileus hemionus sitkensis*) or raccoon (*Procyon lotor vancouverensis*) were deliberately introduced for meat and fur. Several eradication programs have been undertaken to control non-native species that were threatening the archipelago's biodiversity. Some of these programs proved a remarkable success, such as the Norway rat (*Rattus norvegicus*) removal on Langara Islands (Taylor et al., 2000). However, two centuries after their introduction, some invasive non-native species continue to threaten the ecosystems of Haida Gwaii (Golumbia, 1999). Among them, Sitka black-tailed deer represent a major concern. First introduced in 1878, Sitka black-tailed deer have colonised most of the archipelago (Golumbia et al., 2008). Good swimming abilities, the absence of competitors, little hunting or disease and absence of predation pressure have all contributed to this successful colonisation. From seven individuals on one island in 1878, they are estimated today to comprise between 113 000 and 250 000 individuals and to occupy 99.99% of the archipelago's land area (Martin and Baltzinger, 2002). Their density has been estimated at about 37 deer/km² on some islands (Stockton 2005).

The first mention of a deer problem on Haida Gwaii appeared in the early 1980s, through vegetation studies (Pojar et al., 1980). In a paper published in 1980, J. Pojar evokes the "virtual epidemic of deer" that may lead to the "elimination" of certain plant species and to "increasing damage" to others (Pojar et al., 1980). Among the threatened plants some, such

as western redcedar and the Pacific crab apple (*Malus fusca*), have cultural significance for the Haida nation. Certain, like redcedar, are also important economically for the timber industry.



1.5.2 – The deer and the forests of Haida Gwaii: 30 years of research aboveground

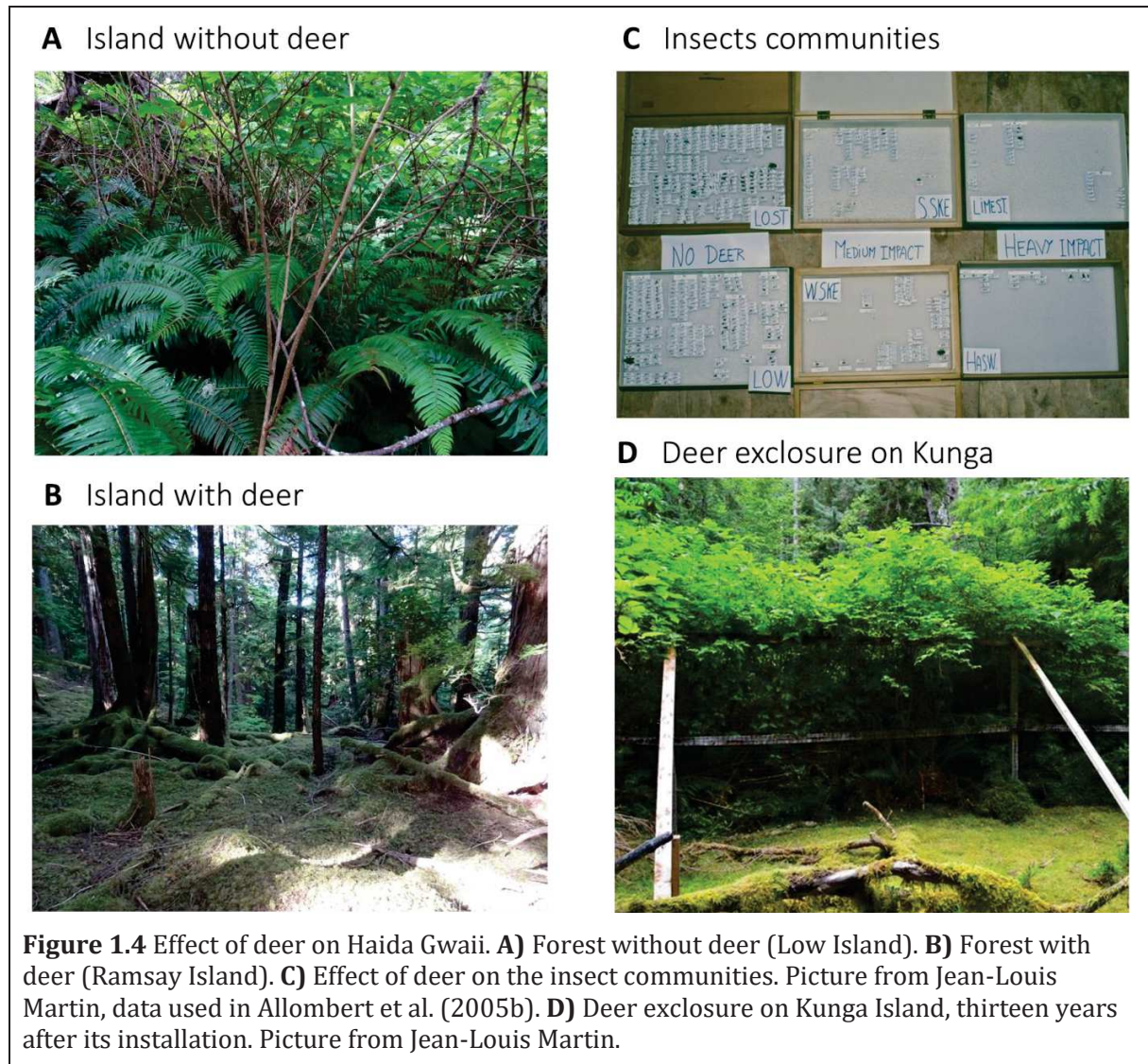
In 1989 the observation was made that some small islands on Haida Gwaii had higher songbird species density than did larger islands of the archipelago (Martin et al., 1995). This observation contradicted the predictions of the island biogeography theory (MacArthur and Wilson, 1967), and was correlated with the absence of deer on the smaller islands (Martin et al., 1995). Distance from the large islands and strong marine currents might have prevented deer colonising these small islands, hence preserving their natural vegetation and bird communities. Such islands represented a state of reference, and provided rare information on how the forest would look in the absence of uncontrolled deer populations. Eight uncolonized islands in the archipelago were identified (Golumbia et al., 2008). The study of fraying scars on tree bark further highlighted a gradient of deer colonisation among islands, with some islands colonised for less than 35 years as of today, while others were colonised for more than 70 years, as of today (Vila et al., 2004a, 2004b). This gradient of islands varying in browsing histories provided a unique quasi-experimental context to test hypotheses on ecosystem changes driven by deer colonisation. The comparison of the vegetation between these different islands documented a profound restructuring of the plant community in the presence of deer, confirming and strengthening predictions made in other temperate forests of the world that lacked true references, but were experiencing increasing deer populations (Figure 1.4 A and B) (Stockton et al., 2005). The cover and diversity of vascular plants in the understory significantly decreased with increasing length of deer presence (Stockton et al., 2005). Although un-colonised islands were significantly smaller than colonised islands, this difference in vegetation was proven to be the result of deer browsing rather than an island size effect (Gaston et al., 2006). The reduction of vascular plant cover by deer favoured the expansion of bryophytes. Bryophyte density and cover were two and eleven times higher on colonised islands than on un-colonised islands (Chollet et al., 2013b). The modifications to the forest vegetation structure reverberated on other trophic layers. Bird abundance and diversity were also dramatically lower in presence of deer (Allombert et al., 2005a; Chollet et al., 2016; Martin and Joron, 2003). Songbird abundance was 51 % lower after 50 years of deer presence at the time of study, with a reduction as high as 93% for birds dependent on

the understorey for food and habitat (Allombert et al., 2005a). Insects were also drastically affected by the vegetation shift caused by deer. Fifty years of deer colonisation reduced insect abundance and diversity in the understorey vegetation by eight and six-fold, respectively (Figure 1.4C, Allombert et al., 2005b). The comparative study of these islands, therefore, showed that uncontrolled deer populations exerted a top-down regulation on the other components of the ecosystem leading to the simplification of forest communities (Martin et al., 2010).

To assess the resilience of Haida Gwaii's forests to the impacts of deer over-abundance, a set of 20 exclosures, distributed in pairs at 10 locations on Graham Island, were installed in 1997 by the Research Group on Introduced Species (RGIS). Each exclosure had an area of 25m². All exclosures were located in mature, undisturbed forests with stands dominated by western red cedar. Three larger exclosures (20m x 20m) were installed on Kunga and on East Limestone Islands (Figure 1.4 D). The comparison of the ecosystem properties inside and outside of these exclosures allowed measurement of the potential resilience of the vegetation after deer exclusion. Eight years of deer exclusion on Graham Island showed that red cedar seedlings only survived inside the exclosures. Subsequent monitoring showed that the very slow growth rate of the protected seedlings in the low light conditions typical of the dense canopy of old-growth forests left seedlings susceptible to deer browsing for over a decade, explaining the total absence of red cedar regeneration in unprotected old-growth stands in presence of deer (Stroh et al., 2008). Thirteen years of deer exclusion on Kunga and Limestone Islands allowed a significant recovery of shrubs and ferns (Chollet et al., 2016). Twenty years after their installation, the vegetation in the exclosures on Graham Island, and in the 3 out of 6 exclosures that had survived winter storms on East Limestone and Kunga Islands, had dramatically recovered (Chollet et al, unpublished data).

The resilience of the vegetation after deer removal was further confirmed by means of an experimental deer cull initiated in 1997/98 by the RGIS with assistance from Parks Canada on Reef and S^Gang Gwaay Islands. Thirteen years of regular, subsequent culls on these islands maintained a much reduced deer population. The vegetation and songbirds were monitored

prior to the culls and at regular intervals thereafter, allowing documentation of the restoration trajectory of the vegetation and the positive response of the bird community to the deer culls (Chollet et al., 2016).



1.6 – Deciphering the effect of deer on soil in temperate forests

The aim of this thesis is to decipher the impact of deer on soil properties, microorganisms and functioning in temperate forests, and to search for an explanation to the idiosyncrasies observed within studies in the literature on deer impacts on soil ecology. To avoid any confounding effects that soil heterogeneity might bring, we focused on a single forest type and a single soil horizon. We chose to focus on the middle horizon of the forest floor, the F horizon, which is the organic soil layer where active litter decomposition occurs. To overcome the temporal and spatial limitations associated with the use of a single study system, we compared the response of soil to deer pressure among three complementary study systems, differing in time of deer presence or removal. To do so, this research in this thesis takes advantage of the unique configuration of the Haida Gwaii archipelago, and of the exceptional characterization of the system provided by 30 years of knowledge on the effects of deer aboveground.

1.6.1 – Three complementary study systems

The deer colonisation gradient – We selected a set of seven islands differing in deer colonisation history. These islands were located in the Laskeek Bay and in the adjacent Juan Perez sound on Haida Gwaii (Figure S1.1). They represent a gradient of deer colonisation from never colonised to colonised for more than 70 years at the time of our study. Low, Lost and Tar Islands were never colonized by deer, presumably due to their distance from the coast and difficult access. The colonisation of South Skedans and West Skedans Islands by deer was estimated to have occurred less than 35 years before our study (Vila et al., 2004a). Louise and Lyell Islands have the longest colonisation history, with deer being present for more than 70 years at the time of our study (Vila et al., 2004b). Deer densities on these islands were estimated to range between 21 and 37 deer/km² (Stockton et al., 2005).

The deer exclosures – We took advantage of the set of twenty deer exclosures installed by RGIS in 1997. We compared soil properties, organisms and functioning inside and outside

each enclosure to assess the soil resilience after twenty years of deer exclusion. The enclosures were distributed on Graham Island in the north of Haida Gwaii (Figure S1.1).

The Ilgaaygwü sdiihlida: Restoring Balance project – In response to the negative effects of deer on plants and songbird communities, Parks Canada launched the “restoring balance” project in the summer 2017. The aim of this ambitious project is to remove deer completely from several islands in Juan Perez Sound [Murchison (400 ha), Faraday (348 ha), House (44 ha), Hot Springs (21 ha) and Ramsay Islands (1623 ha)]. All these islands are assumed to have been colonised by deer over 70 years ago based on extrapolations to previous studies by RGIS (Vila et al., 2004b). At the time of writing, this project resulted in a reduction of over 80% of the deer populations on these islands. The cull took place during spring-summer 2017 and hunts have been scheduled in 2019 to aim for a complete removal of deer. Subsequently, regular culls are planned to prevent deer re-colonisation. We took advantage of this deer cull to assess the potential short-term resilience of soil organisms and soil processes after deer exclusion. We studied soil properties the year before the cull to have a reference, a month after the cull and a year after the cull to assess any short-term changes in soils on Ramsay Island (Figure S1.1). We further followed soil properties on two control islands: Tar Island, which has never been colonised by deer, and Lyell Island, which has been colonised by deer for more than 70 years and where no cull has ever occurred (Vila et al., 2004b).

Finally, we added Reef Island in Laskeek Bat to this study system as a situation where repeated culls between 1997 and 2010 resulted in an island with deer present for more than 70 years, but exposed to a lower browsing pressure for the 20 past years, which has resulted in a partial, but significant, recovery of the understory plant and bird communities (Chollet et al., 2016; Vila et al., 2004a).

Together, these three complementary systems enable a study of the response of the soil to deer colonisation, and the short and long-term resilience of the soil to deer exclusion.

1.6.2 – Three complementary experiments: a multi-pronged approach

Deer may modify edaphic properties through various direct and indirect pathways. Modification of edaphic properties may, in turn, restructure soil communities. Because soil communities, and particularly soil microbial communities, are essential actors in carbon and nutrient cycling, modifications of these communities can have important implications for ecosystem functioning.

In this thesis, we addressed three questions using three complementary experiments to determine the effect of deer on the forest floor F-layer, and the consequences of any changes on the functioning of the forest.

Are deer modifying decomposition activity via a reduction in litter quality and a change in decomposer ability?

To answer this question, we investigated the impact of deer on decomposition processes through changes in litter composition, decomposer community ability and soil properties, and dung addition. We compared litter mass loss during decomposition among three treatments: an ecosystem without deer (Low Island), an ecosystem colonised for more than 70 years (Louise Island), and an ecosystem partially recovered after deer removal (Reef Island). We measured litter decomposition over a year using litterbags. To do so, we collected fresh, senescent litter from the three ecosystems and compared their decomposition after a year between the three treatments. We also translocated the litter between islands to investigate the role of soil properties on decomposition, independently of litter quality. Finally, we also compared the decomposition of Sitka spruce litter (*Picea sitchensis*) with and without deer excrement on the three island types. For these experiments, we used two mesh sizes in the litterbags to separate the decomposition by microbes from the decomposition by meso- and macro-fauna. We predicted that **1)** decomposition of litter collected from the deer-free island would be faster than decomposition of the litter collected from islands with deer (the effect of litter quality change); **2)** in the absence of home-field advantage, decomposition rates on the islands with deer would be higher than on the islands without deer due to an increase in decomposer

ability in microbial communities in response to more recalcitrant litter (effect of change in decomposer ability); **3)** presence of deer excrement, rich in available carbon and nutrient (Ruess and McNaughton, 1987), would locally speed up the decomposition of plant litter; **4)** the decomposition pattern observed on the islands where deer were culled should fall in between those observed on the islands with and without deer.

What are the effects of deer on soil properties and how are these effects reverberating on soil prokaryotic communities?

To answer this question, we investigated the change in forest floor properties and prokaryotic community structure following deer presence and removal. We measured physical (penetration resistance, moisture content) and chemical (pH, carbon content, nitrogen content, phosphorus content, ammonium and nitrate concentrations) properties of the forest floor for each treatment and for the three systems detailed above. We focused on soil prokaryotes, which are important contributors to soil nutrient cycling. We used qPCR and Illumina sequencing of the 16S rRNA gene to measure the potential abundance, diversity and composition of the soil prokaryotic community (bacteria and archaea). We predicted that **1)** short-term modifications in forest floor properties are driven by the direct interaction ungulates have with the soil, through trampling or dung and urine deposition; **2)** long-term modifications of forest floor properties are driven by the indirect effects of ungulates via vegetation structure; **3)** deer will increase prokaryotic α and β -diversity in the short-term as a result of increased spatial heterogeneity due to dung and urine deposition, and decrease the prokaryotic α and β -diversity in the long-term as a mirror of the aboveground homogenisation; **4)** deer will select different soil prokaryote species via the changes in soil physical and chemical properties by deer.

What are the consequences of changes in soil properties and organisms by deer for the nitrogen cycle?

To answer this question, we investigated the effect of deer on gross nitrogen cycling rates and processes using a ^{15}N -isotope tracing method (Masse et al., 2016; Müller et al., 2004). This method allows measurement of the gross and net rates of processes in the nitrogen

cycle. We compared the effect of deer on nitrogen cycling among the three study systems. We first compared the effect of long-term deer colonisation on the nitrogen cycle using seven plots on two islands without deer (Tar and Low Islands) and seven plots on two islands colonised for more than 70 years (Louise and Lyell Islands). We also investigated the long-term resilience of nitrogen cycling to deer exclusion in a subset of 4 exclosures on Graham Island. Third, we investigated the short-term resilience of nitrogen cycling to deer removal. For this, we measured nitrogen cycling a month after, and a year after, a deer cull on three plots on an island without deer (Tar Island), three plots on an island colonised for more than 70 years (Lyell Island), and three on one island with deer for over 70 years and where the deer cull occurred (Ramsay Island) in Juan Perez Sound. We predicted that **1)** deer will promote nitrogen cycling through the inorganic pathway in the short-term through the addition of ammonium (urine) to the ecosystem; **2)** deer will promote nitrogen cycling through the organic pathway in the long-term through modification of the plant community towards poorer quality litter.

In this thesis, only a subset of the nitrogen cycling data is presented. This subset is composed of the studies of nitrogen cycling in plots from Louise and Low Islands from the island comparison system.

Each of these questions forms the subject of a chapter in this thesis:

Chapter 2 – Deer slow down litter decomposition by reducing litter quality in a temperate forest

Chapter 3 – Abundant deer modify soil properties and prokaryotic communities in a temperate forest

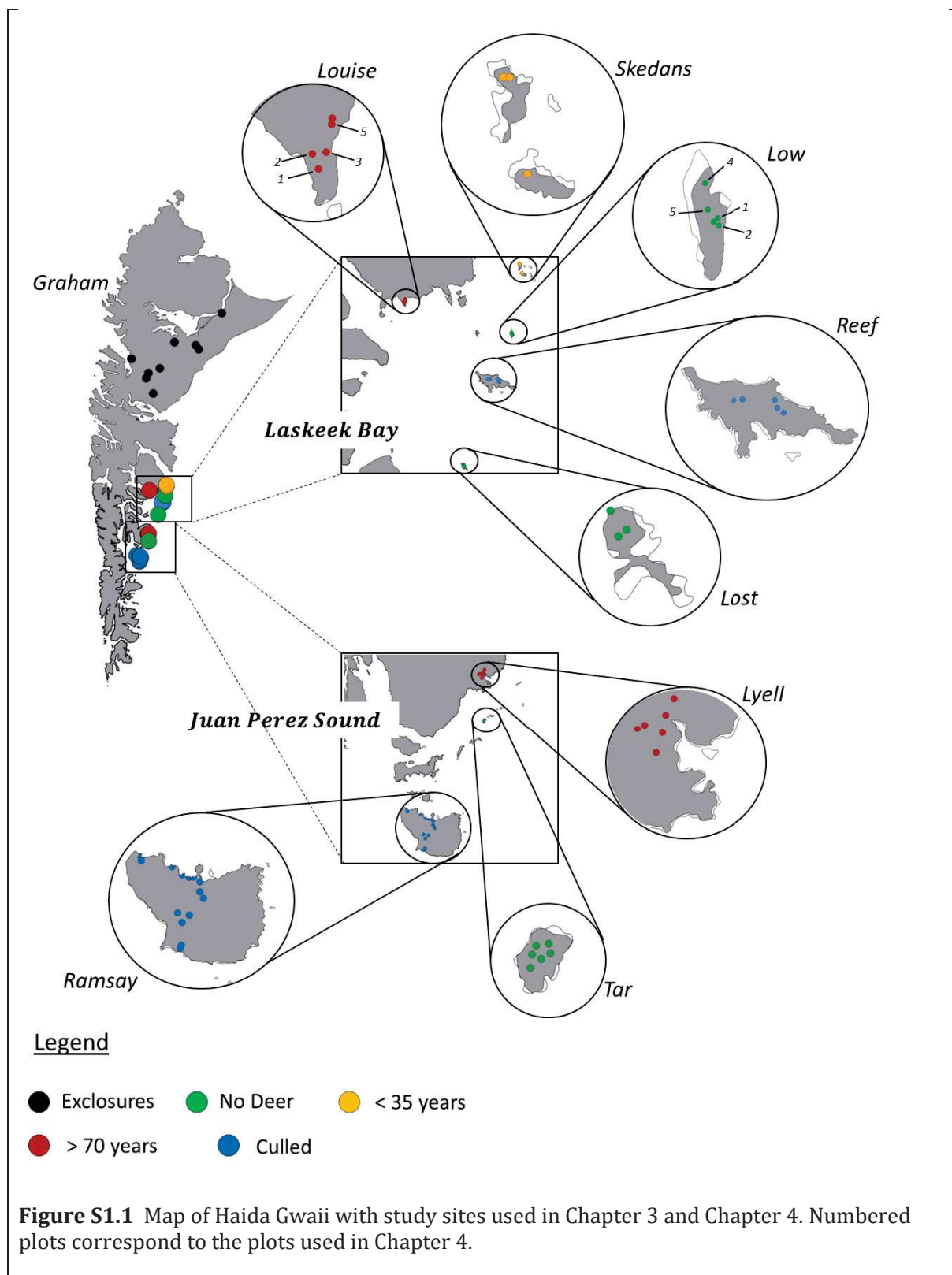
Chapter 4 – Abundant deer do not change nitrogen cycling processes in a temperate forest.

Table S1.1 – Studies on the effects of deer belowground in temperate forests. Min = mineral.

| | Method | Study Length | Location | Forest type | Soil type | Soil sampling |
|----------------------------------|-------------|--------------|---------------|-------------|--------------------|---------------|
| (Harrison et al. (2004) | Exclosures | 14 yrs | Europe | Deciduous | Podsols | 0-10cm |
| Carline et al. (2005) | Defoliation | 2 yrs | Europe | Deciduous | Acid Alfisol | - |
| Mohr & Topp (2005) | Exclosures | 9 yrs | Europe | Deciduous | Acid soil | 0-5cm (A) |
| Mohr et al. (2005) | Exclosures | 2 yrs | Europe | Deciduous | Acid doil | 0-5cm |
| (Niwa et al. (2008) | Defoliation | 3 weeks | Asia | Deciduous | Andosol | A |
| Prietzl et al. (2008) | Exclosures | 30-40 yrs | Europe | Mixed | Calcareous | O and Ah |
| Kumbasli et al. (2010) | Pres./Abs. | 40 yrs | Europe | Deciduous | Alfisol | 0-5cm |
| Stritar et al. (2010) | Exclosures | 13 yrs | North America | Deciduous | Mollisols /Alfisol | 0-15cm |
| Niwa et al. (2011) | Enclosures | 3 yrs | Asia | Deciduous | Andosol | 0-10cm |
| Wardle et al. (2001) | Exclosures | 20-50 yrs | Oceania | | <i>diverse</i> | F and H |
| Gass & Binkley (2011) | Exclosures | 15 yrs | North America | Deciduous | Inceptisol | 0-15cm |
| Suzuki and Ito (2014) | Exclosures | 5 yrs | Asia | Evergreen | NA | topsoil |
| Relva et al. (2014) | Exclosures | 7 yrs | South America | Conifer | Andic | 0-15cm |
| Furusawa et al. (2016) | Exclosures | 9 yrs | Asia | Mixed | NA | 3 min. layers |
| Sabo et al. (2017) | Exclosures | 10-20 yrs | North America | Mixed | <i>diverse</i> | E |
| Iida et al. (2018) | ≠ density | 25-30 yrs | Asia | Deciduous | NA | - |
| Burke et al. (2019) | Exclosures | 11.5 yrs | North America | Deciduous | Silt loam | 0-5cm |

Table S1.1 Suite. Compact. = Soil compaction, MB = Microbial biomass, N miner = Nitrogen mineralization

| | Moisture | Compact. | pH | C:N | N | NH ₄ | NO ₃ | MB | N miner. | C activity |
|------------------------|----------|----------|--------|-----|--------|-----------------|-----------------|--------|----------|------------|
| Harrison et al. (2004) | 0 | - | 1 | 0 | - | -1 | -1 | Idios. | -1 | 0 |
| Carline et al. (2005) | 1 | - | 0 | - | - | -1 | 1 | 0 | 1 | 0 |
| Mohr et al. (2005) | - | - | - | - | Idios. | - | - | - | - | - |
| Mohr et al. (2005) | - | - | -1 | 1 | 1 | - | - | 0 | - | -1 |
| (Niwa et al. (2008) | - | - | - | - | - | 1 | 1 | 1 | 1 | - |
| Prietz et al. (2008) | - | - | - | 1 | -1 | - | - | - | - | - |
| Kumbasli et al. (2010) | - | 1 | -1 | - | - | - | - | - | - | - |
| Stritar et al. (2010) | - | - | 0 | - | -1 | 1 | 1 | -1 | - | Idios. |
| Niwa et al. (2011) | 0 | - | - | 0 | 0 | -1 | 0 | - | 0 | - |
| Wardle et al. (2001) | - | - | Idios. | 1 | 0 | - | - | Idios. | - | Idios. |
| Gass et al. (2011) | -1 | 1 | - | 0 | -1 | - | - | 0 | -1 | - |
| Suzuki et al. (2014) | - | 0 | - | - | - | - | - | - | - | - |
| Relva et al. (2014) | - | 0 | 0 | - | 0 | - | - | - | 0 | 0 |
| Furusawa et al. (2016) | - | 0 | 0 | 0 | 0 | 1 | 1 | - | 1 | - |
| Sabo et al. (2017) | - | 1 | 0 | - | 0 | - | - | - | - | - |
| Iida et al. (2018) | - | 1 | - | - | - | - | - | - | - | 1 |
| Burke et al. (2019) | 0 | 0 | 0 | 0 | - | 0 | - | - | - | - |



Chapter 2: Deer slowdown litter decomposition by reducing litter quality in a temperate forest

Chollet Simon, Maillard Morgane*, Schörghuber Juliane, Grayston Sue, & Martin Jean-Louis*

* Both authors contributed equally to this work

ABSTRACT

In temperate forest ecosystems, the role of deer in litter decomposition, a key nutrient cycling process, remains debated. Deer may modify the decomposition process by affecting plant cover and thus modifying litter abundance. They can also alter litter quality through differential browsing and affect decomposer ability by changing soil abiotic properties and the nature of decomposer communities. We used two litterbag experiments in a quasi-experimental situation resulting from the introduction of Sitka black-tailed deer *Odocoileus odocoileus sitkensis* on forested islands of Haida Gwaii (Canada). We investigated the effects of deer on decomposition through their impacts on litter quality and on decomposer ability. After one year, the effect of deer on litter quality resulted in a lower rate of mass loss in litter from litterbags. This mass loss mainly reflected a 21 and 38 % lower rate of carbon (C) and nitrogen (N) loss, respectively. Presence of deer resulted in lower decomposer ability for the rate of carbon loss, but not for nitrogen loss. The level of C loss after one year was 5% higher for litter decomposing

on an island without deer. But the change in the rate of carbon loss explained by the effect of deer on decomposer ability was outweighed by the effect deer had on litter quality. Additional effects of deer on the decomposition process through feces deposition were significant but minor. These results question the role the large increase in deer populations observed in temperate forests at continental scales may play in broad scale patterns of C and N cycling.



Yellow-spotted millipede (*Harpaphe haydeniana*) on a coarse mesh litter bag, Reef Island, Haida Gwaii.

2.1 – Introduction

2.1.1- Deer and the functioning of temperate forests

Until recently, the role of large herbivores in nutrient cycling processes has been relatively neglected (Tanentzap and Coomes, 2012). In temperate forests in Europe and eastern North America, dominated by coniferous or by broadleaved trees, this may be partly because ungulates, essentially deer, became largely missing as a result of hunting and/or loss of favorable land cover (McShea et al., 1997; Apollonio et al., 2010a). The extirpation of their natural predators, followed in the second part of the 20th century, on both continents, by changes in hunting regulations and in land-uses, such as increased planting of winter crops by agriculture or, in some areas, farm abandonment and reversion to forests (see e.g. Côté et al., 2004; Fuller and Gill, 2001; Milner et al., 2006) resulted in a dramatic rebound in deer populations that brought them back to the forefront of ecological thinking (Terborgh and Estes, 2013). The initial emphasis of research was on the consequences of deer recovery on forest vegetation, beginning with impacts on tree regeneration and growth (Gill, 1992), and, more recently, on aboveground understory community functioning (Horsley et al., 2003; Royo et al., 2010), including cascading effects on different segments of the trophic network [invertebrates, birds (e.g. Chollet and Martin, 2013; Foster et al., 2014)].

While our grasp of deer effects on forest aboveground communities has dramatically improved, their repercussions on belowground patterns and processes are still insufficiently understood (Bardgett and Wardle, 2003; Hobbie and Villéger, 2015). These belowground effects will be partly mediated by the effects deer have on litter decomposition and its pivotal role at the interface between aboveground primary production and belowground processes (Chapin et al., 2011). In temperate forest ecosystems, contrary to grasslands or boreal forest, there are still only a few studies on how large herbivores affect litter decomposition.

2.1.2 - Deer and litter decomposition

Deer may modify belowground processes by affecting two of the main parameters that control decomposition: litter quality and decomposer ability (Keiser et al., 2014). Through plant removal, combined with selective foraging, deer modify plant community composition, plant stoichiometry, as well as the relative contribution of canopy and understory vegetation to litter composition (Côté et al., 2004). These changes in litter quantity and quality will affect decomposition processes and nutrient cycling (Bardgett and Wardle, 2003) .

Deer are also susceptible to modify belowground processes through the alteration of decomposer ability. This alteration can result from changes in edaphic properties such as increased soil temperature and salinity that follow exposure of bare soil after vegetation removal by browsing, or from soil compaction caused by trampling and its effects on soil water and oxygen content (Schrama et al., 2013b). Deer also release dung and urine, a source of organic matter more easily decomposable than recalcitrant plant litter (Ruess and McNaughton, 1987), and a source of inorganic nitrogen for soil decomposers that enhances their development (Sitters et al., 2017). These effects may affect the structure and functioning of decomposer communities [soil fauna (Andriuzzi and Wall, 2017) and microorganisms (Cline et al., 2017; Eldridge et al., 2017)] with effects on the rate of litter decomposition (Handa et al., 2014).

Recent evidence indicates that decomposition sometimes occurs more rapidly when litter is placed under the plant species from which it originated (Gholz et al., 2000; Ayres et al., 2009; Austin et al., 2014). This “home-field advantage” (HFA) is attributed to decomposer specialization. Home-field advantage may compensate the aforementioned potential changes in decomposition caused by deer. But studies explicitly testing this hypothesis are scarce, and provided contrasting results (see Olofsson and Oksanen, 2002; Penner and Frank, 2018).

There is recognition of the multiplicity of pathways through which deer may affect litter decomposition (see Bardgett and Wardle, 2003 for a conceptual model), but our knowledge is mainly based on the independent study of each pathway, which led to

apparent contradictions in results. To better identify the mechanisms behind the effect of deer on litter decomposition we designed a study that combined approaches able to disentangle the relative effects of these different pathways on the process.

2.1.3 - A quasi-experimental context

This study is part of a long-term effort to use the introduction of Sitka black-tailed deer *Odocoileus odocoileus sitkensis* at the end of the 19th century to the Haida Gwaii archipelago (British Columbia, Canada, see Golumbia et al., 2008) as an unplanned experiment on trophic interactions. Native to the coastal forests of British Columbia, Sitka black-tailed deer colonized most, but not all, islands, resulting in a quasi-experimental situation with, side by side, islands colonized by deer, and a limited number of small isolated islands never colonized. All these islands are forested. The occurrence of reference islands without deer made it possible to demonstrate that, on islands where deer were present, independent of island size, deer herbivory was the main factor structuring plant, invertebrate and songbird communities, overwhelming other biotic or abiotic factors (i.e. island area, soil and micro-habitat diversity (Table S2.1 in Supporting Information and Chollet et al., 2013c; Gaston et al., 2006a; Martin et al., 2010a; Martin and Baltzinger, 2002a). Recurrent experimental culls on some islands allowed to document the potential for recovery of the aboveground vegetation and songbirds (Chollet et al., 2016). As a result, islands can be segregated today into three categories of browsing histories and their associated vegetation patterns. Islands without deer are characterized by a diverse and lush understory vegetation dominated by broad-leaved shrubs and ferns, producing a diverse and abundant litter. Islands where deer have been present for over 70 years are characterized by an open understory dominated by bryophytes where litter is dominated by conifer leaves (Martin et al., 2010; Stockton et al., 2005). Finally, on islands where deer have also been present for over 70 years but that have been subjected to recurrent deer culls over the past two decades, the understory is characterized by an intermediate cover of vegetation (Chollet et al., 2016).

We designed litterbag experiments based on these three browsing treatments, deer absent (no browsing, our reference), deer culled (intermediate browsing), and deer present

(severe browsing), to analyze how the prolonged presence of abundant deer affected litter decomposition.

First, we assessed the effect of deer presence on litter decomposition at the scale of an island through a reciprocal litterbag translocation experiment involving litter representative of the three browsing treatments: “no”, intermediate” and “severe” browsing. The objective was to discriminate between the effects deer have on litter decomposition rate, either through their impact on litter quality or through their effect on decomposer ability (including effects on soil properties and on decomposer community composition), and this by explicitly including an assessment of home-field advantage using the approach proposed by Keiser et al. (2014). This approach allows us to assess home-field advantage through the quantification of its role in decomposition rate relative to the role of litter quality per se (i.e. irrespective of the decomposition environment), and relative to the role of decomposer ability per se (i.e. irrespective of litter quality).

Second, we assessed how the deposition of high-quality litter in the form of feces (Ruess and McNaughton, 1987), affected the rate of litter decomposition at a narrow local scale by adding deer feces in a set of litterbags.

We predicted 1) that decomposition of litter collected from the deer-free island would be faster than decomposition of the litter collected from islands with deer (the effect of litter quality change); 2) that, in absence of home-field advantage, decomposition rates on the islands with deer would be higher than on the islands without deer due to an increase in decomposer ability in microbial communities in response to more recalcitrant litter (effect of change in decomposer ability); 3) that, presence of deer excrement would locally speed up the decomposition of plant litter; 4) that, the decomposition pattern observed on the islands where deer were culled should fall in between those observed on the islands with and without deer.

2.2- Methods

2.2.1 -Study sites and plot selection

Haida Gwaii is characterized by a humid temperate-oceanic climate, with mean annual temperature of 8.5°C and precipitation that varies greatly from 1,350 mm on the east coast, where this study took place, to 7,000 mm on the west coast (Banner, 2014). The archipelago is covered by temperate rainforests dominated, at low elevation, by western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Sitka spruce (*Picea sitchensis*). The selected study sites belonged all to the Coastal Western Hemlock Wet Hypermaritime subzone [Biogeoclimatic Ecosystem Classification, code CWHwh1, (British Columbia Ministry of Forests, Lands, and Natural Resource Operations, 2010)] which covers 49% the archipelago, and ranges from sea-level to 350m in elevation (Banner, 2014). The bedrock geology of the selected study sites was volcanic and sedimentary, together with intrusions of granitic rock (Sutherland Brown, 1968). The soil type was organic and classified into the Folisol order (Soil Classification Working Group / Groupe de travail sur la classification des sols, 1998). Soil macro-fauna on Haida Gwaii include earthworms, millipedes, centipedes, beetles, ants and spiders. Soil meso-fauna include mites, pauropods, collembolan, protura, symphylan, pseudoscorpion and dipluran.

We selected three islands in Laskeek Bay (52°53'12"N, 131°35'20"W, Figure S2.1 in Supporting Information). We chose sampling and experimental sites with similar parent material, and that were representative of the patterns of deer impacts we documented at the scale of the archipelago (Chollet et al., 2015; Martin et al., 2010) (see Table S2.1 in Supporting Information for a synthesis of previous studies). The three islands were Low Island, 9.6 ha, that had never been colonized by deer, Louise Island, 25,000 ha, that has had deer for over 70 years (Vila et al., 2004b) and had a current deer density estimated at 30 deer / km², and Reef Island, 249 ha, that had also been colonized by deer more than 70 years ago, but its deer population had been regularly culled between 1997 and 2010. At the time of study Reef Island had a deer population density estimated at about 15 deer / km² and a partially recovered understory vegetation (Chollet et al., 2016). Low, Reef and Louise Islands

were therefore representative of three distinct deer herbivory treatments: absence of current or past browsing, intermediate browsing pressure, and severe browsing pressure, respectively. Our emphasis on selecting sites similar in parent material and similar in forest types prevented us to control also for island size. To take this into account we selected all sampling plots in the coastal area on the three islands. On each island we established fifteen 10 m x 10 m forest interior plots, leading to a total of 45 plots. Adjacent plots were separated by at least 100 m.

2.2.2 -Above and belowground characteristics in relation to deer presence

In each plot we sampled the vegetation by estimating the percent cover of vascular plants and bryophytes using the Londo scale (Londo, 1976). We measured soil bulk density at the surface of the forest floor with five replicate measures per plot. For this, we collected soil with a 5.4 cm depth x 4.1 cm diameter (71.29 cm^3) copper core hammered into the soil using a mallet. We took care to not change the structure of the soil while sampling. We removed any coarse woody debris from core samples and subtracted their volume from the volume of the core. We then dried soil at 105°C for 24h to obtain a value for bulk density expressed as g of dried soil per cm^3 of fresh soil.

We used data on soil pH, C:N and organic horizon depth collected in the course of a sister study in plots located in the same area on these same islands (Maillard et al. unpublished data). This data was collected from five plots on Low Island, five plots on Louise Island and six plots on Reef Island. We sampled soil within these plots with a 2.5 cm diameter x 30 cm depth core collecting approximately 100 cores per plot. They were mixed and sieved with a 5 mm sieve to ensure homogenization as recommended for soil with high content of organic matter (Haynes and Swift, 1990). We measured soil pH in a 0.01 M CaCl_2 solution using a 1:10 ratio (air dried soil: solution). We determined soil C:N ratio from 3 mg of freeze-dried and ground soil using an Elementar Vario El Cube Analyzer. In each plot we measured the depth of the soil's organic horizon from a soil pit dug within the plot.

2.2.3- Experimental design and protocol

We measured litter decomposition rates using the litterbag method. We made 15 cm * 15 cm bags using polypropylene mesh with two different mesh sizes. We used the litterbags with a 0.2 mm mesh size to target the decomposition solely due to soil microfauna and microorganisms. We used litterbags with a 3.7 x 4.45 mm mesh size to assess the additional effect of mesofauna and macrofauna on litter decomposition. For these large mesh litterbags, we used a 0.2 mm mesh size on the bottom of the bag to avoid litter spillage.

To obtain our litter samples, we collected summer senescent leaves from plant species with a percent cover on the plots greater than 5 %. In total, we sampled 18, 20 and 17 plant species respectively on the island with no browsing, on the island with an intermediate level of browsing, and on the island with a severe level of browsing (Table S2.2 in Supporting Information). We dried these litter samples at 30°C for a week before using them.

We developed two complementary experiments in order to study the various mechanisms by which deer could affect the decomposition process.

Experiment 1 - To investigate litter decomposition rate in relation to the three browsing treatments we produced, for each plot, three identical litterbags for each of the two mesh-sizes. Each of these six litterbags contained, in the same proportion as in the plot, plant material from all plant species covering more than 5 % of the plot area. We fixed the total mass of litter per litterbag at 4 g. Hence, the mass of litter from each plant species in a given litterbag was calculated according to its relative abundance in the plot. For each mesh size we placed one of the three litterbags on the plot the litter came from ("home"), and placed the two remaining bags on one plot on each of the two remaining islands ("away"). This translocation allowed us to independently test for the relative effects of home-field advantage, of decomposer ability (soil properties and decomposer community composition), and of litter quality on litter decomposition (Figure S2.1).

Experiment 2 - To investigate the influence of deer feces on litter decomposition rate within a litterbag we used a standardized litter. We chose litter from one of the dominant tree species on all islands, *P. sitchensis*. To avoid any biases from potential inter-treatment differences in spruce litter quality, we used, for this set of litterbags, a mix of *P. sitchensis* litter collected from all three islands. In order to standardize feces quality we collected fresh deer feces from another island with deer, also situated in Laskeek Bay (East Limestone, 48 ha). We used this material to place on each of the 45 plots one fine-mesh litterbag containing 2 g of deer feces and 2 g of the standardized litter and two fine-mesh litterbags containing controls, one filled only with 5 g of deer feces and one filled only with 2 g of *P. sitchensis* litter. We repeated this with coarse-mesh litterbags.

Thus, to implement these two experiments, we placed a total of 12 litterbags on each plot including 6 fine-mesh litterbags [3 for experiment 1 (1 home and 2 away) and 3 for experiment 2 (1 feces only + 1 *P. sitchensis* only + 1 with feces and *P. sitchensis*)] and a similar set of 6 litterbags for the coarse-mesh litterbags. As a result we had a total of 540 litterbags (12 bags * 45 plots) for the full design. All litter bags were identified and numbered with aluminum tags and placed randomly on the surface of the forest floor on each plot. We used U pins at each corner of the bag to hold them in place. We placed litterbags on the three islands in July 2017, and collected them one year later in July 2018. The remoteness of the islands and associated logistics prevented designing an experiment with a partial collection of litterbags during the year to better assess the kinetics of decomposition. After collection (all but 35 bags were retrieved) we dried the litterbag contents at 70°C for 48h prior to weighing the contents and then performing chemical analyses.

2.2.4- Mass, Carbon and Nitrogen loss in litterbags

To assess litter mass loss over a year in each litterbag we subtracted the final mass of the bag's content from its initial mass. We weighted the quantity of foreign material accumulated after a year in a control coarse-mesh litterbag displaced in each plot. We then subtracted this mass of foreign material from the final mass in coarse mesh litterbags, in order to correct for contamination. To assess C and N loss over a year, we calculated C and N

concentrations of samples with an Elementar Vario El Cube Analyzer (Elementar, Langenselbold, Germany) using 3.5 mg of ground material. We first calculated the initial C and N concentrations of dried litter using eight individuals of each plant species (vascular and bryophytes) from each island/browsing treatment. Based on these values, and on the relative proportion of each litter in the bags, we calculated the initial C and N concentrations for each litterbag. We also measured initial C and N concentrations of eight deer pellet groups that were previously dried at 70°C for 48h.

At the end of the experiment we finely ground the dried litter from the fine-mesh litterbags only, to remain within our budget limitations, and measured C and N concentrations. We calculated carbon and nitrogen loss by subtracting the amount of carbon and nitrogen remaining in litter after one year of decomposition from the initial estimates based on our calculations of initial C and N concentrations of the litter material.

2.2.5 - Statistical analysis

In order to evaluate the effect of deer herbivory on plant community composition we used a Correspondence Analysis on our data of plant species cover per plot, and performed a between class analysis (Dray and Dufour, 2007). We evaluated the significance of the class effect with a permutation test. To assess the initial litter C:N ratios at each plot we calculated the Community Weighted Mean (CWM) of this initial litter C:N ratio using the formula

$$CWM_i = \sum_1^j (C_j \times p_j) / \sum_1^j (N_j \times p_j)$$

where i represents the plot, j the plant species on this plot, C_j and N_j the C and N content of the corresponding litter, and p_j the relative abundance of the corresponding plant species on the plot.

We used one way ANOVA with permutation tests to compare litter CWM C:N ratio, soil bulk density, soil pH, soil C:N ratio and organic horizon depth among the three islands.

We used the multiple comparison post-hoc test with the function *kruskalmc* from the package *pgirmess*.

To assess differences in the rate of litter mass loss and of C and N loss in both experiments, we calculated the percent differences among treatments in litter mass loss and in C and N loss relative to the no-browsing treatment using the formula:

$$100 - (x_{browsing} \times 100) / x_{no\ browsing}$$

Where $x_{browsing}$ is the litter, C, or N mass loss in litterbags from either the intermediate or the severe browsing treatment, and $x_{no\ browsing}$ the litter, C, or N mass loss in litterbags from the no browsing treatment.

Analyses of decomposition experiment 1 - We used a two way ANOVA to compare litter mass loss, carbon loss and nitrogen loss from litter after one year among the three origins of litter (from island with no, intermediate or severe deer browsing) and among treatment categories (island with no, intermediate or severe deer browsing).

To disentangle the relative importance of the two main ways deer may modify C and N decomposition, namely litter quality and decomposer ability, we used the Decomposer Ability Regression Test proposed by Keiser et al. (2014) using SAS 9.4 (SAS Institute, Cary, NC). This method statistically discriminates among effects of litter quality (here defined as how rapidly a litter is decomposed regardless of decomposition site), ability [i.e. how rapidly a litter is decomposed at one site regardless of litter quality (includes the effect of soil abiotic conditions and the ability of the decomposer communities)] and home-field advantage [i.e. the acceleration of litter decomposition when litter is placed in the site it comes from (home) and where it can potentially benefit from a local specialization of the decomposer community]. The regression model defines the rate of decomposition of observation *i* (Y_i) by three parameters: litter quality ($Litter_i$), soil ability ($Soil_s$), and HFA ($Home_h$) which are dummy variables that equal 1 or 0, respectively, depending on the presence or absence of the litter mixture, soil community and home combination (in observation *i*). The parameters to be estimated are β_l , γ_s and η_h (Keiser et al., 2014). The average decomposition across all data (*i*)

in a dataset, after controlling for litter, soil and home combinations, is represented by the intercept (α), and the error term is defined by ϵ . The β_l and γ_s are restricted to prevent collinearity.

$$Y_i = \alpha + \sum_{l=1}^N \beta_l \text{Litter}_{l_i} + \sum_{s=1}^M \gamma_s \text{Soil}_{s_i} + \sum_{H=1}^K \eta_h \text{Home}_{h_i} + \epsilon_i$$

Compared to the classically used Structural Equation Modeling (SEM), the Decomposer Ability Regression Test offers the additional possibility to explicitly test home-field advantage and maximizes the information extracted from the litter transplant experiment (Keiser et al., 2014).

To explore the reasons for differences in litter quality caused by deer we performed linear models between C or N loss and litter CWM C:N ratio.

Analyses of decomposition experiment 2 (feces manipulation) - We used a two way ANOVA to compare mass loss, C loss, and N loss of *Picea sitchensis*, feces and the combination of both.

For all analyses in which homoscedasticity and normality of the distribution of the residues were not respected, we used ANOVA with permutation tests instead of classical ANOVA ImPerm package (Wheeler, 2010).

We used the R 3.4.1 environment (R Core Team, 2017) for all statistical analyses (except Decomposer Ability Regression Test).

2.3 – Results

2.3.1 - Deer modify aboveground and belowground characteristics

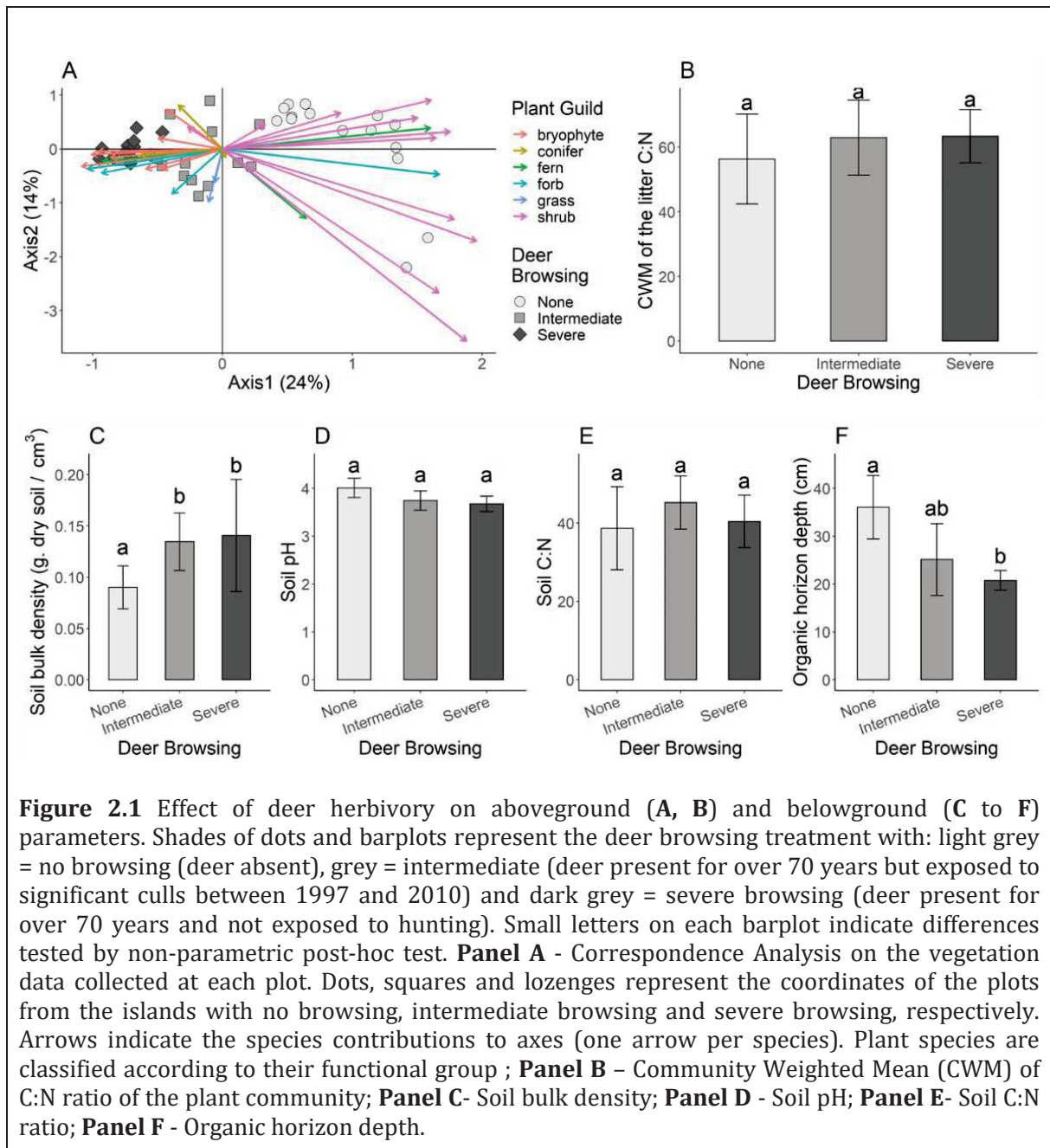
The first axis of the Correspondence Analysis significantly discriminated the plant species composition and abundance in the plots according to the intensity of deer browsing (Fig. 2.1A, Monte-Carlo permutation test: $p < 0.001$). In the absence of deer, vegetation cover was higher and there was greater shrub diversity (Table S2.2 in Supporting Information). The

vegetation from plots with severe deer browsing was characterized by a high cover and diversity of bryophytes (Fig. 2.1A). Plots under intermediate deer browsing showed intermediate plant species diversity and cover. We found no significant difference in the initial C:N ratio of the plant litter among deer browsing treatments (Fig. 2.1B, p-value = 0.2). Soil bulk density was significantly higher on plots from islands with deer (Fig. 2.1C, p-value < 0.001). Soil pH decreased significantly with increasing deer browsing pressure (Fig. 2.1D and F, p-value = 0.037 and 0.005 respectively, however statistical power was not sufficient to discriminate which treatment is different in the post-hoc test). Soil C:N was not significantly different among treatments (Fig. 2.1E, p-value = 0.32). Depth of the organic horizon measured in the plots decreased by 44 % with increasing browsing intensity.

2.3.2 - Litter mass loss in experiment 1

Litter mass loss was highest in litterbags with litter from the island with no deer and lowest in litterbags with litter from the island with severe browsing pressure. In fine-mesh litterbags average litter mass loss was 55% in litter from the islands without deer, 43% for litter from islands with intermediate browsing pressure and 34% in litter from islands with severe browsing pressure (Fig. S2.2A in Supporting Information). This pattern hold whatever the island category litterbags were placed on (Fig. S2.2A). Variation due to the context in which a given category of litter was placed had only a little influence, there was no significant effect of home-field advantage (Figs. S2.2A and S2.2B). Decomposers were not more efficient in decomposing litter when it originated from their own environment rather than from other sites. Mass loss was significantly affected by the place of decomposition (Table S2.3, $F = 113.36$, p-value = 0.05). This pattern was due to a significantly better ability of the micro-fauna and microorganisms from plots on the island without deer to decompose litter (Figs S2.2A and S2.2C, p-value = 0.0045). Litter quality, understood here as the rate of decomposition independent of decomposer ability and home-field advantage [calculated using the method developed by Keiser et al., (2014)], was the main driver of carbon loss (Fig.S2.2D). Litter mixes originating from the island with no deer had the best quality index (highest loss after one year, first three bars on Fig. S2.2A), followed by the litter mixes

originating from the island with intermediate deer browsing and then by litter mixes originating from the island with severe deer browsing (Figs. S2.2A and S2.2D).



In coarse-mesh litterbags the overall patterns of decomposition was similar to those from fine-mesh litterbags (compare Figs. S2.2A to S2.2D and Figs. S2.2E to S2.2H in Supporting Information), although variability among plots within sites was greater (Figs. S2.2A and S2.2E). As a result, mass loss in litter mixes originating from islands with intermediate and severe deer browsing were respectively 25% and 39% lower than in litter mixes originating from the plots on the island without deer (p-value < 0.001) (Fig. S2.2C). There was no evidence for home-field advantage in large mesh litter bags (Fig. S2.2F). Place of decomposition significantly affected litter mass loss (Figure S2.3, $F = 4.54$, p-value = 0.01). The ability of the micro-fauna and microorganisms to decompose litter was significantly lower for litter placed in plots exposed to severe browsing (Fig. S2.2G, p-value = 0.009). As observed for fine-mesh litterbags, litter quality had a significant effect on litter decomposition in coarse-mesh bags (Fig. S2.2H, p-value < 0.001).

2.3.3 - Carbon and Nitrogen loss in fine-mesh litterbags in experiment 1

The loss of carbon in the litterbags after one year was highest for litter representative of the vegetation on islands without deer and lowest for litter representative of the vegetation on islands with the most severe browsing pressure (Fig. 2.2A, $F = 108.78$, p-value = < 0.001) a pattern consistent with the pattern of litter mass loss. When compared to the carbon loss observed in litter originating from the island with no deer, carbon loss after one year was 12% lower in litter collected from the island with intermediate browsing, and 30% lower in litter collected from the island with severe browsing, this independently of the incubating (i.e. island) context (Fig. 2.2A). Home-field advantage was not significant (Fig. 2.2B), indicating that decomposers were not more efficient in decomposing litter carbon when it originated from their own environment rather than from other sites. The ability of the micro-fauna and microorganisms to decompose carbon was significantly higher for litter placed in plots without deer than in plots on islands with deer (Fig. 2.2C, p-value = 0.008). Indeed, carbon loss after one year was 5% lower in litterbags incubated in plots on the island with severe deer browsing than carbon loss observed in plots from the island without deer. Litter quality, understood here as the rate of decomposition independent of decomposer ability and home-field advantage [calculated using the method developed by (Keiser et al., 2014)],

was the main driver of carbon loss. Litter mixes originating from the island with no deer had the best quality index (highest loss after one year), followed by the litter mixes originating from the island with intermediate deer browsing and then by litter mixes originating from the island with severe deer browsing (Figs 2.2A and 2.2D).

Litter mixes from the island without deer had significantly higher nitrogen loss than litter mixes from islands with deer (Fig. 2.2E). However, unlike C loss, nitrogen loss was lower for litter mixes originating from the island with intermediate deer browsing than that originating from the island with severe deer browsing (45% and 30% respectively, Fig. 2.2E, $F = 17.53$, $p\text{-value} < 0.001$). We detected no home-field advantage for N loss (Fig. 2.2E). In addition, none of the decomposer communities were better at decomposing and releasing nitrogen (Fig. 2.2F). Similarly than for carbon, litter quality (sensu Keiser et al. 2014) was the main driver of nitrogen loss in litter bags. However, conversely to carbon, the lowest rate of N loss after one year was observed in litter from the island with intermediate browsing pressure (Figs 2.2E and 2.2H).

Carbon and nitrogen loss in litterbags after one year were significantly and negatively related to the initial C:N ratio Community Weighted Mean (CWM) of the litter (Fig. 2.3). For litter carbon loss, the initial litter C:N CWM explained only 10% of its variation (Table S2.2). Conversely, litter nitrogen loss was strongly linked to the initial litter C:N CWM, which explained 50% of its variability (Table S2.4 in Supporting Information).

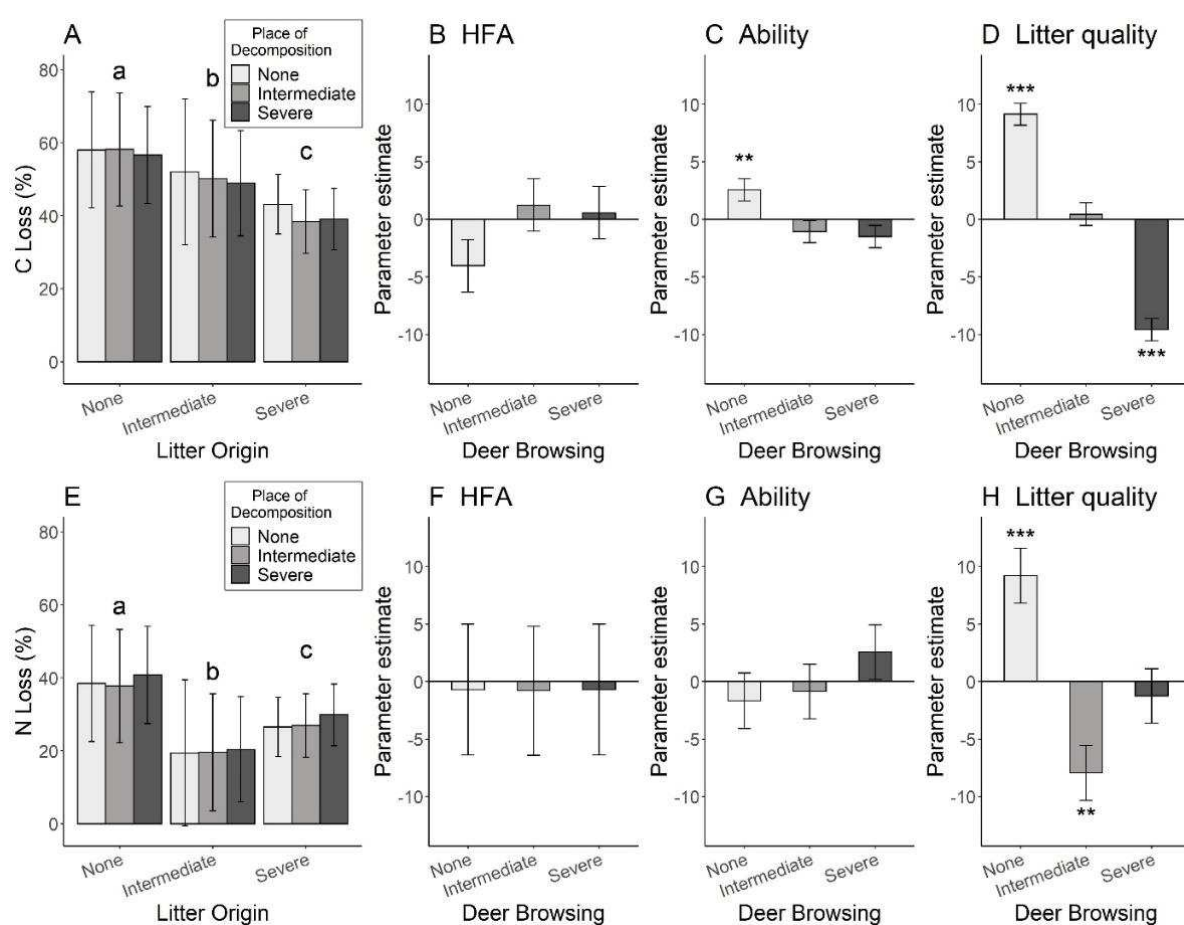
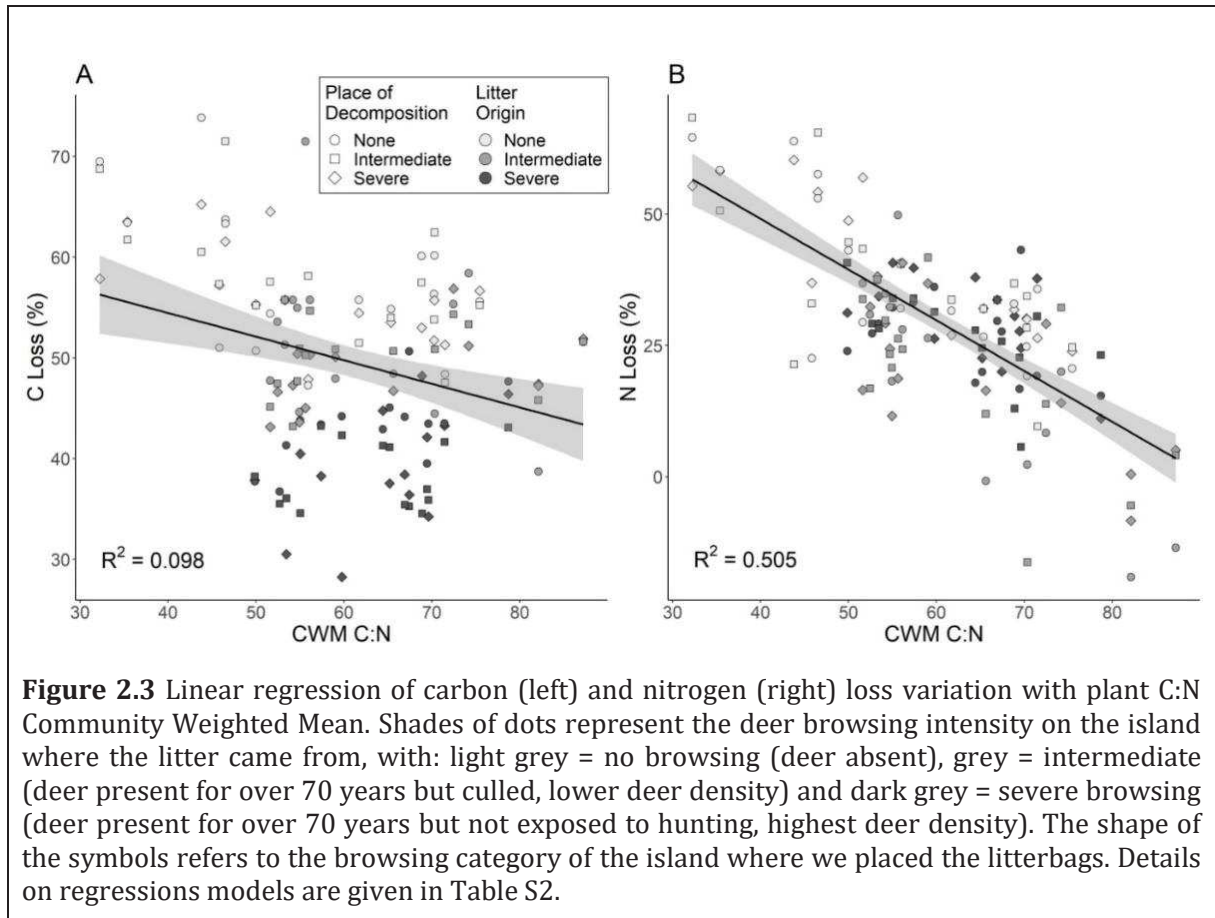


Figure 2.2 Decomposition rate of the plant community litter among deer browsing categories for carbon (top) and nitrogen (bottom) in fine-mesh litterbags in the translocation experiment. Shades of barplots represent the deer browsing intensity with: light grey = no browsing (deer absent), grey = intermediate (deer present for over 70 years but deer density reduced by culls between 1997 and 2010) and dark grey = severe browsing (deer present for over 70 years but not exposed to hunting, highest deer density). Asterisks indicate estimates significantly different from zero with * <0.05 , ** <0.01 , *** <0.001 . Fine letters in each bar plots indicate differences tested by post-hoc test. **Panel A** and **Panel E** represent carbon and nitrogen loss after one year among treatments respectively with bars grouped according to litter origin and shades corresponding to the category of deer browsing of the location where the litter bags were placed. **Panel B-D** and **F-H** represent the parameter estimates (\pm SE) calculated using the Decomposer Ability Regression Test proposed by Keiser et al. (2014).

2.3.4 - Feces decomposition in experiment 2

In fine-mesh litterbags place of decomposition had no effect on feces mass loss (Fig. S2.3A). In coarse-mesh litterbags mass loss from feces after one year was 15% higher on islands with deer than on islands without deer (Fig. S2.3B, p-value < 0.001). The addition of feces enhanced the mass loss in *P. sitchensis* litter by 29% in fine-mesh (p-value = <0.001, Fig. S2.3C) and by 20% in coarse-mesh litterbags (p-value = 0.047, Fig. S2.3D), on island with intermediate or no browsing (Fig. S2.3D). There were no differences among treatments in carbon and nitrogen loss from feces after one year ($F = 1.386$, p-value = 0.26 and $F = 0.416$, p-value = 0.66 respectively, Figs. 2.4A and B). Feces addition significantly increased the C and N loss in *P. sitchensis* litter after one year, by 31% for carbon and 47% for nitrogen ($F = 175.62$, p-value < 0.001 and $F = 66.39$, p-value < 0.001 respectively, Fig. 2.4C and D). The ability of the decomposer community (i.e. decomposition place) had no effect on carbon loss ($F = 0.752$ and p-value = 0.47, Fig. 2.4C). However, for nitrogen loss in *P. sitchensis* litter after one year, the presence of deer feces significantly improved the decomposition ability of the decomposer community in the plots from the islands with deer ($F = 20.10$, p-value < 0.001, Fig. 2.4D).



2.4 – Discussion

2.4.1 - Deer slow down decomposition through modification of the understory plant communities

To our knowledge, our study is among the few studies on the effect of large forest herbivores on decomposition processes that have attempted to dissociate the relative effect of changes in plant community composition (community litter quality) from the effects of changes in abiotic soil properties and decomposer community (decomposer ability, Andriuzzi and Wall, 2017). Previous research focused on decomposer ability and, when integrating litter quality, considered only the plant specific responses to herbivory (i.e. changes in plant chemical composition associated to browsing), but neglected the change in plant species composition and relative abundance. In our study, we demonstrated that herbivores change

the overall quality of litter reaching the forest floor, and that this is the overriding factor governing litter decomposition, rather than soil properties, composition of the soil decomposer community, or home-field advantage (Fig. 2.2). This difference in overall litter quality was not exclusively attributed to a modification of the litter C:N CWM (Figs. 2.1B & 2.3). This suggests that other parameters of litter quality not measured in our study, such as lignin or anti-herbivore compounds, were also implied in overall litter quality decline in presence of deer. Thereafter we refer to the term 'litter quality' as a composite variable calculated as proposed by Keiser et al. (2014), and representing the rate of decomposition independent of decomposer ability and home-field advantage. The major shift in litter quality caused by deer browsing resulted, after one year, in an overall reduction in litter mass loss in presence of deer. This translated after one year into a 25% lower carbon and a 27% lower nitrogen loss in litter from the island with severe browsing compared to C and N losses in litter from the island with no browsing (Figs. 2.2A and 2.2E). This strong control of litter quality on C, and especially on N loss, contrasts with the previous assumption that vegetation changes may affect nitrogen and phosphorus dynamics less than the dynamics of carbon (Bryant et al., 1983; Wardle et al., 2002).

The prevailing importance of change in litter quality that resulted from deer herbivory on decomposition in the temperate forests we studied is in agreement with the microcosm study of Harrison and Bardgett (2003) who showed that decomposition of birch (*Betula pubescens*) litter, originating from inside deer exclosures (unbrowsed) in the Scottish Highlands decomposed faster than litter from outside of the exclosures (browsed), irrespective of the origin of the soil (inside or outside of exclosures). Conversely, Olofsson and Oksanen (2002), in a field translocation experiment assessing the decomposition of four plant species dominating the vegetation of lightly and heavily grazed tundra demonstrated a positive effect of reindeer herbivory on decomposition rate.

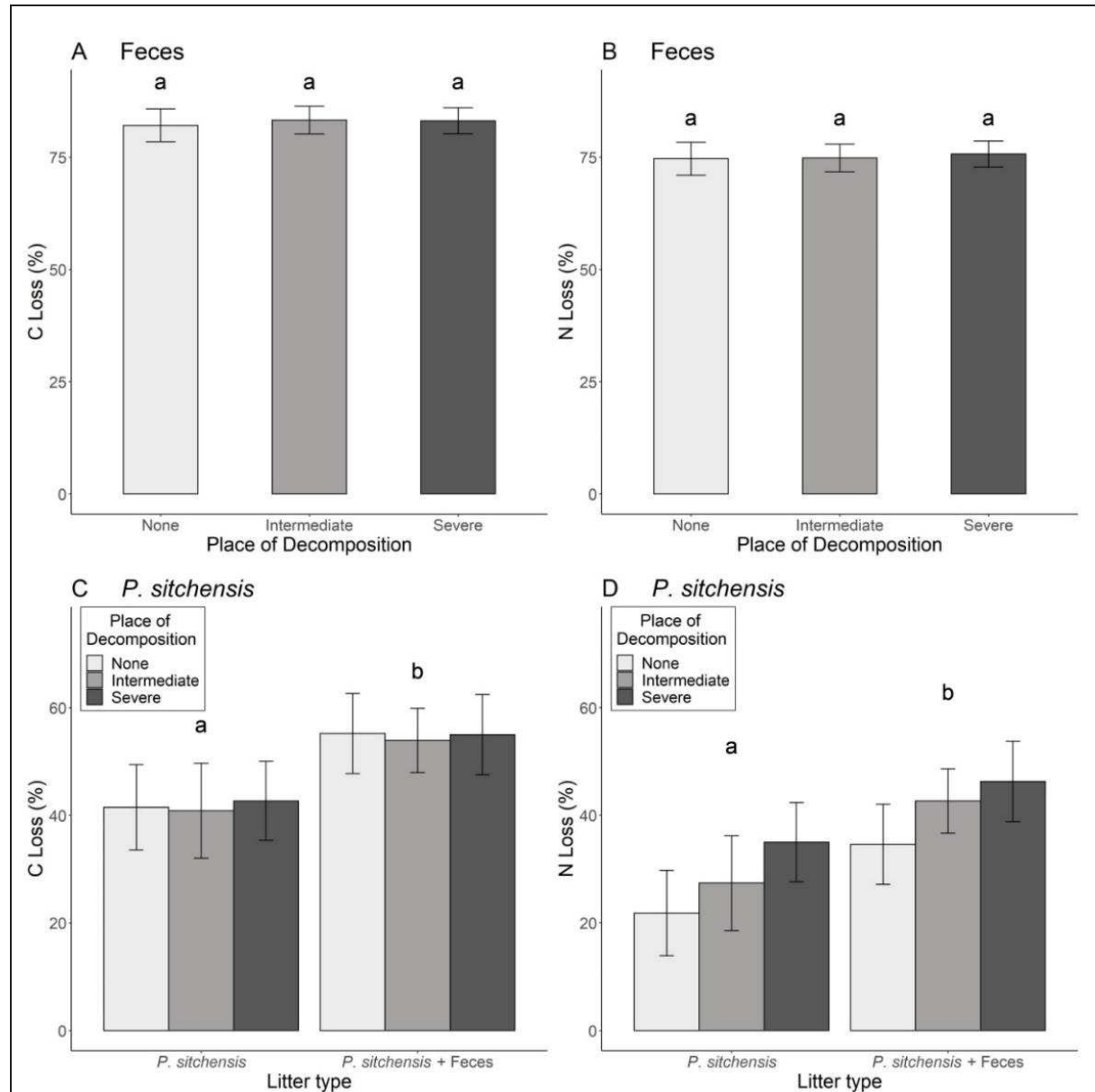


Figure 2.4 Carbon and nitrogen loss in feces in relation to browsing treatment (top) and effect of feces addition on carbon and nitrogen loss in *Picea sitchensis* litter (bottom) in fine-mesh litterbags. Shades of barplots refer to the deer browsing category of the place of decomposition with: light grey = no browsing (deer absent), grey = intermediate (deer present for over 70 years but not exposed to hunting, highest deer density) and dark grey = severe deer browsing (deer present for over 70 years but not exposed to hunting, highest deer density). **Panel A** – Carbon loss in feces; **Panel B** – Nitrogen loss in feces; **Panel C** – Carbon loss in *P. sitchensis* litter with and without the addition of feces; **Panel D** – Nitrogen loss in *P. sitchensis* litter with and without the addition of feces. Small letters above each barplots indicate differences tested by post-hoc test.

The dramatic change in litter quality found in our study is the result of an alteration in the understory plant community (Figs. 2.1A, 2.2D and 2.2H). Intense and prolonged deer browsing dramatically changed the understory plant composition and cover, resulting in an up to 90% reduction in understory shrub cover (Table S2.1 Fig. 2.1A), and in a shift in litter quality. We interpret this change in litter quality on islands with as the main cause for the dramatic reduction in litter decomposed after one year (Figs S2.2D and S2.2H and Figs. 2.2D and 2.2H). These modifications not only confirm the severe impact of deer on the understory vegetation of Haida Gwaii (Chollet et al., 2013b; Martin et al., 2010) but are consistent with results in other temperate forests (Côté et al., 2004; Boulanger et al., 2018).

We found that the reduction in litter quality, and the associated modifications in the decomposition pattern, were partly driven by the variation in the litter CWM C:N ratio (Fig. 2.3) although we found no overall difference in litter CWM C:N ratio among islands (Fig. 2.1B). The decline in litter quality affected carbon and nitrogen cycles differently. For carbon, litter quality decreased as deer browsing intensity increased (Fig. 2.2D). For nitrogen, litter quality was poorer in the intermediate deer browsing than on the island with severe browsing (Fig. 2.2H). The decline in litter C loss could be explained by the shift from an understory dominated by more decomposable species (shrubs) towards an understory of less decomposable species (conifers and bryophytes) as the level of deer browsing intensity increased. Conifers and bryophytes are known to have slow decomposition rates due to low N content and high concentrations of structural carbohydrates and aromatic compounds (Cornwell et al., 2008; Turetsky et al., 2008). The presence of these secondary compounds may largely explain the lower decomposition of the litter from islands with deer and thus the slight effect of CWM of litter C:N ($\approx 10\%$ of variation explained, Fig. 2.3). The contrasting result we obtained for N loss from litter after one year suggests that the vegetation shift caused by deer had different consequences for nitrogen mineralization. Although there was no overall significant difference in CWM litter C:N ratio among deer herbivory treatments, the intermediate treatment had the highest values of C:N (Fig. 2.3), which explains the lowest N loss values observed in this treatment ($\approx 50\%$ of the variation is explained by CWM litter C:N).

2.4.2 - Deer also modify decomposer ability

Although the change in litter quality (sensu Keiser et al. 2014) caused by deer herbivory was identified as the main driver of the rate of mass loss during decomposition, several other changes in the soil decomposer communities affected nutrient cycling. Decomposers from the island without deer had a greater ability to decompose the carbon present in litter, but not nitrogen (Figs. 2.2C and 2.2G). The contrast between C and N decomposition among islands when using fine-mesh litterbags, and the similarity in C and N decomposition when using coarse-mesh litterbags (Fig. S2.2), suggests that the observed decomposition patterns are more likely explained by biotic differences in soils (i.e. differences in decomposer community) than by the effect of abiotic modifications such as higher soil compaction (Fig. 2.1C). A possible explanation for the observed contrasts in litter decomposition may be a switch in the bacterial:fungal ratio in presence of deer. In fact the disappearance of base-rich shrubs and their replacement by species with high concentrations of phenolic compounds (e.g. bryophytes) as a result of deer browsing may have increased the dominance of fungi which require less calcium and magnesium for growth (Prescott, 2010). This change in decomposer community structure would favor the formation of a mor humus, in which up to 30% of the litter mass is converted to humus rather than decomposing (Prescott, 2010). In addition the dramatic reduction in shrub cover may have reduced root exudation which stimulates bacterial activity (Ekberg et al., 2007). Contrary to carbon, the ability of decomposers to decompose nitrogen in litter did not vary among islands with different patterns of deer herbivory (Fig. 2.2G). This may be explained by the selection of microorganisms better able to exploit N in environments where this element is the most limiting ("nitrogen mining hypothesis", (Craine et al., 2007) compensating for the switch in bacterial:fungal ratio. This hypothesis is supported by our control experiment, where we used a standardized quality of litter (*Picea sitchensis*), and found a greater ability of decomposers to decompose N in litter samples incubated on sites with deer (Fig. 2.4D).

Interestingly, we also found that the inclusion of the soil macro and mesofauna (hereafter fauna) in litter decomposition via the use of coarse-mesh litterbags affected litter mass loss (Fig. S2.2E). Specifically, we found that litter decomposition was reduced on the island with

the highest deer density, suggesting a negative effect of high deer density on the faunal decomposer communities. Previous studies documented negative effects of large herbivores on the abundance and diversity of the soil fauna (see review by Andriuzzi and Wall, 2017), but the consequences on litter decomposition were not studied. This negative effect could be due to a reduction in both the abundance and the activity of the soil fauna through several mechanisms. Directly, through soil trampling by deer which might reduce soil fauna habitat through soil physical compaction and its reduction of soil pore size (Beylich et al., 2010). In addition, the reduction of litter quality by deer might be responsible for an indirect slowing down of soil faunal abundance and activity for which litter quality is known to be a controlling factor (García-Palacios et al., 2013; Hendriksen, 1990). As most previous studies on the effects of large herbivores on decomposition focused mainly on the role of microbes, we feel more attention needs to be paid to the role of the soil fauna in order to better understand ecosystem nutrient cycling.

Soil fauna also plays an important role in the decomposition of feces, with evidence of home-field advantage (HFA). Indeed deer feces decomposition in our study was more rapid on islands with deer (home) than on the island without (away), but only when including the effects of fauna (coarse-mesh bags, Fig. S2.3B). We infer that deer have a positive effect on macrofauna decomposing dung. Such a positive effect of large herbivores on this specialized fauna has been recently demonstrated in Japan where (Iida et al., 2018) found a positive relationship between the populations of dung beetles and deer density. In our study, we demonstrated that, litter fauna, but not microorganisms, were selected for decomposition of a particular litter type. This is an important result as most of the literature on HFA only considered microorganisms, and this suggests a potential underestimation of fauna on HFA.

A large proportion of what we know on the effect of high quality litter deposition (dung and urine) by large herbivores on nutrient cycling comes from the study of domestic animals and/or grassland ecosystems (McNaughton et al., 1997; Frank and Groffman, 1998; Christenson et al., 2010). We demonstrate that in the temperate forests we studied dung decomposed faster, and released a larger proportion of nitrogen, than observed for plant

litter. Also, the addition of feces, whatever the mesh size, increased the rate of *Picea sitchensis* decomposition, increasing C loss by 31% and N loss by 47% (Fig. 2.4C and 2.4D). This may be explained by the presence of labile nutrients in dung, which enhance the development of microbial communities, increasing rates of nutrient cycling (Bardgett et al., 1998). However, despite these results, we found that dung deposition did not affect overall decomposer ability (no higher decomposer ability on islands with deer dung/urine, Fig. 2.2C and 2.2G). This results differ from recent studies which demonstrated that feces deposition enhanced plant productivity and soil nutrient availability (Barthelemy et al., 2015; Wang et al., 2018). The explanation for the lack of effect in the forests we studied likely rests with the patchy distribution of solitary deer, in contrast to herding species like reindeer or livestock, and thus reflects the patchy, and limited, amounts of dung deposited locally, amounts that appear not to be sufficient to influence the nutrient cycling at the ecosystem level (Pastor et al., 1988).

2.5 – Conclusion

Our results show that in temperate forests abundant deer can play an important role in ecosystem functioning, modifying aboveground, as well as belowground, characteristics, and reducing nutrient cycling. In the last few decades, the awareness and knowledge of the effects of overabundant deer on aboveground communities has been growing worldwide (Côté et al., 2004; Takatsuki, 2009). Our study suggests that these aboveground changes are probably at the root of major modifications in nutrient cycling in temperate forest ecosystems. In addition, it has to be emphasized that our results are likely an underestimation of effects as we did not take into account the dramatic effect deer have on the quantity of litter reaching the forest floor. For example, in Western Europe the current 10 million roe deer *Capreolus capreolus*, in addition to the increasing populations of other ungulates, represent a standing biomass estimated at 0.75 billion kg that consumes ≈20 million tons of green vegetation each year (Apollonio et al., 2010a). Consequently, there is critical need to expand our results to other temperate forests to assess the overall

consequences increasing deer populations have on broad scale nitrogen cycling in soils (Hobbie and Villéger, 2015) and their potential influence on global carbon storage (Tanentzap and Coomes, 2012).

2.6 – Acknowledgements

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2.7 – Supporting Informations

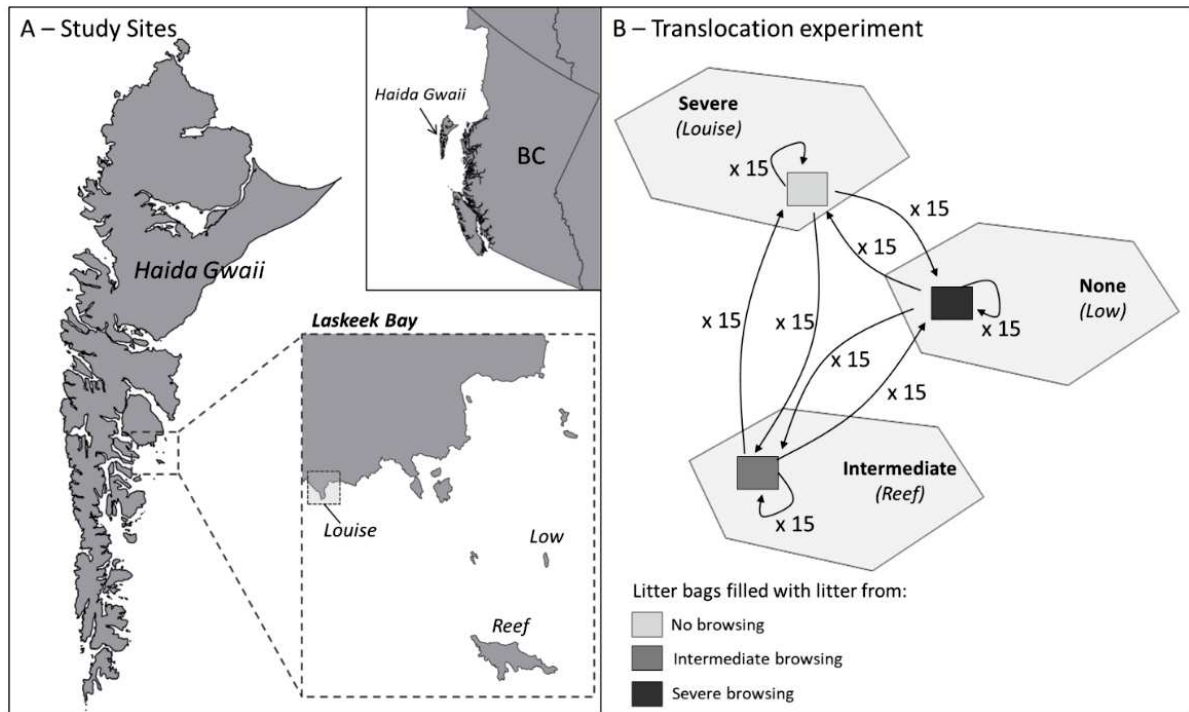


Figure S2.1 Study area and experimental design. **A)** Map of the study sites, **B)** Translocation pattern in the experiment 1. None = no deer browsing, intermediate browsing = deer present for over 70 years but exposed to significant culls between 1997 and 2010, severe browsing = deer present for over 70 years but not exposed to culls nor hunting

Table S2.1. Table synthesizing previously published results on the effect of deer on aboveground ecology of Haida Gwaii on islands covering the entire range of island sizes present in the archipelago

| Reference | Method/protocol | Main results |
|---|--|---|
| Martin et al 1995, Oikos | Songbird and vegetation sampling, 65 islands ranging from 1 to >300,000 ha | Except for the smallest most remote islands never colonized by deer, deer presence is the key factor explaining plant and animal distribution and community structure |
| Engelstoft 1995, Master's thesis | Vegetation sampling on Graham (6,361 km ²) and Moresby Islands (3,399 km ²) | Deer have dramatically reduced the understory vegetation and keep the sparse understory from recovering. Deer will also have profound affects on the overstory by eliminating recruitment of Western Redcedar |
| Martin & Baltzinger 2002, Can. J. For. Res. | Graham (6,361 km ²) and Moresby Islands (3,399 km ²): in different contexts of deer hunting pressure | Regeneration of western redcedar (<i>Thuja plicata</i>) is drastically reduced in presence of deer |
| Allombert et al. 2005, Conservation Biology | Six small islands of Laskeek Bay with different browsing histories (no deer vs deer present) | Insect abundance in the vegetation decreased eightfold and species density sixfold on islands with deer |
| Stockton et al. 2005, Biological Conservation | Seven small islands of Laskeek Bay with different browsing histories (no deer vs deer present) | Vegetation cover exceeded 80% in the lower vegetation layers on islands without deer and was less than 10% on the islands with deer |
| Gaston et al. 2006, Ecoscience | Ten islands of Laskeek Bay with different browsing histories (no deer vs deer present) ranging from 4.5 to 395 ha | Reversal of the normal species number-island area relationship as a result of deer browsing. Conclude that deer are a major factor structuring the island plant communities |
| Stroh et al. 2008, Forest Ecology & Management | Graham (6,361 km ²): deer exclosure | Protected seedlings survived better, were higher, presented more leafed shoots, and had less stems than unprotected individuals |
| Chollet et al. 2015, Biological Invasions | 57 islands ranging from 1 to 425 ha with different browsing histories | Deer are the main factor explaining the abundance of understory vegetation and understory songbirds on the islands except for the few small isolated islands never colonized by deer. |

Table S2.2. List of plant species recorded in the three browsing treatments. All species had a percent cover higher than 5 % in at least one plot. Mean shannon index (Sh.) and richness (Rich.) are given for each plant guild. Mean percent covers are given for each species.

| No browsing | Intermediate browsing | Severe browsing |
|--|---|---|
| Bryophyte (Sh. = 0, Rich. = 0.13) <i>Kindbergia oregana</i> (0.15%) <i>Polystichum munitum</i> (2.92%) | Bryophyte (Sh. = 0.47, Rich. = 2.07) <i>Kindbergia oregana</i> (0.45%) <i>Marchantia</i> sp (0.41%) <i>Plagiomnium undulatum</i> (3.62%) <i>Polystichum munitum</i> (3.40%) <i>Porella navicularis</i> (0.22%) <i>Rhizomnium glabrescens</i> (2.83%) <i>Rhytidiadelphus loreus</i> (0.35%) <i>Scapania bolanderi</i> (0.22%) | Bryophyte (Sh. = 0.73, Rich. = 2.93) <i>Dicranum scoparium</i> (0.09%) <i>Hyloconium spendens</i> (3.98%) <i>Kindbergia oregana</i> (4.88%) <i>Plagiomnium undulatum</i> (0.81%) <i>Rhizomnium glabrescens</i> (0.69%) <i>Rhytidiadelphus loreus</i> (17.95%) <i>Scapania bolanderi</i> (0.10%) |
| Conifer (Sh. = 0.42, Rich. = 1.73) <i>Picea sitchensis</i> (19.78%) <i>Thuja plicata</i> (4.86%) <i>Tsuga heterophylla</i> (5.53%) | Conifer (Sh. = 0.60, Rich. = 2.07) <i>Picea sitchensis</i> (24.92%) <i>Thuja plicata</i> (6.1%) <i>Tsuga heterophylla</i> (36.70%) | Conifer (Sh. = 0.73, Rich. = 2.4) <i>Picea sitchensis</i> (18.58%) <i>Thuja plicata</i> (9.70%) <i>Tsuga heterophylla</i> (41.33%) |
| Fern (Sh. = 0.17, Rich. = 1.00) <i>Pteridium aquilinum</i> (1.97%) | Fern (Sh. = 0, Rich. = 0.47) <i>Blechnum spicant</i> (0.11%) | Fern (Sh. = 0, Rich. = 0.07) <i>Blechnum spicant</i> (0.10%) |
| Forb (Sh. = 0, Rich. = 0.47) <i>Maianthemum dilatatum</i> (3.74%) | Forb (Sh. = 0, Rich. = 0.13) <i>Listera caurina</i> (0.07%) <i>Moneses uniflora</i> (0.43%) | Forb (Sh. = 0, Rich. = 0.07) <i>Galium triflorum</i> (0.12%) |
| Grass (Sh. = 0, Rich. = 0) - | Grass (Sh. = 0.07, Rich. = 0.47) <i>Calamagrostis nutkaensis</i> (5.83%) <i>Luzula parviflora</i> (0.53%) | Grass (Sh. = 0.04, Rich. = 0.13) <i>Bromus sitchensis</i> (0.30%) <i>Luzula parviflora</i> (0.12%) |
| Shrub (Sh. = 1.03, Rich. = 3.53) <i>Alnus crispa</i> (2%) <i>Gaultheria shallon</i> (42.47%) <i>Lonicera involucrata</i> (3.74%) <i>Malus fusca</i> (0.99%) <i>Rosa nutkana</i> (0.94%) <i>Rubus parviflorus</i> (0.82%) <i>Rubus spectabilis</i> (4.79%) <i>Salix scouleriana</i> (0.91%) <i>Sambucus racemosa</i> (0.07%) <i>Symphoricarpos albus</i> (0.32%) <i>Vaccinium parvifolium</i> (3.95%) | Shrub (Sh. = 0.42, Rich. = 1.40) <i>Alnus rubra</i> (0.65%) <i>Gaultheria shallon</i> (10.11%) <i>Merzeansia feruginea</i> (0.27%) <i>Vaccinium parvifolium</i> (2.81%) | Shrub (Sh. = 0.03, Rich. = 0.60) <i>Gaultheria shallon</i> (0.09%) <i>Merzeansia feruginea</i> (0.24%) <i>Vaccinium parvifolium</i> (0.92%) |

Table S2.3. – ANOVA tables of the models explaining the mass, carbon and nitrogen loss according to the litter composition and the decomposition place.

| Model | F-value | p-value |
|--|----------------|------------------|
| Mass loss in fine-mesh bags ~ | | |
| Composition | 117.36 | <0.001 |
| Decomposition place | 3.02 | 0.05 |
| Composition * Decomposition place | 1.08 | 0.37 |
| Mass loss in coarse-mesh bags ~ | | |
| Composition | 9.49 | <0.001 |
| Decomposition place | 4.54 | 0.01 |
| Composition * Decomposition place | 0.80 | 0.53 |
| C loss ~ | | |
| Composition | 108.78 | <0.001 |
| Decomposition place | 2.44 | 0.09 |
| Composition* Decomposition place | 1.03 | 0.40 |
| N loss ~ | | |
| Composition | 17.53 | <0.001 |
| Decomposition place | 1.15 | 0.32 |
| Composition * Decomposition place | 0.03 | 1 |

Table S2.4. – ANOVA tables of the models explaining carbon and nitrogen loss according to the decomposition place and the CWM litter C:N.

| Model | F-value | p-value | R ² |
|--------------------------|---------|---------------------------|----------------|
| C loss ~ | | | |
| CWM | 11.49 | 9.4 e⁻⁴ | |
| Decomposition place | 0.99 | 0.38 | 0.098 |
| CWM* Decomposition place | 0.09 | 0.92 | |
| N loss ~ | | | |
| CWM | 119.75 | 2e⁻¹⁶ | |
| Decomposition place | 1.77 | 0.18 | 0.505 |
| CWM* Decomposition place | 0.96 | 0.38 | |

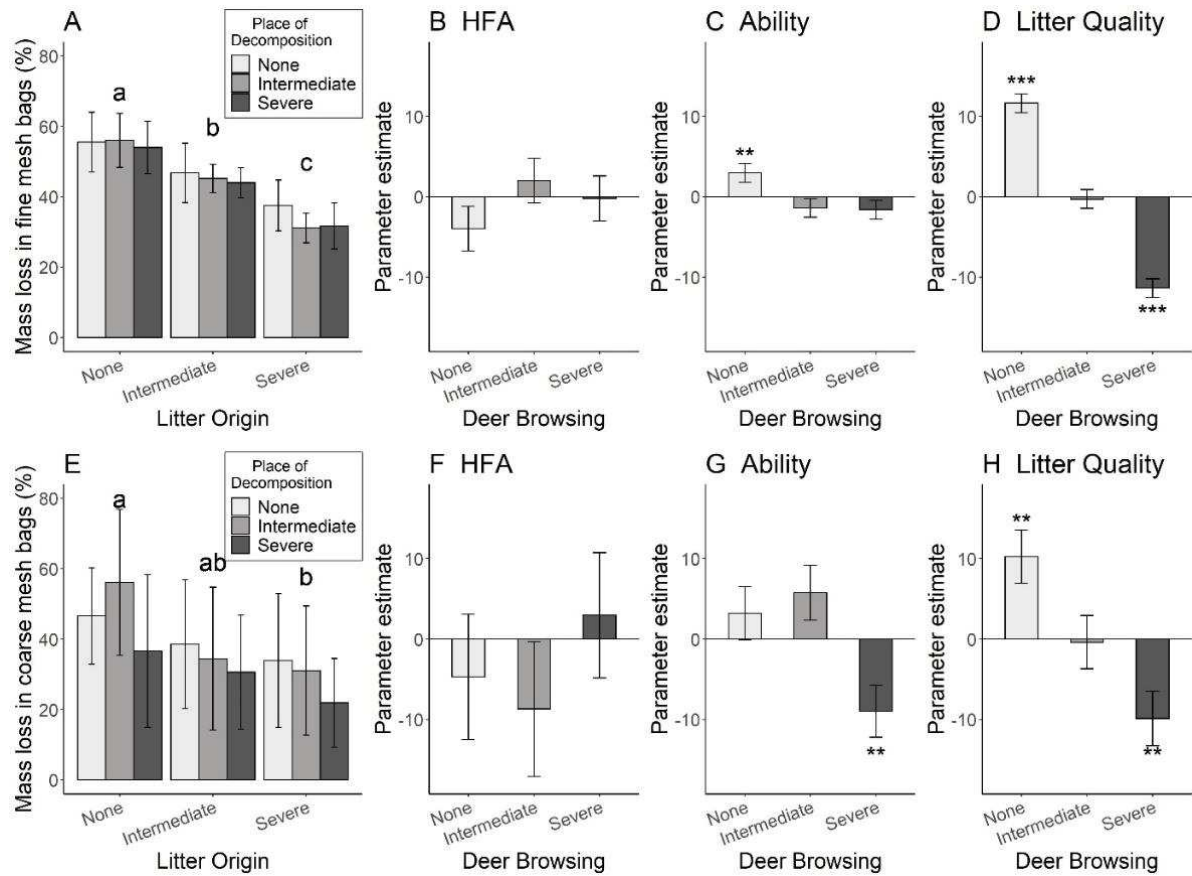


Figure S2.2 Mass loss after one year of the plant litter among herbivory treatments for fine-mesh litterbags (top) and coarse-mesh litterbags (bottom) observed in the translocation experiment. Shades of barplots represent the herbivory treatment with: light grey = no browsing (no deer), grey = intermediate (deer present for over 70 years but exposed to significant culls between 1997 and 2010) and dark grey = severe browsing intensity (deer present for over 70 years but not exposed to hunting). Asterisks indicate estimates significantly different from zero with * < 0.05, ** < 0.01, *** < 0.001. **Panel A** and **Panel E** represent mass loss among treatments in fine and coarse-mesh litter bags respectively with bars grouped according to litter origin (X axis) and shades corresponding to the place of decomposition. **Panel B** to **D** and **F** to **G** represent the parameter estimates (± SE) calculated using the Decomposer Ability Regression Test proposed by Keiser et al. (2014)

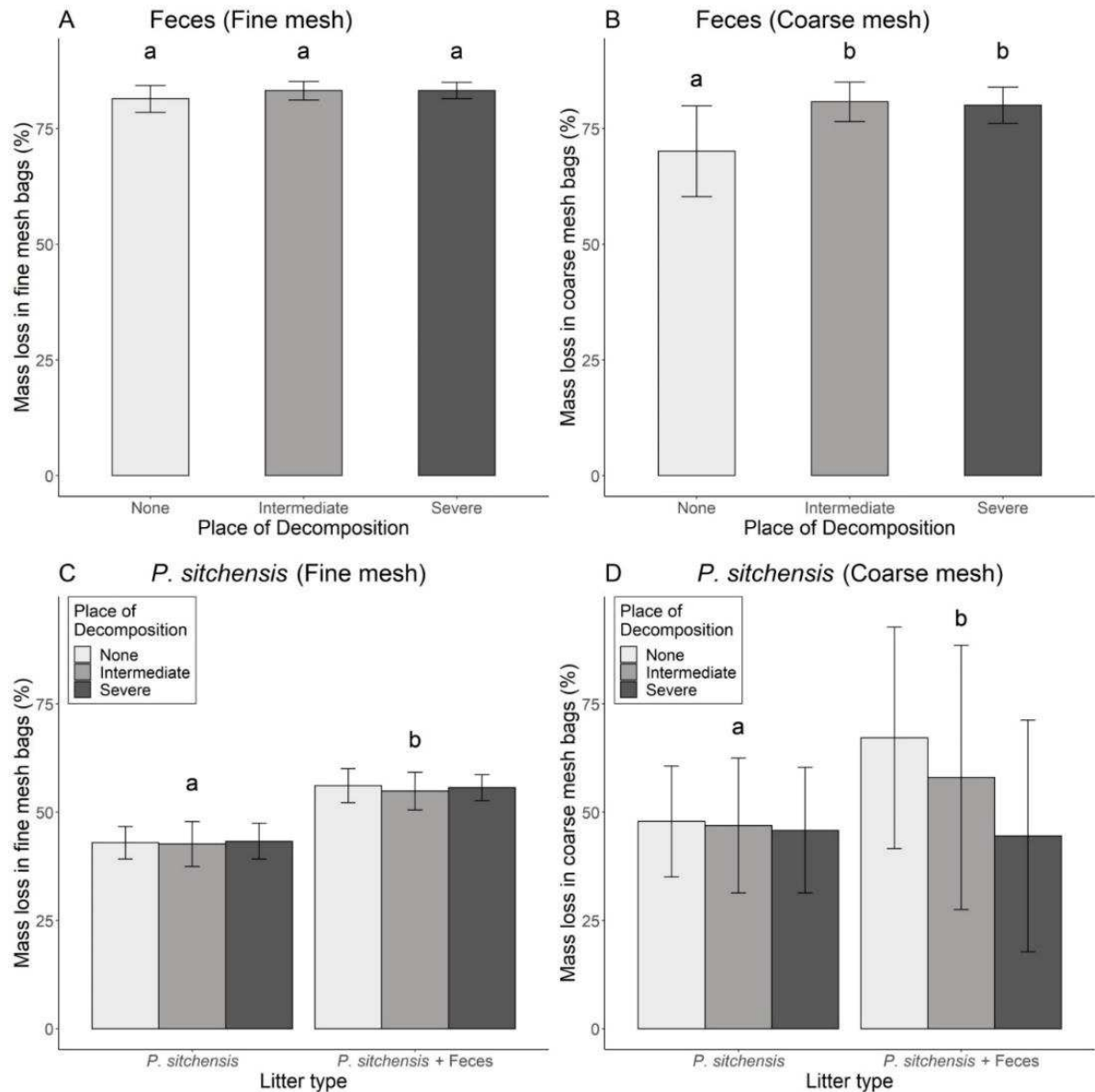


Figure S2.3 Decomposition of feces (top) and effect of feces addition on *Picea sitchensis* decomposition (bottom) for fine-mesh (left) and coarse-mesh (right) litterbags. Shades of barplots represent the browsing intensity of the place of decomposition with: light grey = no browsing (no deer), grey = intermediate (deer present for over 70 years but exposed to significant culls between 1997 and 2010) and dark grey = severe browsing (deer present for over 70 years but not exposed to hunting). **Panel A** – Mass loss in feces in fine-mesh litter bags; **Panel B** – Mass loss in feces in coarse-mesh litter bags; **Panel C** – Mass loss in *P. sitchensis* litter with and without the addition of feces in fine-mesh litter bags; **Panel D** – Mass loss in *P. sitchensis* litter with and without the addition of feces in coarse-mesh litter bags.

Chapter 3: Abundant deer modify soil properties and prokaryotic communities in a temperate forest.

Maillard Morgane, Martin Jean-Louis, Chollet Simon, Léna Simon & Grayston Sue

ABSTRACT

Although negative effects of deer on soil properties and ecosystem functioning have been predicted in forest ecosystems, current studies in temperate forests have found inconsistent results within, and across, systems. These inconsistencies may be the result of a time-dependence of the soil response to deer presence. Short-term modifications belowground may reflect the direct interactions of deer with edaphic properties (i.e. trampling and waste deposition), while long-term modifications may reflect both direct and indirect (i.e. vegetation shift) interactions of deer with soil. We compared the effect of overabundant deer on ecosystem properties and on soil prokaryotic communities in the temperate forests of Haida Gwaii, using three systems varying in both length and scale of deer presence or exclusion. We found that one year of deer removal had no effect on edaphic properties and prokaryotic community structure. 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 increased soil compaction, reduced total soil phosphorus content and soil prokaryotic diversity, and modified soil prokaryotic community composition. This shift in soil prokaryotic community composition suggested important implications for carbon and nutrient cycles. Detection of changes in soil chemical and biological properties in presence of deer therefore required long-term studies longer than those currently available in the literature.



Male Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), Haida Gwaii. Picture from Jean-Louis Martin.

3.1- Introduction

The past century witnessed a dramatic increase in deer abundance at continental scales in North America and Western Europe (Côté et al., 2004; Fuller and Gill, 2001). This massive increase has triggered important changes in the structure of temperate forests (Côté et al., 2004; Ramirez et al., 2018). High deer abundance has been shown to prevent forest regeneration and to dramatically reduce understory vegetation cover and composition (Gill, 1992; Horsley et al., 2003; Stockton et al., 2005). Selective browsing toward more palatable (i.e. nutrient-rich and poor in structural carbohydrates) plant species further shifted the dominance in the forest vegetation to less palatable (i.e. nutrient-poor and rich in structural carbohydrates) plant species (Pastor et al., 1993; Tamura, 2016). These modifications in the forest vegetation structure and diversity had negative consequences for other trophic layers such as birds and insects (Cardinal et al., 2012; Martin et al., 2010; Nuttle et al., 2011; Takada et al., 2008). These consequences are not likely to be limited to the aboveground characteristics of forests. Forests rely on dynamic and constant interactions between their aboveground subsystem, vegetation and fauna, and their belowground subsystem, the soil. Plant growth is indeed sustained by the recycling of organic matter into inorganic nutrients by soil microbial communities. The structure and activity of the latter depend on soil chemical and physical properties (Fierer et al., 2009; Fierer and Jackson, 2006; Karimi et al., 2018). Because of their central role in carbon and nutrient recycling, any modifications affecting soil communities can have important feedbacks on ecosystem functioning and aboveground organisms (Wardle et al., 2004). Understanding the interactions between over-abundant deer and the belowground ecosystem, and being able to predict their effects on the soil properties and activities, is therefore essential for a comprehensive understanding of ecological processes in temperate forests.

Deer can modify soil properties via several pathways. Soil compaction by trampling can modify the soil habitat by reducing pore size, increasing water retention and temperature, and decreasing oxygen levels (Cambi et al., 2015). Reduction in plant litter quantity due to browsing reduces organic nutrients entering the soil to feed faunal and

microbial communities (Bardgett and Wardle, 2003). Similarly, the shift in the plant community towards a community dominated by nutrient-poor plant species can result in lower inputs of organic nutrients to soil communities (Forsyth et al., 2005; Pastor et al., 1993). This reduction in litter quality is strengthened by the dominance of plant species rich in structural carbohydrate (i.e. lignin) and by increased plant production of chemical defences, such as tannins and terpenes, in response to herbivory (Tallamy and Raupp, 1991). These structural and defensive compounds make litter less easily decomposable for soil micro-organisms (Grime et al., 1996). Deer can also modify soil properties through waste deposition. Through digestion, feces are a source of organic matter more easily decomposable than recalcitrant plant litter (Bardgett et al., 1998). By excreting urine, deer release organic ammonium directly into a mineral ammonium form. This by-passes nitrogen mineralisation steps in soil and can speed up decomposition and nitrogen cycling (Bardgett et al., 1998; Molvar et al., 1993).

Although negative effects of deer on soil properties and ecosystem functioning have been predicted in forest ecosystems, current studies in temperate forests have found inconsistent results within, and across, systems (Bardgett et al., 1998; Bardgett and Wardle, 2003; Harrison and Bardgett, 2008). Effect of deer on the soil in temperate forests was found to be significant (*e.g.* Bressette et al., 2012; Gass and Binkley, 2011; Niwa et al., 2011), neutral (Relva et al. 2014), or idiosyncratic (Wardle et al., 2001; Harrison and Bardgett, 2004). The method of choice to study the effect of deer on ecosystems was through their exclusion using fenced areas. Comparison of the ecosystem inside and outside these exclosures provided information on the ecosystem's resilience following deer exclusion and, therefore, on the pressure deer have exerted on the ecosystem. Duration of exclusion varied widely across studies but generally were in the range of a decade (Andriuzzi and Wall, 2017). Exclusions allowed a good characterisation of the effect of deer aboveground, results belowground, however, were more contrasted (Frerker et al., 2014; Wardle et al., 2001). Exclusions did not provide information on the above and belowground forest ecology in the absence of past deer browsing. Furthermore, mechanisms through which deer interact with soil are not all operating at the same temporal and spatial scale. It takes years for the plant

community to be restructured at the ecosystem-scale, while deposition of dung and urine is a local, and an instantaneous process. Time since deer exclusion must, therefore, play an important role in exclosure studies. We predict that the short-term modifications of the belowground subsystem are driven by the direct interaction of deer with edaphic properties, through trampling or dung and urine 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 studies (Murray et al., 2013; Schrama et al., 2013b). Conversely, the indirect effects of ungulates via changes in vegetation structure will be longer-term processes that operate at the ecosystem scale. Revealing its effects on the belowground system will, therefore, require studies that last longer (Bardgett et al., 2005). We further hypothesised that deer effects on soil increase the prokaryotic α and β -diversity in the short term as a result of the spatial heterogeneity of deer main initial effect (i.e. dung and urine deposition), but decrease the prokaryotic α and β -diversity in the long-term as a result of the aboveground homogenisation caused by deer after several decades (e.g. Martin et al., 2010). Finally, we also predicted that deer should induce a shift in prokaryote composition through the modification of soil physical and chemical properties.

To test these predictions, we compared the effect of overabundant deer on ecosystem properties and on soil prokaryotic communities in a temperate forest using three systems varying in both length and scale of deer presence or exclusion. We designed these three complementary systems to be able to assess respectively the long-term, intermediate- and short-term effects of deer on the soil ecosystem. We took advantage of the context of the Canadian archipelago of Haida Gwaii, where introduced Sitka black-tailed deer *Odocoileus hemionus sitkensis* colonised most of the islands. This resulted in a unique situation with, side by side, 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., 2004b). We complemented this quasi-experimental and unique system of islands with two islands that had been colonised by deer for less than 35 years at the time of this study (Vila et al., 2004b), and an island where thirteen years of regular culls between 1997 and 2010 resulted, for the whole period and up to the time of this study (i.e. 20 years), in lower

deer densities. We had also access to nineteen 20-year old exclosures distributed in a portion of the archipelago where deer have been present since the very early 20th century, offering a comparative study situation of 20 years of total deer exclusion compared to prolonged presence of an abundant deer population. Finally, we contrasted the above long-term impacts of deer with the rapid (months) to short-term (year) responses of the vegetation and soil to a very severe deer cull on Ramsay Island located in the same portion of Haida Gwaii than our other study islands.

3.2 - Materials and Methods

3.2.1 - Site descriptions

Haida Gwaii is a Canadian archipelago located off the west coast of British Columbia, Canada (latitude 53.255, longitude -132.087). Sitka black-tailed deer were first introduced to these islands in 1878 by Europeans for hunting. In the absence of natural predators, deer populations increased rapidly, modifying the aboveground ecosystem (Allombert et al., 2005a, 2005b; Martin et al., 2010). The presence of islands varying in browsing histories offered a remarkable context for the long-term accumulation of empirical and experimental data on these above-ground consequences. This 30 year-long accumulation of data provides a situation of choice to study the impact of deer on soil processes. The East of the archipelago is located within the wet hypermaritime subzone of the Coastal Western Hemlock biogeoclimatic zone (Meidenger and Pojar, 1991). All our study sites were located at low elevation in the submontane wet hypermaritime subzone (biogeoclimatic unit: CWHwh1). The climate of this subzone is cool, temperate and oceanic. Mean annual temperature and precipitation are 7.6°C and 1349 mm, respectively (Meidenger and Pojar, 1991). Low altitude Haida Gwaii is covered with a coastal temperate rainforest that is dominated by western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Sitka spruce (*Picea sitchensis*). Soil bedrock geology is volcanic and sedimentary, together with intrusions of granitic rock (Sutherland Brown, 1968). Soil types range from organic soils

that are classified into the Folisol order, to podzols, brunisols and gleysols (The Canadian System of Soil Classification, 3rd ed.).

The three complementary systems we used to assess the long-term, intermediate and short-term effects of overabundant deer on soil ecosystems consisted of:

A deer colonisation system: We selected five islands all covered in mature forests – Low, Lost, Tar, Louise and Lyell Islands – that differed in presence or absence of deer. Low, Lost and Tar Islands have never been colonized by deer due to their distance from the coast and difficulty of access. Louise and Lyell Islands have a long colonisation history, with deer being present for more than 70 years (Vila et al., 2004b). Deer density on these islands was estimated to range between 21 and 37 deer/km² (Stockton et al., 2005). We compared these two sets of islands to study the long-term response of the ecosystem to deer presence. We further added to these islands two additional islands, South Skedans and West Skedans, that had been colonized for less than 35 years at the time of study (Vila et al., 2004b) and one island, Reef Island, where deer had been experimentally culled by the Research Group on Introduced Species (RGIS) to study the ability of the vegetation and fauna to respond to a reduction in browsing pressure (Chollet et al., 2016). The over 80 % initial reduction in deer population and subsequent culls created a period of 20 years of reduced browsing pressure followed by a dramatic recovery of the plant and songbird communities (Chollet et al., 2016). These eight islands are situated in the adjacent areas of Laskeek Bay and Juan Perez Sound in the central part of the east coast of Haida Gwaii. For this study system, we sampled vegetation and soils during the summer of 2017.

A deer exclosures system: Twenty deer exclosures distributed by pairs in 10 sites across Graham Island, in the northern half of the archipelago, were built by the Research Group on Introduced Species (RGIS) in 1997, i.e. 20 years prior to this study. Deer densities on Graham Island have been estimated at 13deer/km² (Engelstoft, 2001). Each exclosure was 5m x 5m in size and consisted of a 2.4 m high, large-mesh wire fence that prevented deer to access the vegetation. We used this experimental set-up to study the small-scale resilience of

vegetation and soil after 20 years of deer exclusion. For this system, we sampled vegetation and soil during the summer of 2017.

A recent deer cull system: In response to the documented 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 Llgaygwü sdiihlda: Restoring Balance project” in 2017. The aim of this project was to remove deer completely from several islands in Juan Perez Sound (Murchison, Faraday, House, Hot Springs and Ramsay Islands) in order to restore the ecosystems of this world-class protected area. We took advantage of this initiative to study the short term response of the ecosystem one year after a very severe deer cull, estimated in excess of 80% of the initial deer population. We sampled the vegetation and soil prior to (summer 2016), a couple of months after (summer 2017), and one year after (summer 2018) the cull on Ramsay Island. As controls, we used Tar Island that had never been colonised by deer, and Lyell Island that had been colonised for more than 70 year, as did Ramsay, but where no culling had occurred.

3.2.2 - Plot characteristics and sampling

Deer colonisation and deer cull systems: We established plots randomly on each island with a minimum distance of 100 m from the shoreline. Each plot was 20m x 20m in size. We sampled soil using a 2.5 cm diameter x 30 cm long soil core. We sampled approximately 100 cores within each plot and composited them to cover plot heterogeneity.

Deer exclosure system: We defined two plots per exclosure – one placed inside and one outside – to compare the vegetation and soil characteristics with and without deer exclusion. We set the size of the plots to 4 m x 4 m to take into account edge effects in the exclosure. We sampled and composited into one sample the soil from 5 small pits randomly dug inside the plot. One exclosure had been destroyed by tree-fall a few months before field work, leaving 19 exclosures to be sampled. The number of plots per treatments and per system is given in Table 3.1.

We surveyed the percent cover of vascular plant species in every plot using a modified Braun-Blanket scale (Braun-Blanquet, 1932) (Table S3.1). We sampled the cover of bryophyte species by placing 20 times randomly on the forest floor, within each plot, a 20 x 20 cm quadrat (deer colonisation and recent deer cull system) or a 5 x 5 cm quadrat (deer exclosure system), and surveyed bryophyte species in each 20 iteration. We estimated the percent cover of each species as the number of occurrences of the species divided by 20 and multiplied by the total bryophyte cover on the plot. We assigned a percent cover value of 0.01 % to the bryophytes present on the plot but that never occurred in the quadrat. In the recent deer cull system tree cover was sampled in year one and kept constant between years of sampling.

We sampled all the soil samples exclusively from the F layer of the forest floor according to the Canadian system of soil classification (The Canadian System of Soil Classification, 3rd ed.), which is biologically the most active soil horizon. Soil samples were kept cool at 4°C for transport back to the laboratory within one month. Soil samples were then sieved to ensure homogenization and kept frozen at -20°C prior to chemical analyses.

3.2.3 - Soil physical and chemical properties

We estimated the depth of the organic soil horizon (forest floor) in the deer colonisation system by averaging the values recorded for the five soil pits dug per plot. We measured the F-layer bulk density in this system based on ten measurements per plot. For this, we used a copper cylinder to collect soil from the F-layer in a volume of 5.4 cm depth x 4.1 cm diameter (71.29cm³). We took care not to change the structure of the soil while sampling. We removed any coarse woody debris from these core samples and subtracted their volume from the volume of the core. We then dried the soil at 105°C for 24h. Bulk density was calculated as grams of dry soil per cm³. We measured forest floor penetration resistance, as a proxy of soil compaction, using a hand-held penetrometer. We recorded 50 penetration resistance measurements per plot to take into account soil heterogeneity. A logistical mishap prevented us assessing soil penetration resistance the first year of the study for the short term response to cull (2016, one year before the cull). Soil water content was measured by

drying the fresh soil at 105° until constant weight was achieved (~48 hours), and subtracting the dry weight from the fresh weight. We measured soil pH in duplicates on air dried soil in a 0.01M CaCl₂ solution using a 1:10 (air dry soil : solution) ratio. We measured total soil carbon and nitrogen content (g / g dry soil) on 3.5 mg of freeze-dried soil using an Elementar Vario El Cube Analyzer (Elementar, Langenselbold, Germany). We calculated carbon and nitrogen stocks in the soil organic layers in the deer browsing history system as the amount of carbon (nitrogen) contained per m² according to the organic horizon depth and bulk density of the soil. We measured total soil phosphorus content (µg P/ g dry soil) in 0.1g 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 (NH₄) and nitrate (NO₃) (µg N/ g dry soil) 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 NH₄ quantification (Weatherburn, 1967) and the VCl₃ reduction method for NO₃ quantification (Hood-Nowotny et al., 2010).

3.2.4 - 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 10µL with 500nM of primers, 0.5µL of DNA template, 3µL of H₂O and 5µL of PowerUpTM SYBRTM 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 1min 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 reactions were 0.998 and 91.12 % respectively. All the measurements were made in triplicates. Illumina sequencing of the 16S RNA gene took place at the Integrated Microbiome Resource platform in Halifax (NS, Canada) using the primer pair 515F (Parada) – 806R (Apprill) (Apprill et al., 2015; Parada et al., 2016). We used the pipeline DADA2 with the package *dada2* and the software R to analyse these sequences (Callahan et al., 2016; R Core Team, 2018). We filtered and trimmed reads using the function *filterAndTrim*. We used the standard filtering parameters of the function, and trimmed the reads after the 250 and the 200 nucleotides for the forward and reverse reads, respectively. Error rates were calculated for both forward and reverse reads using the function *learnErrors*, and used to calculate the number of true sequence variants using the sample inference algorithm of DADA2. The denoised forward and reverse reads were then merged using the function *mergePairs*. Chimeras were removed using the function *removeBimeraDenovo* with the method "consensus". At the end of the reads cleaning, we retained a total of 6186, 10505 and 17291 Operational Taxonomic Units (OTUs) for the deer colonisation, deer enclosure and recent deer cull systems respectively. Rarefaction curves are given on Figure S3.1. One sample ("OB1OUT") from the deer enclosure system had a low sequencing depth; we therefore removed this enclosure from the analysis (Figure S3.1). We rarefied samples to the minimum read count in each system using the function *rarefy_even_depth* from the package *phyloseq* in R. Rarefaction did not change the results of the analysis. We assigned taxonomy with the SILVA database to genus level (Quast et al., 2013).

3.2.5 - Data analysis

We calculated vascular plant, bryophyte and prokaryotic alpha diversities using the Shannon index. We used Principal Component Analysis (PCA) to visualise the effect of deer on the environmental factors measured (plant and soil characteristics) for the three systems. We performed PCA on normalised data using the function *prcomp* from the package *stats* on R (R Core Team, 2018). Significant differences in the deer colonisation system were tested

between the plots from islands without deer and the plots from islands colonised for more than 70 years only, due to the low sample size in the two other treatments. We assessed differences in aboveground properties, belowground properties, microbial diversity and abundance between treatments with a Wilcoxon test for the deer colonisation system, a paired Wilcoxon test for the deer exclosures system, and the *npard* function with a F1-LD-F1 design for the deer cull system (Noguchi et al., 2012). This last method is suitable for nonparametric analysis of paired data in factorial experiments with one whole-plot factor and one sub-plot factor design (Brunner et al., 2001).

OTUs were Hellinger-transformed prior any further analyses of the microbial community structure. To visualise the microbial community structure and β -diversity we used a Canonical Analysis of Principal coordinate (CAP) ordination. This ordination consists of a Principal Coordinate Analysis (PCoA) followed by a canonical discriminant analysis to identify the micro-organisms responding to deer presence (Anderson and Willis, 2003). We used the *CAPdiscrim* function from the BiodiversityR package in R to perform the CAP ordination (Kindt and Coe, 2006), and the *cmdscale* function from the stats package to plot the PCoA graphs. For each system, we ran the PCoA using both the Jaccard and the Bray Curtis metric in order to assess the differences due to community composition and abundance. We assessed the significance of the difference in microbial community structure among treatments with a PERMANOVA using the function *adonis* from the package *vegan* in R (Oksanen et al., 2019). We calculated the dispersion of the prokaryotic community across treatment with the function *betadisper* of the package *vegan*, using the group centroid analysis (Oksanen et al., 2019). We assessed the difference in dispersion across treatments with a Wilcoxon test for the deer colonisation system, paired Wilcoxon test for the deer exclosures system, and the *npard* function with a F1-LD-F1 design for the deer cull system (Noguchi et al., 2012). We drew the heatmap of the relative abundance of the genera significantly correlated with the CAP axes using the function *heatmap.2* from the package *gplot* (Warnes et al., 2019).

We used a Redundancy Analysis (RDA) to investigate the correlation between the plant and soil data and the microbial community. Prior to any variable selection for (RDA), we

performed an overall test on all the explanatory variables as recommended by Blanchet et al. (2008). For this, we performed an ANOVA with 999 permutations on the model resulting from the RDA on all the explanatory variables. We then ran a forward selection on all the explanatory variables using the function *forward.sel* from the package *adespatial* on R (Dray et al., 2019). We corrected p-values for multiple testing using the function *p.adjust* from the package *stats*, and with the method 'holm'. We performed a RDA on the selected variables and the prokaryotic OTUs using the function *rda* from the package *vegan* in R. We calculated the percent variation explained by the selected variables with the function *varpart* from the package *vegan*.

We assessed the potential functional capabilities of the prokaryotic community by Predictive Metagenomic Profiling (PMP) using the algorithm Tax4Fun developed by (Aßhauer et al., 2015). We used the function Tax4Fun from the package Tax4Fun in R, and computed the algorithm using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa and Goto, 2000).

Table 3.1 Sampling locations and details for the three study systems.

| System | Island | Island size (ha) | Deer presence | # plots |
|------------------------------|-------------------|---------------------|---------------------------|------------|
| Deer colonisation | <i>Low</i> | 9.6 | Never colonised | 3 |
| | <i>Lost</i> | 7.3 | Never colonised | 5 |
| | <i>Tar</i> | 6 | Never colonised | 6 |
| | <i>W. Skedans</i> | 8.2 | Colonisation < 35 yrs | 4 |
| | <i>S. Skedans</i> | 5.6 | Colonisation < 35 yrs | 1 |
| | <i>Reef</i> | 249 | Culled from 1998- 2010 | 6 |
| | <i>Louise</i> | 35,000 | Colonisation > 70 yrs | 4 |
| | <i>Lyell</i> | > 17,300 | Colonisation > 70 yrs | 6 |
| Deer exclosures | <i>Graham</i> | 636,100 | Inside/Outside. | 19 exclos. |
| Recent deer cull | <i>Tar</i> | 6 | No Deer | 6 |
| | <i>Lyell</i> | > 17,300 | Deer | 6 |
| | <i>Ramsay</i> | 1,622.8 | Culled | 13 |

3.3 – Results

3.3.1 - Effect of deer aboveground and belowground

Effects of deer aboveground – We found a consistent modification of the vegetation by deer among the three study systems. In the deer colonisation system, the first axis of the PCA fully discriminated the vegetation data according to deer colonisation history on the islands (Figure 3.1A). Vascular plant diversity was lower and bryophyte diversity was higher on the islands colonised for over 70 years than on the islands without deer (Table S3.2 and Figure S3.2; $W = 114$, $p\text{-value} = 0.04$; and $W = 9.5$ and $p\text{-value} = 2.3e^{-4}$, respectively). In presence of deer, shrub and pteridophyte cover were respectively eleven- and twelve-fold lower, and bryophyte, graminoid and conifer cover were, respectively, seven-, six- and two-fold higher when compared to covers observed on islands without deer (Figure 3.1A, Table S3.2, Figure S3.2). Islands with a shorter period of deer presence (< 35 years category) or where deer abundance had been experimentally reduced for 20 years showed an intermediate pattern of plant cover and diversity (Figure 3.1A, Figure S3.2).

We found a similar pattern of deer effects on plant community structure in the deer exclosure system (Figure 3.1A). Vascular plant diversity was significantly higher inside the 20-year old deer exclosures (Table S3.2 and Figure S3.3, $W = 183$, $p\text{-value} = 7e^{-05}$). Shrub and forb cover were significantly higher in the exclosures, by two- and eight-fold, respectively (Table S3.2 and Figure S3.3, $W = 179$, $p\text{-value} = 2.1e^{-04}$; $W = 146$, $p\text{-value} = 0.001$, respectively). Conversely, bryophyte cover was significantly lower under deer exclusion (Table S3.2 and Figure S3.3, $W = 13.5$, $p\text{-value} = 0.002$). On the first axis of the PCA, plots from the exclosure system had intermediate coordinates between those from islands without deer and plots from long-term colonised islands. The vegetation from the plots inside the exclosures was more similar to the vegetation from the plots on islands without deer, whereas the vegetation from the plots from outside the exclosures was more similar to the vegetation from plots on islands where deer were present for over 70 years (Figure 3.1A).

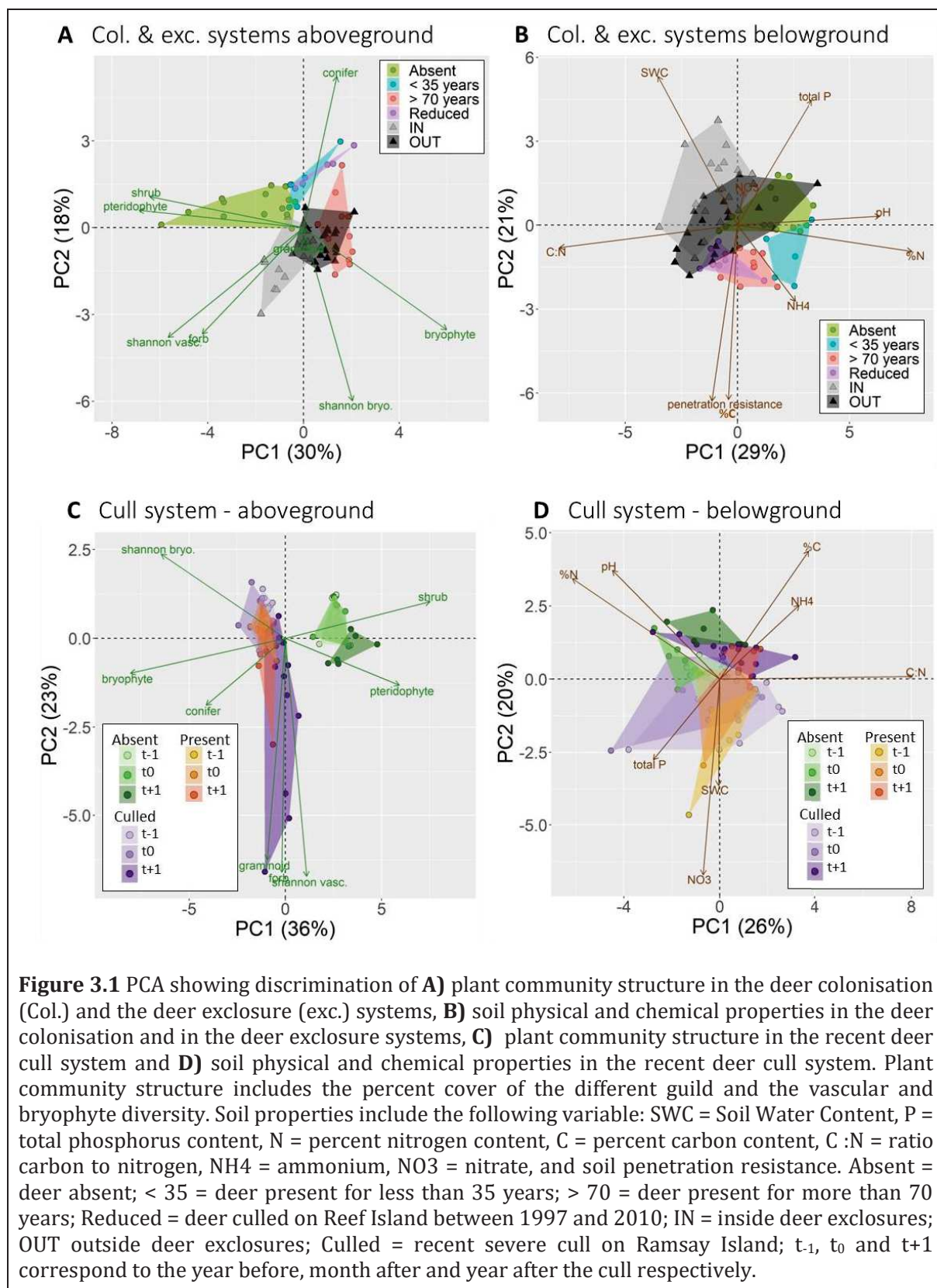
In the recent deer cull system, the first axis of the PCA discriminated the plots from the islands that have or had deer present ('present' and 'culled' treatments) from plots on islands without deer ('absent' treatment) (Figure 3.1C). The second PCA axis discriminated between years of sampling, with the lower values corresponding to the year after the cull (t_{+1}). Interaction between treatments and year of sampling was significant and expressed the response of the vascular plant and bryophyte diversity, and forb cover to the cull (Table S3.2 and Figure S3.4). The increase in vascular plant diversity and forb cover was greater in plots with a low canopy cover (Figure S3.5). Bryophyte diversity was lower the year after the cull (Table S3.2 and Figure S3.4).

Effects of deer belowground – In the deer colonisation system, soil properties discriminated plots across treatments on the second axis of the PCA (Figure 3.1B). Samples from islands with long-term deer presence had significantly lower water content, pH and total phosphorus (Table S3.2 and Figure S3.2, $W = 5$, $p\text{-value} = 1e^{-05}$; $W = 152$, $p\text{-value} = 5e^{-05}$ and $W = 118$, $p\text{-value} = 0.02$, respectively). Soil penetration resistance was three times higher on islands with long-term deer presence (Table S3.2 and Figure S3.2, $W = 0$, $p\text{-value} = 3e^{-05}$). On islands where deer were present for more than 70 years the depth of the soil organic horizon was only half its depth on islands without deer (Figure 3.2A and 3.2B, $W = 149$, $p\text{-value} = 9e^{-05}$). Bulk density was significantly higher on islands with long-term deer presence compared to islands without deer (Figure 3.2C, $W = 9$, $p\text{-value} = 4.4e^{-05}$). Soil carbon and nitrogen stock in the soil organic layer were significantly lower on the islands with long-term deer presence than on islands without deer (Figure 3.2D and 3.2E, Table S3.2, $W = 125$, $p\text{-value} = 2.2e^{-03}$ and $W = 131$, $p\text{-value} = 7.5e^{-05}$ respectively). On the PCA graph, plots from the islands with shorter deer presence (< 35 years) had values between those of plots from islands without deer and those from islands with long-term deer presence. The plots from the island where deer have been culled had coordinates that clustered with those from the non-culled islands with deer for over 70 years (Figure 3.1B). Plots from the islands with a < 35 years deer presence had the thinnest soil organic horizon, lowest C and N stock, and an intermediate bulk density between the one of plots from no-deer islands and plots from islands with deer for over 70 years (Figure 3.2C, D and E). Soil taken from plots on the island where deer had

been culled had the highest bulk density (Figure 3.2C). The depth of the organic horizon and the C and N stocks in the soils from the culled island varied between those from islands without deer and those from the islands with deer for over 70 years (Figure 3.2A, D and E).

In the exclosure system, soils taken from inside and outside exclosures were segregated by the PCA axes (Figure 3.1B). This segregation was due to soil penetration resistance, which was significantly higher outside exclosures (Table S3.2, $W = 0$, $p\text{-value} = 1.4\text{e-}04$), and to total carbon content which, although not significant, was higher outside of the exclosures (Table S3.2, $W = 51$, $p\text{-value} = 0.08$). The other soil properties did not differ significantly between the inside and outside of the exclosures (Table S3.2).

In the recent deer cull system, plots from the island without deer and those from the island with deer were discriminated along the first axis of the PCA (Figure 3.1D). The samples collected before the cull and one month after the cull overlapped with the plots from the island with long-term deer presence. The second axis of the PCA discriminated plots between years of sampling, with lower values observed for the sampling done the year before and one month after the cull, and higher values observed in the sampling done one year after the cull, with plot coordinates closer to those of plots from the island without deer. The interaction between year of sampling and treatment was significant for soil pH and total phosphorus (Table S3.2), but was not correlated to the cull (Figure S3.4). The interaction was marginally significant for soil ammonium, and corresponded to a decrease in soil ammonium the month following the cull (Figure S3.4, $W = 70$, $p\text{-value} = 0.077$).



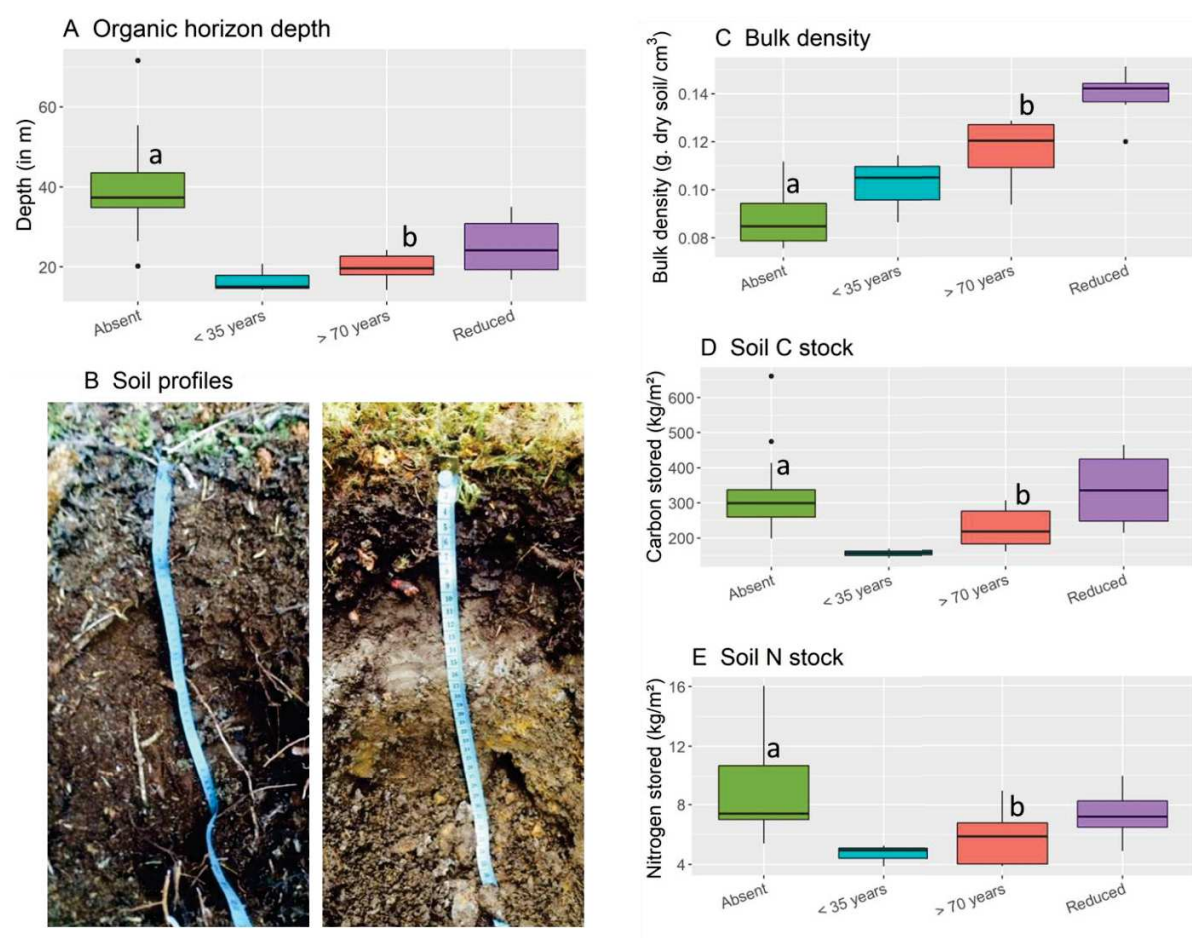


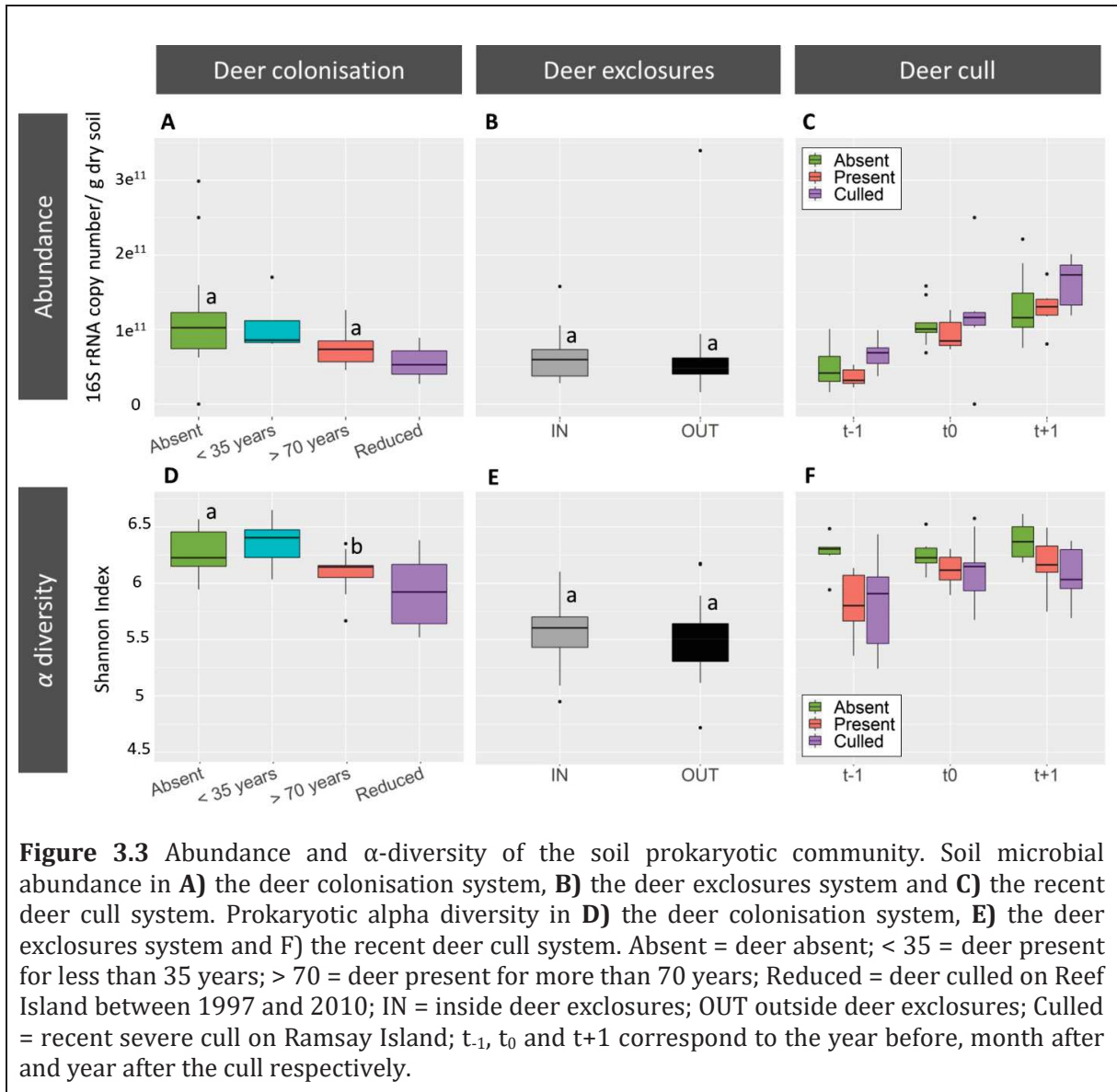
Figure 3.2 In the deer colonisation system, effect of deer on **A)** Organic horizon depth, **B)** Soil profiles from a plot on a deer-free island (left picture) and from a plot on a long-term colonised island (right picture), **C)** Soil bulk density, **D)** Soil carbon stock and **E)** Soil nitrogen stock. Statistical differences were calculated between the islands without deer and with deer for more than 70 years, and results are indicated with letters on top of boxplots. Absent = deer absent; < 35 = deer present for less than 35 years; > 70 = deer present for more than 70 years; Reduced = deer culled on Reef Island between 1997 and 2010

3.3.2 – Structure of the prokaryotic community

We retained a total of 19,717 unique Operational Taxonomic Units (OTUs) after filtering and rarefaction across the three systems, with 82.1% of the total OTUs shared among the three study systems (Figure S3.7A). On average, 99.3 % of the OTUS belonged to the Bacterial

kingdom. They were classified into 623 genera from 231 families, 69 classes and 32 phyla. The actinobacteria, planctomycetes and proteobacteria phyla were consistently among the ten most abundant genera in each sample. On average, only 0.23 % of the OTUs belonged to the Archaeal kingdom. The alignment using the SILVA database identified 4 genera belonging to 6 families, 11 classes and 6 phyla. The archaeal family *Nitrososphaera* from the Thaumarchaeota phylum largely dominated the archaeal population with an average representation of 88 % across treatments and systems. It was followed by the archaeal family *Methanobacteriaceae* from the Euryarchaeota phylum, with an average representation of 38 % 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*.

Bacterial abundance – The variation in soil bacterial abundance in relation to deer presence varied with the study system. Difference in soil bacterial abundance was marginally significant between islands without deer and islands with deer for over 70 years (Figure 3.3A, $W = 44$, $p\text{-value} = 0.075$), with a higher bacterial abundance in soil samples from the islands without deer. Islands that have been colonised for less than 35 years showed a bacterial abundance similar to those of islands without deer (Figure 3.3A). The island with deer present for over 70 years but with reduced deer abundance following a period of deer culls, showed the lowest bacterial abundance (Figure 3.3A). We found no significant differences in soil bacterial load after 20 years of deer exclusion (Figure 3.3B, $W = 103$, $p\text{-value} = 0.768$). In the recent deer cull system, soil bacterial abundance significantly increased with time (Fig3.3C, $F = 59.1$, $p\text{-value} = 1.13 \times 10^{-20}$), but this increase was not significantly different between treatments ($F = 0.37$, $p\text{-value} = 0.74$). Therefore, there is no evidence that the deer cull affected bacterial abundance one year after the cull.



Prokaryotic α diversity – The effect of deer on soil prokaryotic α diversity also depended on the study system. Prokaryotes α diversity was significantly higher in soils from islands without deer than on the islands with deer present for over 70 years (Figure 3.3D, $W = 41$, p -value = 0.051). Soils from island with deer present for over 70 years but with a reduced deer abundance following a period of deer culls had the lowest value for prokaryote α diversity (Figure 3.3D). Soil from islands with deer present for less than 35 years had a similar prokaryotic diversity to the one observed on islands without deer (Figure 3.3D). We found no

significant differences in soil prokaryotic diversity after 20 years of deer exclusion (Figure 3.3E, $W = 89$, $p\text{-value} = 0.899$). In the recent deer cull system soil prokaryotic diversity was also significantly higher in soils from the island without deer, consistently with what we found in the deer colonisation system (Figure 3.3F, $F = 7.12$ and $p\text{-value} = 8.2 \times 10^{-4}$). Although soil prokaryotic diversity also increased significantly with the years of sampling (Figure 3.3F, $F = 8.81$, $p\text{-value} = 4.5 \times 10^{-4}$), the interaction between islands and the year of sampling was not significant, indicating that the cull did not drive soil prokaryotic diversity (Figure 3.3F, $F = 1.83$, $p\text{-value} = 0.15$).

Prokaryotic β diversity – The effect of deer on soil prokaryotic β diversity also varied with the study system. The PCoA showed that the soil prokaryotic community structure was significantly different between the islands colonised for more than 70 years (with or without past culling) and the islands without deer (Figure 3.4A, $F = 7.21$, $p\text{-value} = 0.001$). It also showed that soil prokaryotic communities on the islands colonised for less than 35 years clustered with the ones from the islands without deer (Fig. 3.4A). Conversely, soil prokaryotic communities on the island with deer for over 70 years, but where deer density had been reduced, clustered with the ones from the islands colonised for more than 70 years. The dispersion of the soil prokaryotic community was not significantly different between the islands without deer and the islands colonised for more than 70 years ($W = 104$, $p\text{-value} = 0.15$). The constrained analysis (CAP) identified 115 genera belonging to 64 families and 9 bacterial phyla that significantly contributed to the difference observed between the four treatments (Figure 3.4B and Figure S3.8). On the graph resulting from the CAP analysis, we found that the first axis discriminated treatments according to time since deer colonisation, with low coordinates attributed to plots for sites without deer and for sites with deer for less than 35 years, and high coordinates attributed to plots that have experienced prolonged presence of deer (treatments '>70 years' and 'Reduced') (Figure 3.4B).

We found no significant difference in the soil prokaryotic community structure after 20 years of deer exclusion (Figure 3.4C, $F = 0.781$, $p\text{-value} = 0.297$). Similarly, the dispersion of the soil prokaryotic community was not significantly different between inside and outside deer enclosures ($W = 92$, $p\text{-value} = 0.80$).

In the recent deer cull system, the PcoA discriminated the plots between deer presence and absence on the first axis and between years of sampling on the second axis (Figure 3.4D). This discrimination was confirmed by the PERMANOVA, which showed significant differences in the soil prokaryotic community both between treatment and year of sampling ($F = 10.45$, $p\text{-value} = 0.001$ and $F = 4.24$, $p\text{-value} = 0.001$ respectively). However, the interaction between year and treatment was not significant ($F = 0.84$, $p\text{-value} = 0.831$), indicating that the change over time was the same for the three islands and, therefore, that no change was due to the deer cull. Similarly, the interaction between year and treatment was not significant for the dispersion of the soil prokaryotic community ($F = 1.70$, $p\text{-value} = 0.16$). In the three systems, PcoA was similar when computed on Jaccard and Bray-Curtis dissimilarity matrix, indicating that the observed changes between treatments were driven by a difference in the soil prokaryotic composition rather than its abundance (Figure S3.9). Because we only found an effect of deer presence on the soil prokaryotic community in the deer colonisation system, we focused on this system for the rest of the analyses.

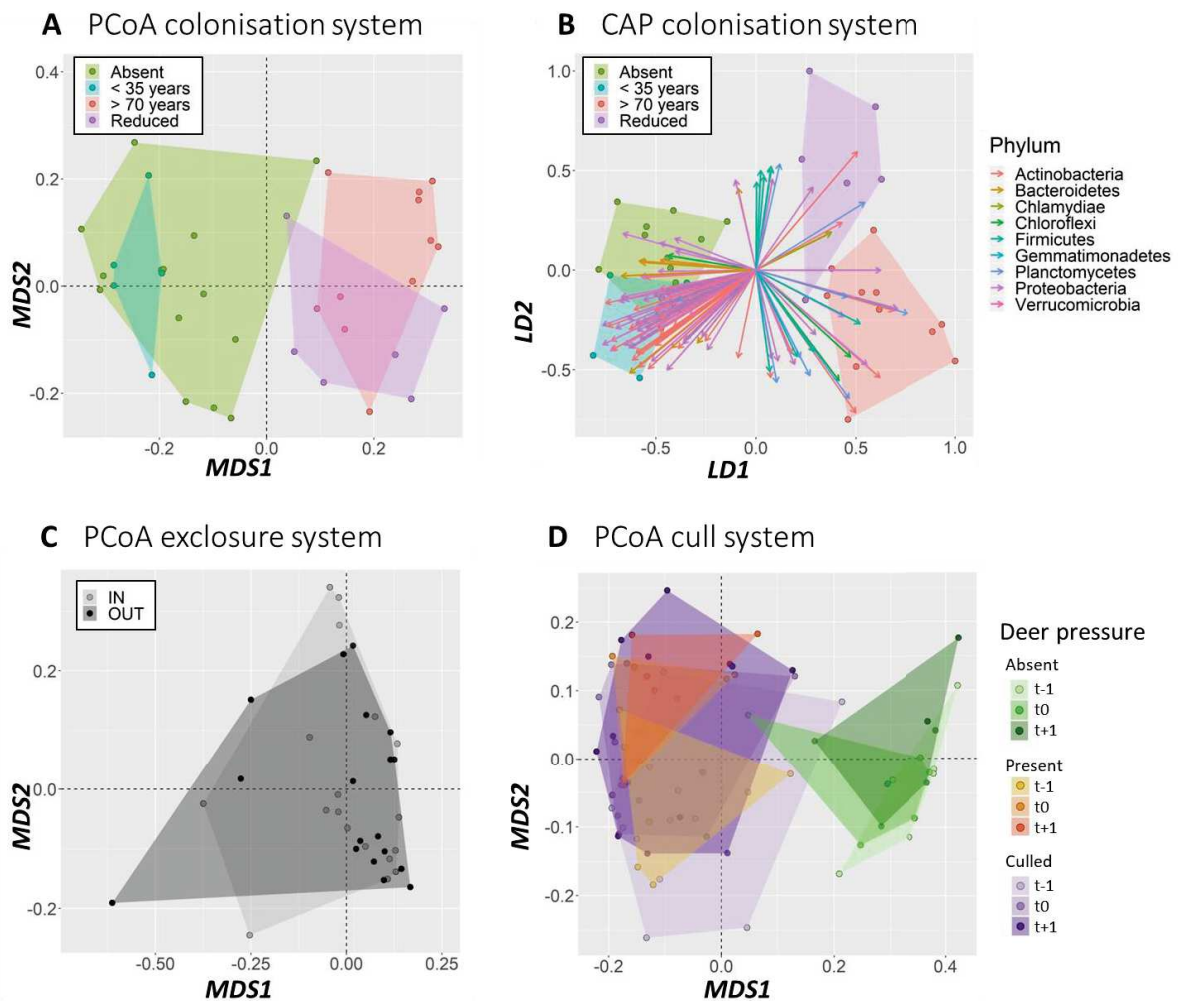
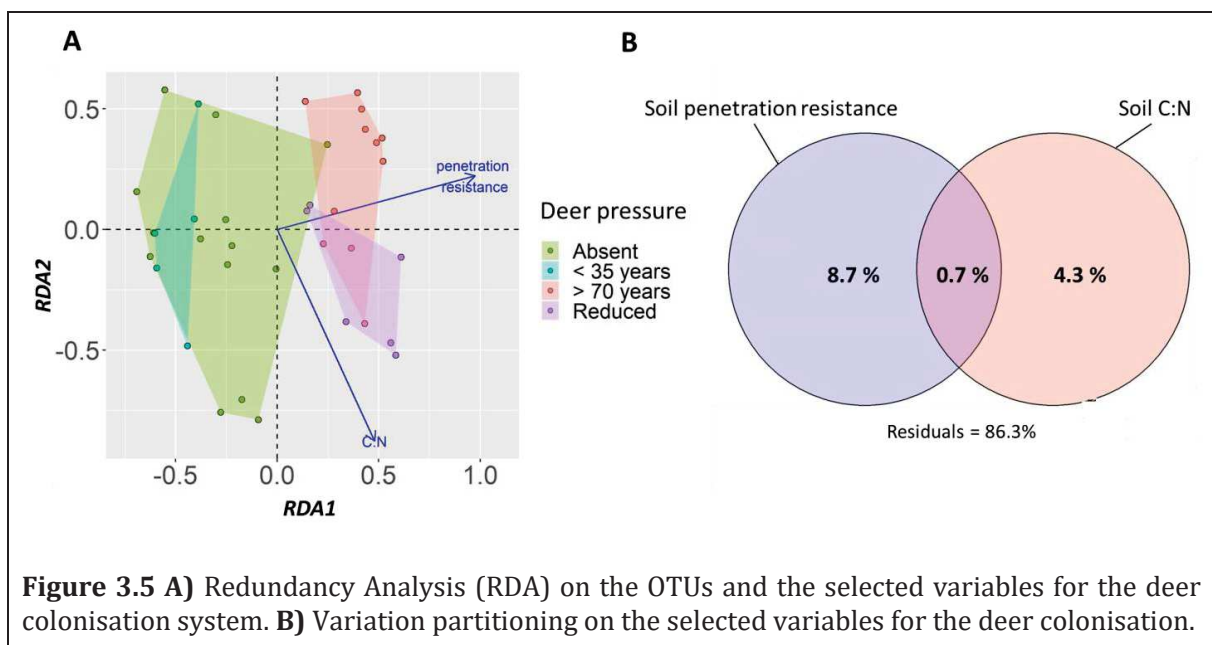


Figure 3.4 β -diversity of the prokaryotic community in the three systems. All the presented PcoA are calculated with the Bray Curtis distance **A)** PcoA of the soil OTU's abundance from the deer colonisation gradient system. Goodness of Fit and R^2 were 0.38 and 0.61 respectively. **B)** Canonical discriminant analysis of the prokaryotic genera from the deer colonisation gradient system. Only the genera significantly correlated with the axes (p -value ≤ 0.05) are represented on the graph **C)** PcoA of the soil OTU's abundance from the deer exclosures system. Goodness of Fit and R^2 were 0.26 and 0.77 respectively. **D)** PcoA of the soil OTU's abundance from the recent deer cull system. Goodness of Fit and R^2 were 0.33 and 0.56 respectively. MDS = MultiDimensional Scaling. LD = Linear Discriminant. Absent = deer absent; < 35 = deer present for less than 35 years; > 70 = deer present for more than 70 years; Reduced = deer culled on Reef Island between 1997 and 2010; IN = inside deer exclosures; OUT outside deer exclosures; Culled = recent severe cull on Ramsay Island; t_{-1} , t_0 and t_{+1} correspond to the year before, month after and year after the cull respectively.

3.3.3 – Relationship between environmental variables and prokaryotic communities

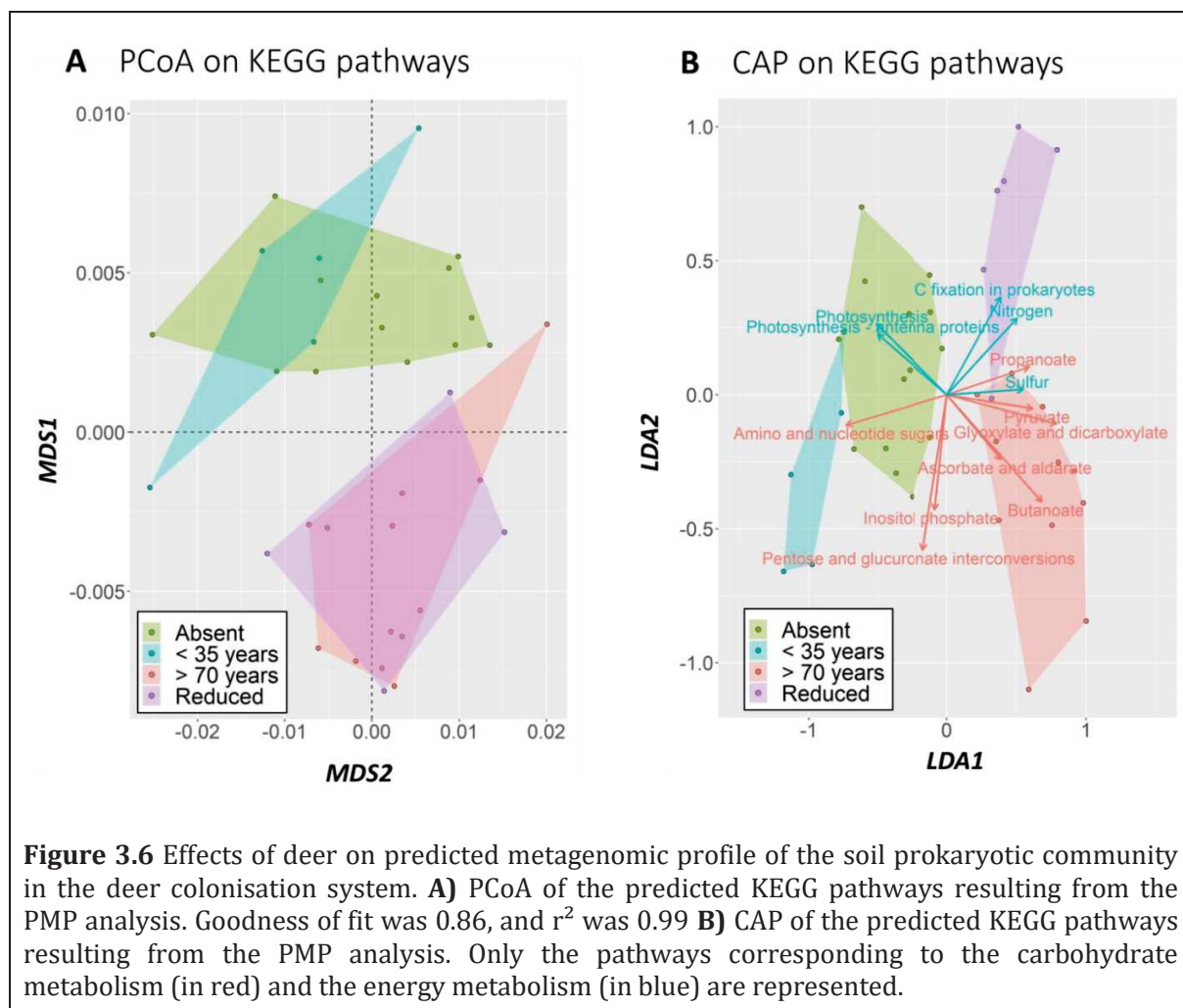
The overall test we performed on all the explanatory variables was significant, with $F = 1.57$ and $p\text{-value} = 0.001$. Therefore, we were able to proceed to the variables selection as suggested by Blanchet et al. (2008). We retained soil penetration resistance, soil C:N and soil NH_4 and Shannon index of the vascular plants after forward selection on the explanatory variables. After adjustment of the p -values for multiple testing, only soil penetration resistance and soil C:N remained significant, with $F = 4.63$, adjusted $p\text{-value} = 0.018$ and $F = 2.69$, adjusted $p\text{-value} = 0.018$ respectively).

Redundancy Analysis (RDA) on the selected variables and the soil prokaryotic OTUs showed that the difference in soil prokaryotic communities between islands without deer and islands colonised for over 70 years correlated with soil penetration resistance (Figure 3.5A). This variable explained 8.92 % of the OTUs variability between plots (Figure 3.5B). Together with the soil C:N, the RDA model built with the selected variables explained 14.01 % of the OTU variability.



3.3.4 – Implications for ecosystem functioning

In the deer colonisation system, the Predictive Metagenomic Profiling (PMP) of the prokaryotic community identified 136 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways from 11 metabolisms within the metabolism category of the KEGG pathways database. PCoA on the occurrence of these pathways in each sample showed that one plot from the island without deer (plot 'TAR01') was responsible for most of the variability in metabolic pathways among plots (Figure S3.10A). When omitting the sample 'TAR01' in the PCoA analysis, separation across treatments appeared more clearly on the second axis (Figure 3.6A). Similarly to the prokaryotic community structure, samples from the islands without deer and those from plots with less than 35 years of deer presence clustered together on the graph, whereas samples from the islands with more than 70 years of deer presence with or without deer culling clustered together. The constrained analysis (CAP) identified 74 pathways from 11 metabolism categories that were significantly correlated with the LDA axes (Figure S3.1B). A focus on energy and carbohydrate metabolisms showed that five and eight pathways, respectively, were significantly correlated with the CAP axes (Figure 3.6B). Within the energy metabolism, the pathways the more represented in soils from islands with deer present over 70 years, with or without population reduction through culling, were nitrogen metabolism, sulphur metabolism and carbon fixation pathways in prokaryotes. The pathways most represented in soils from islands without deer, or from islands with less than 35 years of deer presence, were related to the photosynthesis metabolisms (Figure 3.6B). With respect to the carbohydrate metabolism, soils from islands without deer, or from islands with less than 35 years of deer presence, were associated with amino sugar and nucleotide metabolism. Soils from the islands with deer present for over 70 years, with or without reduced deer density, were also associated with propanoate, pyruvate and glyoxylate and dicarboxylate metabolisms. Soils from the islands with deer present for more than 70 years were also characterized by ascorbate, aldarate, and butanoate metabolisms. Inositol phosphate metabolism and pentose and glucuronate interconversions pathways were more represented in soils from the islands with deer present for less than 35 years and present for more than 70 years (Figure 3.6B).



3.4 – Discussion

We used three different approaches contrasting in time of deer presence and exclusion to investigate the effect of over-abundant deer on the above and belowground properties of a temperate forest ecosystem, with an emphasis on soil properties and soil prokaryotic communities. We confirmed previously reported patterns of understory cover reduction, bryophyte cover increase and vegetation structural simplification that are associated with severe deer browsing (Chollet et al., 2013b; Côté et al., 2004; Martin et al., 2010; Stockton et al., 2005). The aboveground changes observed were remarkably consistent across the three contrasting study systems. Conversely, this consistency of deer effects on aboveground plant

patterns was not reflected belowground. The relationship between deer presence and belowground properties varied across the three systems, reflecting a time-dependant response of the soil to deer pressure.

3.4.1 – Deer modify ecosystem components at different time scales

Deer presence and understory vegetation - Aboveground, we found deer colonisation and exclusion impacted vegetation in a consistent way across the three systems. Deer presence significantly reduced vascular plant abundance and diversity, and significantly promoted the dominance of less-palatable conifers and non-palatable bryophytes. These modifications were stronger and involved more plant guilds the longer deer were present (Figure 3.1A and Table S3.2). Such modification of the plant community composition is in agreement with previous studies on the same islands (Stockton et al., 2005; Chollet et al., 2013) and in other temperate forests of the world (Côté et al., 2004; Gill, 1992; Takatsuki, 2009). In the recent deer cull system, we observed a clear initial shift in the vegetation after one year of severe deer population reduction (Figure S3.4 and Figure 3.7) but that, understandably, was still far from leading to an understory vegetation similar to the one typical of islands without deer, a rate of response also limited by the low light conditions typical of closed canopy forests (Poulson and Platt, 1989; Tripler et al., 2005). The re-colonization of the forest floor by understory plants is further dependent on the presence of refuges, and is, therefore, spatially localised in the first years after deer exclusion or population reduction (Chollet et al., 2016, 2013a). Nevertheless, we found that vascular plant diversity and forb cover had increased one year after the cull, particularly in plots where the canopy was more open (Figure S3.5). Monitoring of the vegetation after an experimental deer cull on two other islands in Haida Gwaii in the late 90' showed that understory recovery is a slow process that probably spans over decades, although this recovery can be remarkable for most species by the end of the first decades following a severe cull (Chollet et al., 2016). The differences in vegetation structure we documented across the three systems therefore reflected different stages in a temporal and consistent response of the vegetation to deer presence.



Figure 3.7 Regeneration of salal (*Gaultheria shallon*) after the deer cull on Ramsay island. Salal regeneration requires both the proximity of a refuge to establish and sufficient light to sustain growth within the first years. Left picture: 1 month after the cull, right picture: 1 year after the cull.

Deer presence and soil physical and chemical properties – We found that response of the soil physical and chemical properties to deer presence and removal differed among our three study systems.

Soil penetration resistance, a proxy for soil compaction, was significantly higher on islands with over 70 years of deer presence than on islands never colonised by deer (Table S3.2, Figure S3.2). The high foot pressure of ungulates can indeed induce an important physical compaction of the soil (Duncan and Holdaway, 1989). In addition, soil water content, total phosphorus and soil pH were significantly altered after 70 years of deer presence (Table S3.2, Figure S3.2). Higher soil water retention has been documented previously as a direct consequence of soil compaction (Cambi et al., 2015). We interpret the lower levels of soil phosphorus on islands with deer presence as the consequences of the higher cover of bryophytes. Mosses have indeed been shown to sequester important level of phosphorus in

coniferous forests (Chapin et al., 1987). The acidification of the soil after long-term deer presence may be explained both by the higher relative abundance of both conifers and moss, which litter have been shown to be acidic (Cornelissen et al., 2006; Finzi et al., 1998). Long-term urine deposition by deer might also explain this acidification, as ammonia input to soil may stimulate nitrification with consequent production of H^+ ions (Ball et al., 1979; Black, 1992). Contrary to what we might have expected in response to the replacement of palatable plants by unpalatable plants (Pastor et al., 1993), we did not observe any modification of the soil C:N. However, this result is consistent with the fact that modification of the plant community composition by deer did not change litter C:N on the same islands (Chapter 2 of this thesis, Chollet, Maillard et al., 2019), and is also consistent with the results of Binkley et al. (2003) after 35 years of deer exclusion using an enclosure system in the Rocky Mountain National Park in United States. Similarly, we did not observe any differences in the amount of soil inorganic and total nitrogen between deer absence and presence, which suggests a resilience of the soil to the local addition or removal of dung and urine inputs. This finding is consistent with the fact that dung deposition did not play a role in carbon and nitrogen decomposition at the ecosystem level on these same islands (Chapter 2 of this thesis). Additional analyses in the deer colonisation system also showed that the soil organic horizon was shallower on islands colonised for more than 70 years, and this thinner organic horizon led to a lower soil carbon and nitrogen stock in the soils of these islands in comparison to islands without deer. This reduction in the organic horizon could be the result of both the reduction in litter quantity due to browsing, and the higher erosion of the soil due to the reduction in protective understory cover (Hartanto et al., 2003; Lei et al., 2010; Tanentzap and Coomes, 2012).

Although we did not statistically study them due to low sample sizes, the observation of the soils on islands colonised by deer less than 35 years prior to this study or from the island where the deer population had been culled, provided an estimation of the transitional response of the soil to deer presence and removal. Results on these islands suggest that 35 years of deer colonisation is sufficient to result in similar soil compaction level than those observed after 70 years of deer colonisation, but that this compaction is not mitigated after

twenty years of deer population reduction (Figure S3.2 and S3.6). They also suggest that increased soil water content, soil acidification and reduced level of soil phosphorus in presence of deer are slow processes that take longer than 35 years to operate and which are not modified by twenty years of deer population reduction (Figure S3.2). Depth of the organic soil horizon had the lowest value on the islands colonised by deer for less than 35 years. This shallow forest floor may result from the small size and the low elevation of these islands, which may confer some shoreline characteristics to the soil rather than strict forest interior characteristics. Sand and shells were indeed found just beneath the forest floor on the plots from these islands colonised by deer for less than 35 years (personal observation). Comparison of the soil inside and outside exclosures showed that a twenty yearlong total exclusion of deer was sufficient to restore compaction levels to the values observed on islands that never had deer. We found that this relapse in soil compaction was not correlated with other changes in edaphic properties (Table S3.2). Particularly, we did not observe differences in soil water content between soil samples from the inside and outside of the exclosures contrary to what we found in the deer colonisation system. This inconsistency between the two systems may be interpreted as the result of an island bias in the deer colonisation system and not as an effect of compaction as first interpreted. Although the islands studied were selected based on their strong and proven effects of deer on the aboveground ecosystem (Gaston et al., 2006), slightly different microclimates could explain the difference in soil water content, as larger islands colonised by deer receive, on average, more rain than the smaller more off shore islands that lack deer. Another explanation for the discrepancy between the two systems could come from the heavy rains that occurred during the soil sampling in the exclosures, and which might have brought the soil samples close to their water holding capacity.

In the study on the short-term response of soils to the recent deer cull, soil ammonium was the only edaphic variable that changed following deer removal. Soil ammonium concentrations were reduced in the month following the deer cull, although this reduction was only marginally significant (Figure S3.4). This decrease can be explained by the sudden cessation of urine input, which constitutes a source of ammonium to the soil. However this

moderate decrease was not reflected by any change in soil ammonium, nitrate or total N in the following year.

Our results suggest that modifications of the ecosystem components by deer are time dependant. Aboveground, changes in the plant community in response to deer presence or removal were relatively fast and consistent, because they are the result of a direct negative interaction (browsing and seedling trampling). Belowground, changes in edaphic properties also depended on the time of deer presence. Consistent with our prediction, short-term and intermediate effects of deer belowground were the results of the direct interactions of deer on the soil, i.e dung and urine deposition and trampling. Long-term effects of deer belowground appeared to be the result of both direct interaction, due to trampling, and indirect interactions due to vegetation shift. We found that increases in soil NH_4 concentrations by deer were not retained one year after deer removal in the recent deer cull system. This demonstrates that the deposition of urine by deer only has a relatively short-term (inferior to one year) influence on soil properties. The lack of difference in total and inorganic soil nitrogen between deer absence and presence at intermediate and long-time scales was supported in our three study systems, suggesting that waste deposition by deer does not impact these forests soils.

Previous studies in temperate forests found no impact of deer on soil compaction for deer exclusion length inferior to 15 years (Burke et al., 2019; Furusawa et al., 2016; Relva et al., 2014; Suzuki and Ito, 2014). However, consistent positive impact of deer on soil compaction have been observed for longer study length (Gass and Binkley, 2011; Iida et al., 2018; Kumbasli et al., 2010; Sabo et al., 2017) . This is consistent with our results, where we found that 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 S3.2). The longest period of deer exclusion in temperate forests has been investigated by Wardle et al. (2001) in New Zealand. The authors found idiosyncratic effects of 20 to 50 years of deer exclusion on soil properties. In our study, the effects of deer on soil chemistry (pH and total phosphorus) were detectable after 70 years of deer colonisation. This result suggests that non idiosyncratic modifications

of soil chemical properties are a long-term process taking several decades to be detected. It also suggests that exclosure studies, the method of choice to study the impact of large herbivores on an ecosystem, might not be an optimal method when investigating the effects of deer on the soil due to the important time lag in soil response to vegetation changes by deer.

3.4.2 – Long-term deer colonization modifies soil prokaryotic community structure through soil compaction by trampling.

We found that the soil prokaryotic community structure was only significantly different in samples from islands with over 70 years of deer presence.

On islands with deer present for less than 35 years or in 20 year old exclosures, soil microbial biomass did not differ from those from islands without deer, similar to the results found in western North American, Patagonian and New Zealand temperate forests (Gass and Binkley, 2011; Relva et al., 2014; Wardle et al., 2001), but contrary to results found in Japanese temperate and North American boreal forests (Niwa et al., 2011; Pastor et al., 1988). In the longer term, however, we found that soil microbial biomass tended to decrease with deer presence, consistently with the results found in boreal and Japanese temperate forests (Niwa et al., 2011; Pastor et al., 1988).

Prokaryotic α diversity was lower in soils from islands with a long-term deer presence, mirroring the simplification observed aboveground. Eldridge et al. (2017) found that grazing by domestic and wild herbivores increased bacterial diversity by excluding Actinobacteria, the competitive microbial phylum, through reduction in soil carbon content. This contrasts with our study, where long-term deer presence reduced prokaryotic diversity, and where modifications were the results of a shift in composition rather than a modification of taxa abundance. Dispersion of the prokaryotic communities on the island colonised for more than 70 years was not significantly different than on the island without deer, refuting our hypothesis of a homogenisation of the prokaryotic diversity on the long term.

Previous studies have found a top-down regulation of the microbial community structure by wild ungulates in a sagebrush steppe (Cline et al., 2017; Peschel et al., 2015), in an alpine

grassland (Yang 2013) and in Australian woodlands (Eldridge et al., 2017). Our results show that such top-down regulation also operates in temperate forests. However, this modification was only observed after over 70 years of deer presence, suggesting that regulation of the soil prokaryotic community is time-dependant in such ecosystems. Our observation can explain the results of Gass and Binkley (2011), who did not find any differences in the soil microbial communities of a grazed and un-grazed temperate forest after 15 years of elk exclusion. In their meta-analysis on the effect of wild herbivores exclusion on the soil, Andriuzzi and Wall (2017) found that time since herbivore exclusion 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.

Therefore, our results suggest that the response of the soil prokaryotic community structure to deer pressure is a slow process in temperate forests.

In our study we attributed the modification of the soil microbial community structure under long-term deer presence mainly to the variation in soil penetration resistance (Figure 3.5). This result suggests that deer modify the microbial community structure via physical compaction through trampling. Soil compaction in forests has been shown to increase soil water retention and soil temperature, and to decrease oxygen levels through reduction in soil porosity (Cambi et al., 2015). Soil compaction has also been linked to a reduction in microbial abundance and the modification of microbial composition towards microbes adapted to low oxygen availability (Hartmann et al., 2014). Similarly, simulated trampling has been shown to decrease soil microbial biomass in sub-arctic grasslands (Sørensen et al., 2009).

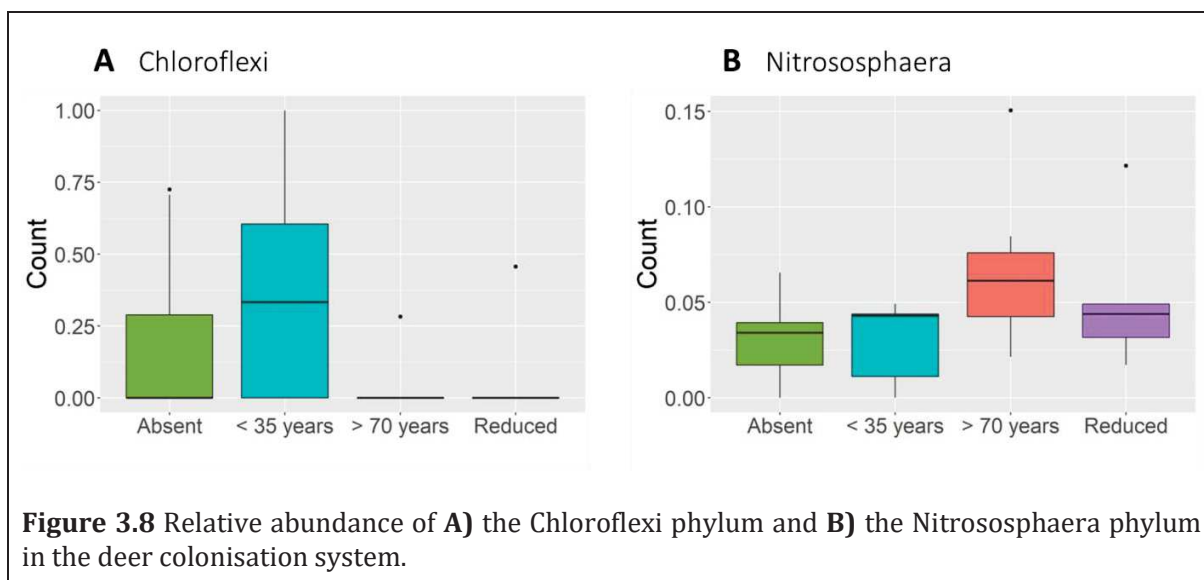
This regulation of soil prokaryotic composition by soil compaction confirms that the observed differences in the soil prokaryotic community structure between the different islands is due to deer presence, rather than to an island effect. The lack of difference in the soil prokaryotic

community structure observed in the exclosures system, where soil penetration resistance appears as being strongly alleviated by deer exclusion, is therefore surprising (Figure S3.6). The small scale of the exclosures may not allow for a marked contrast in soil prokaryotic community between the inside and outside of the exclosures. However, we also found that prokaryotic communities from the islands colonised for less than 35 years were similar to the communities from un-colonised islands, although their level of compaction was higher (Figure S3.6). Together these results suggest that it is not only the level of compaction, but also the length of time compaction lasted, that plays a role in restructuring the soil microbial community. Previous studies have found that short-term compaction of the forest floor had no effect on soil microbial community structure (Kissling et al., 2009; Mariani et al., 2006). Furthermore, changes in soil α and β microbial diversity have also been linked with time since compaction disturbance (Hartmann et al., 2014).

3.4.3 – Modifications of the soil prokaryotic community by deer may affect ecosystem functioning

In our study, investigation of the potential functional capabilities of the soil prokaryotic community across the different treatments suggested that the modifications induced by long-term deer presence may have consequences on ecosystem functioning. Because it assumes that phylogenetically close micro-organisms share similar functions, Predictive Metagenomic Profiling (PMP) can have inaccuracies and its results need to be interpreted with caution. Nevertheless, PMP gives valuable insights to the functional potential of the microbiome investigated (Aßhauer et al., 2015; Langille et al., 2013). Correlation of the photosynthesis metabolism with samples from the islands without deer and from islands colonised for less than 35 years was probably due to the higher level of the photosynthetic genus from the phylum chloroflexi observed on these islands (Figure 3.8A). Reduction in the abundance of this phylum may reflect a decrease in light accessibility to the soil surface because of the thick layer of bryophytes on the islands colonised by deer for more than 70 years. Sulfur metabolism was correlated with long-term deer presence (Figure 3.6B). Bacteria within this functional guild can be associated with anaerobic conditions (Overmann and van

Gemerden, 2000), and a closer look at the functional orthologs (or “KO” for KEGG Orthology) involved in the sulphur metabolism indeed revealed functional orthologs related to the anaerobic sulphur respiration among the KO differentially represented between the deer treatments (e.g. anaerobic dimethyl sulfoxide reductase subunit A and subunit B). Correlation of the sulphur metabolism with long-term deer presence could, therefore, reflect a lower oxygen level due to soil compaction by deer trampling. Nitrogen metabolism was correlated with long-term deer presence and reduced deer density (Figure 3.6B). A focus on the nitrification functional guild showed that members of the Nitrososphaera family were indeed more abundant on these islands (Figure 3.8B). Long-term addition of ammonium to soil due to urine deposition by deer may have selected this nitrifier family. An increased nitrification activity, i.e. the oxidation of ammonium to nitrate, in soils from islands colonised by deer could explain the absence of signal on soil NH_4 and NO_3 observed in our study. Input of NH_4 by urine deposition would indeed be quickly transformed into NO_3 by the nitrifiers, and the produced nitrate would be either assimilated by plants or leached to the hydrosphere. As nitrification activity is responsible for soil acidification, this increase in nitrifier abundance may also explain the lower pH found in soils from islands colonised by deer for more than 70 years (Bolan et al., 1991). Finally, modification in the prokaryotic community structure by long-term deer presence was correlated with pathways involved in carbon metabolism such as carbon fixation and carbohydrate metabolism pathways. This finding suggests that the observed restructuring of soil microbial community by long-term deer presence has consequences on carbon cycle. A decomposition study realised on the same islands indeed found that soil microbial decomposers from an island without deer had a better ability in decomposing carbon than soil microbial decomposers from an island colonised by deer for more than 70 years (Chapter 2, Chollet, Maillard et al. (2019)). Our results therefore suggest that long-term modification of the ecosystem by deer could have important implications for carbon and nutrient cycling.



3.5 – Conclusion

We found that aboveground effects of deer were consistent among the three systems, reflecting a temporal evolution of the vegetation to deer presence that was consistent with plant growth duration and requirements. The effects of deer on soil properties and organisms were time-dependent, and were driven by waste deposition and trampling in the short-term and by trampling and vegetation shift in the long-term. Detection of these changes in soil chemical and biological properties by deer therefore required these long-term studies which are currently lacking in the literature. Conversely to our prediction, soil prokaryotic α and β diversity were not affected by short-term changes in deer pressure. Changes in soil properties to long-term deer presence were associated with a lower soil prokaryotic α diversity and a change in soil prokaryotic composition, consistently with our prediction. However, the dispersion of the prokaryotic communities on the island without deer and with deer for more than 70 years was similar, refuting our hypothesis of homogenisation of the soil prokaryotic diversity in the long term. The response of the soil prokaryotic communities to changes in the soil properties by deer was mainly linked to physical compaction of the soil through trampling. These changes were associated with prokaryotic functional guilds involved in carbohydrate degradation and nitrogen and sulphur

metabolism, which suggests that long-term deer presence may have important implications for soil carbon and nutrient cycling.

3.6 – Supplementary tables and figures

Table S3.1 – 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 |

Table S3.2 Results of the statistical tests in each system and for each variable. Col. = deer colonisation system, Exc. = deer enclosure system and Cull = recent deer cull system. Wilcoxon test, paired Wilcoxon test and F1-LD-F1 nparLD test were used for the three systems respectively. Values in bold and blue correspond to significant p-value < 0.05 that were attributed to a deer effect. Values in bold and black correspond to significant p-value < 0.05, but that were not attributed to any deer effect. Values in bold and green correspond to marginally significant p-value < 0.1 that were attributed to a deer effect.

| Variables | Statistic | | | p-value | | |
|---------------------------|-----------|--------|--------|---------------------------|---------------------------|---------------|
| | 1/Col. | 2/Exc. | 3/Cull | 1/Col. | 2/Exc. | 3/Cull |
| <i>Vegetation</i> | | | | | | |
| Shannon vasc. | 114 | 183 | 3.78 | 0.04 | 7e⁻⁰⁵ | 0.0097 |
| Conifer | 29.5 | 117.5 | - | 0.01 | 0.38 | - |
| Forb | 78 | 145.5 | 6.30 | 0.98 | 0.001 | 0.0043 |
| Graminoid | 17.5 | 11.5 | 1.32 | 5.5e⁻⁰⁴ | 0.74 | 0.27 |
| Pteridophyte | 147 | 62 | 1.34 | 1.3e⁻⁰⁴ | 0.08 | 0.26 |
| Shrub | 154 | 179 | 2.26 | 3e⁻⁰⁵ | 2.1e⁻⁰⁴ | 0.11 |
| Sannon bryo. | 30 | 114 | 4.42 | 0.01 | 0.47 | 0.0035 |
| Bryophyte | 9.5 | 13.5 | 5.20 | 2.3e⁻⁰⁴ | 0.002 | 0.0049 |
| <i>Soil</i> | | | | | | |
| Penetrometer | 0 | 0 | 0.45 | 3e⁻⁰⁵ | 1.4e⁻⁰⁴ | 0.59 |
| SWC | 5 | 127 | 0.15 | 1e⁻⁰⁵ | 0.21 | 0.91 |
| pH | 152 | 107 | 4.39 | 5e⁻⁰⁵ | 0.64 | 0.0067 |
| %C | 50 | 51 | 1.02 | 0.15 | 0.08 | 0.39 |
| %N | 83 | 73 | 1.87 | 0.77 | 0.4 | 0.12 |
| C:N | 56 | 95 | 0.91 | 0.27 | 1 | 0.44 |
| Total P | 118 | 127 | 2.46 | 0.02 | 0.21 | 0.057 |
| NH4 | 70 | 70 | 2.32 | 0.73 | 0.33 | 0.077 |
| NO3 | 61.5 | 37 | 1.65 | 0.30 | 0.58 | 0.19 |
| Depth org. horizon | 149 | - | - | 9e⁻⁰⁵ | - | - |
| Bulk density | 9 | - | - | 4.3e⁻⁰⁵ | - | - |
| C stock | 125 | - | - | 7.5e⁻⁰³ | - | - |
| N stock | 131 | - | - | 2.2e⁻⁰³ | - | - |

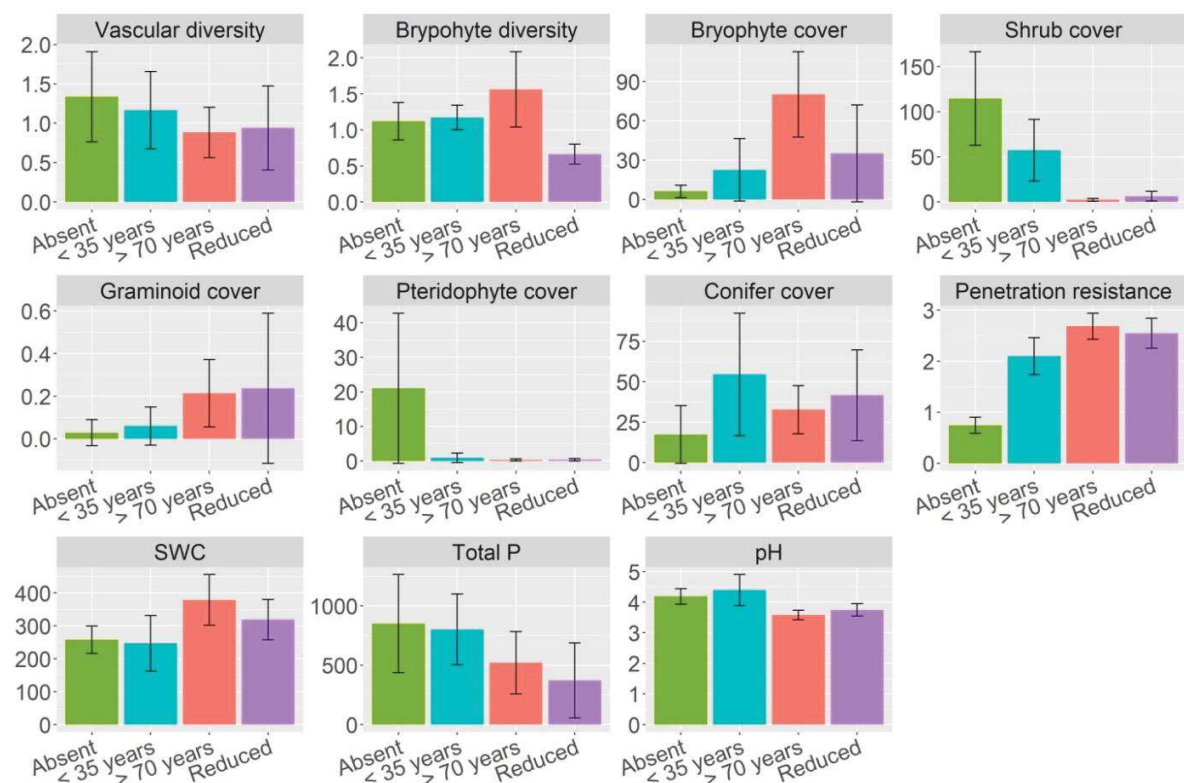


Figure S3.2 Plant and soil variables that differed significantly between treatments without deer and the colonisation longer than 70 years in the deer colonisation system. Plant diversities are represented with the Shannon index. Plant covers are expressed in %. Penetration resistance is expressed in kg/cm². Soil Water Content (SWC) is expressed in percent. Total phosphorus (P) is expressed in µg P/g dry soil.

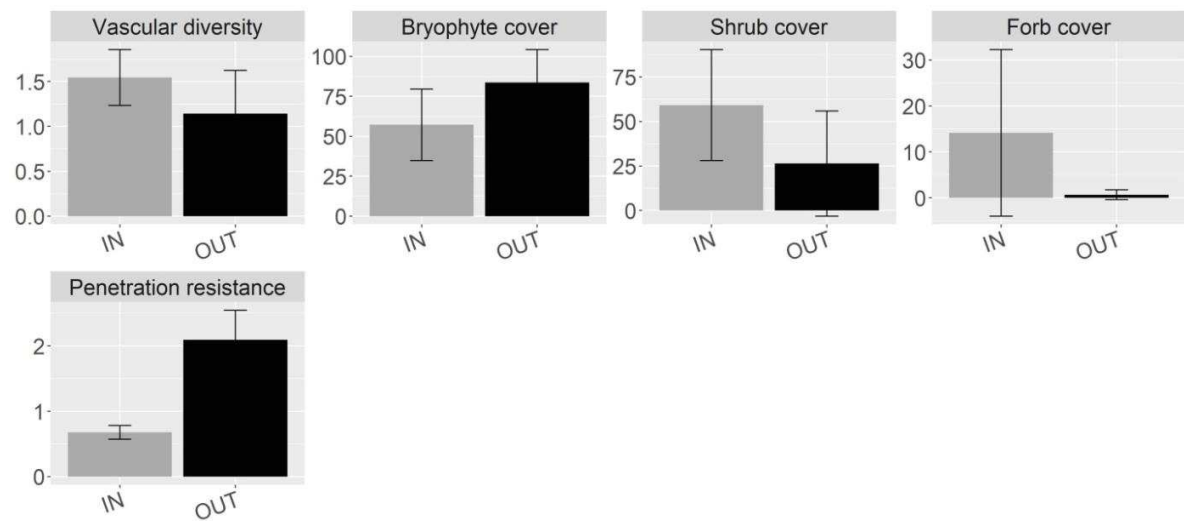


Figure S3.3 Variables found to be significantly different between inside (IN) and outside (OUT) enclosures in the deer enclosures system. Units are the same as in Figure S3.2

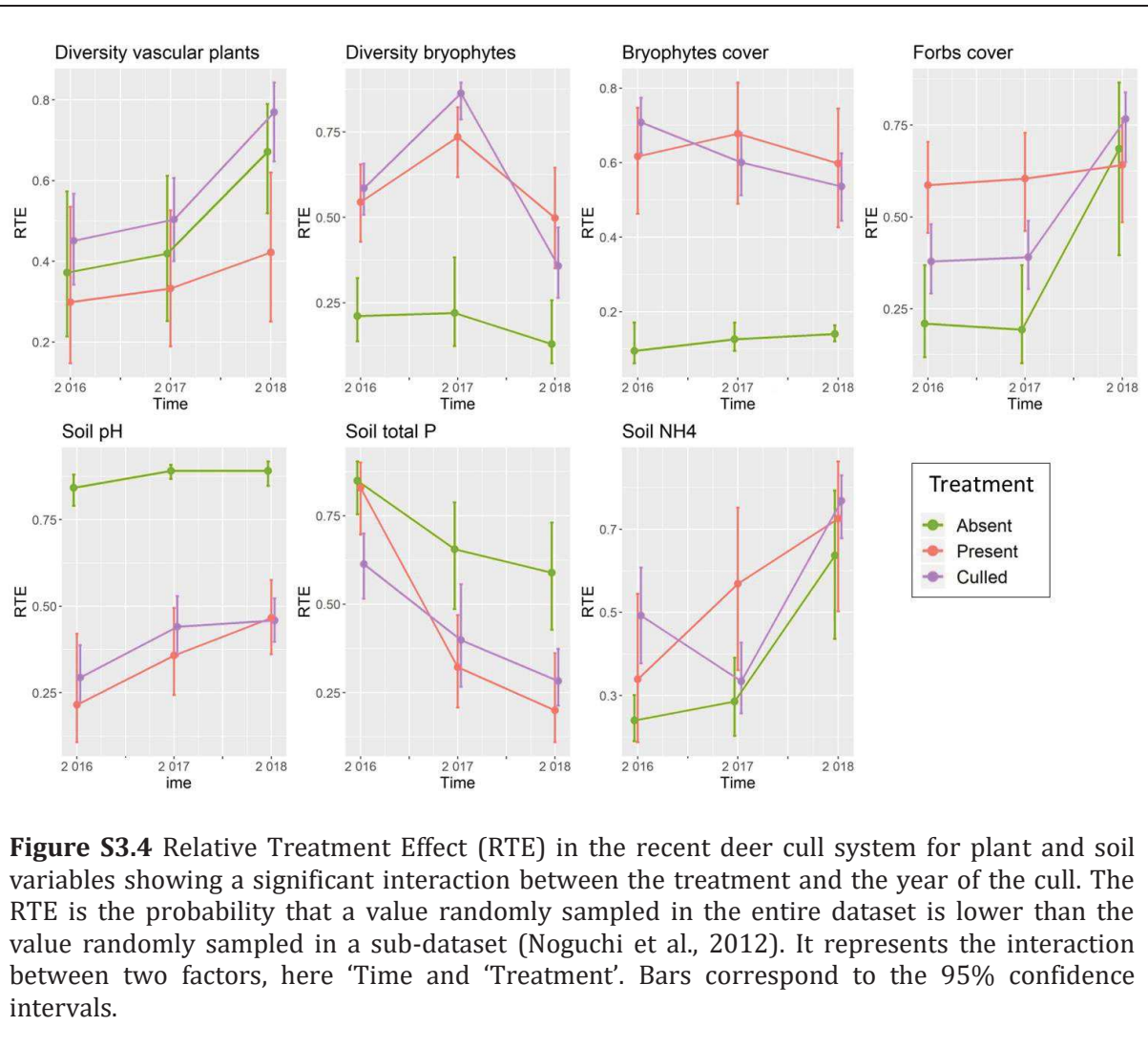


Figure S3.4 Relative Treatment Effect (RTE) in the recent deer cull system for plant and soil variables showing a significant interaction between the treatment and the year of the cull. The RTE is the probability that a value randomly sampled in the entire dataset is lower than the value randomly sampled 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.

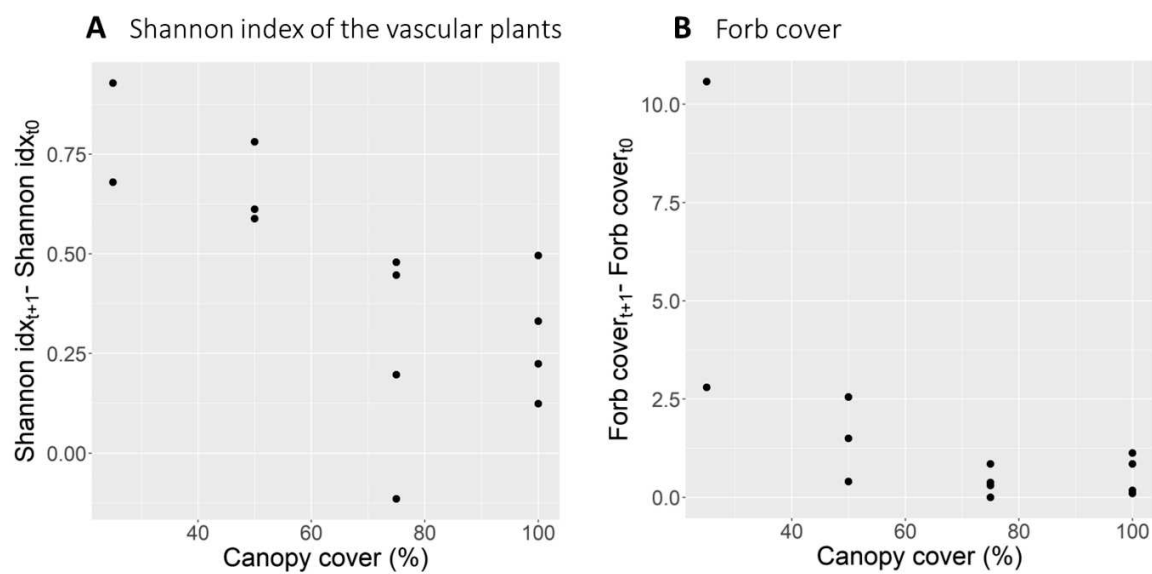
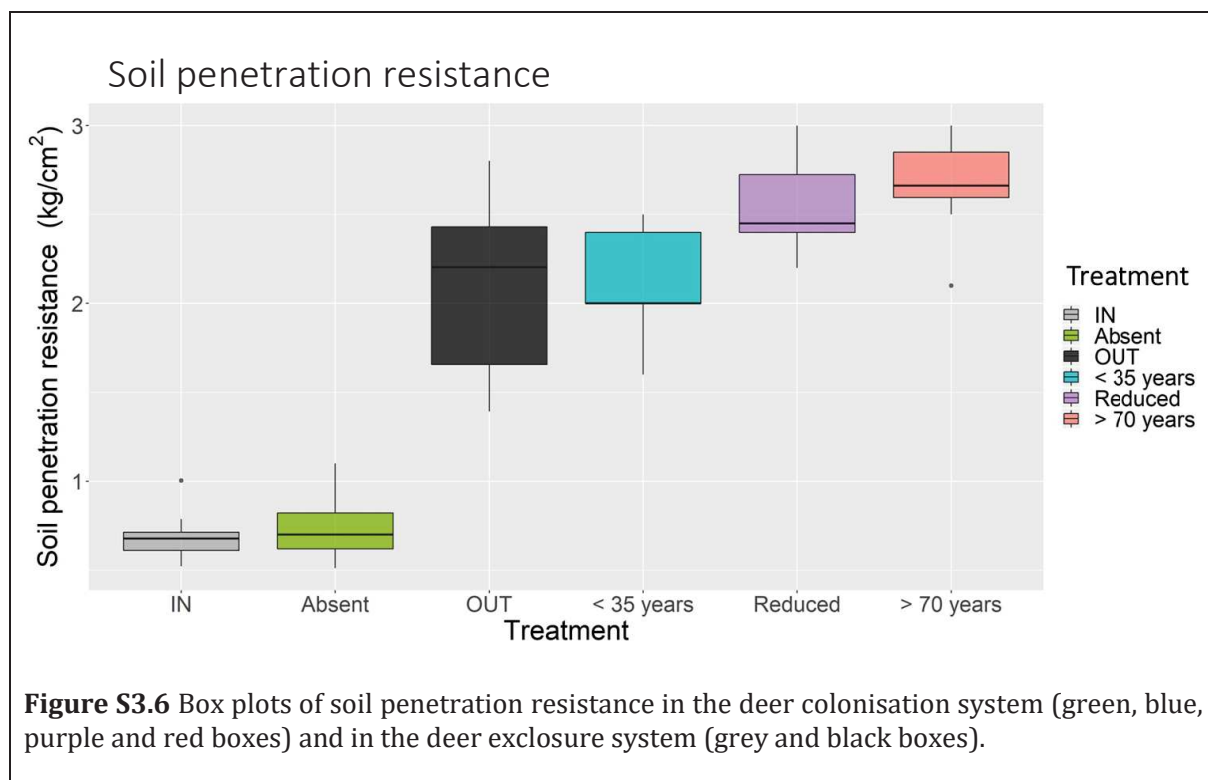


Figure S3.5 A) Relationship between the difference post and pre-cull (1 year – 1 month) in Shannon index of the vascular plant species with plot canopy cover. **B)** Relationship between the difference post and pre cull (1 year – 1 month) in forb cover with plot canopy cover.



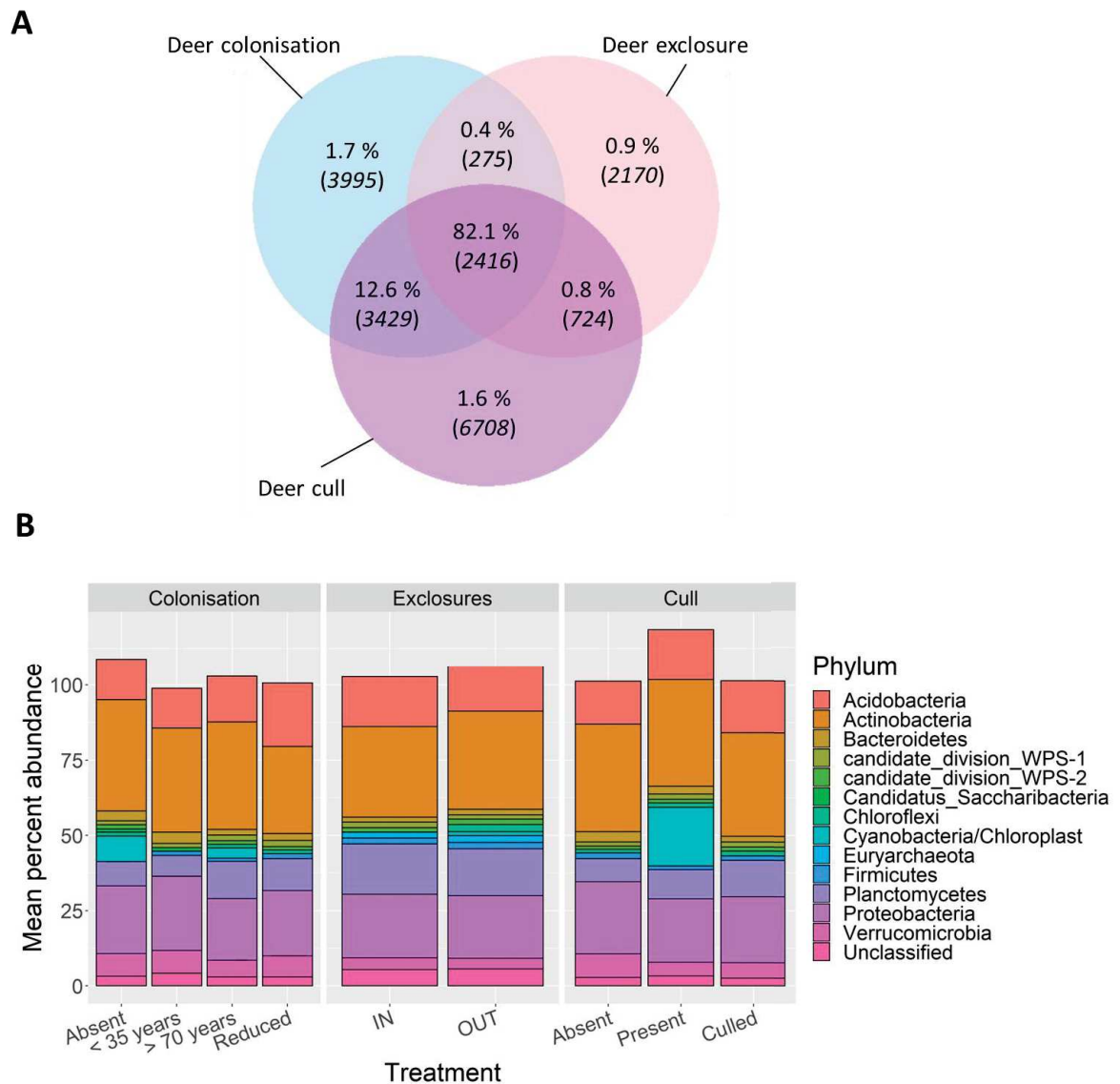


Figure S3.7 A) Venn diagram representing the percent of shared OTUs among systems. Values in bracket correspond to the number of reads. Percent values include the abundance of each reads. **B)** Average proportion of phyla per treatment and per system. Only dominant phyla (proportion > 1%) are represented.

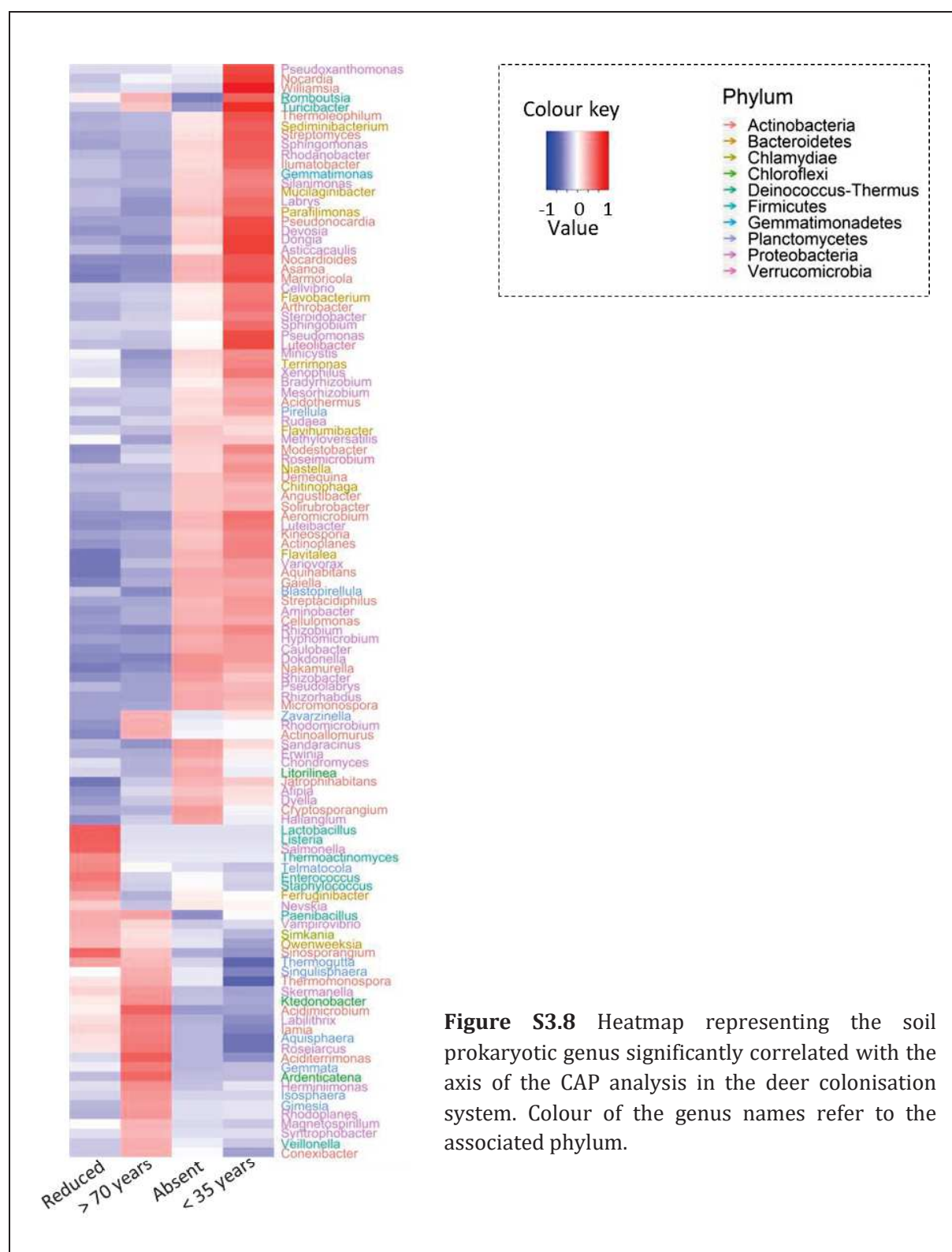
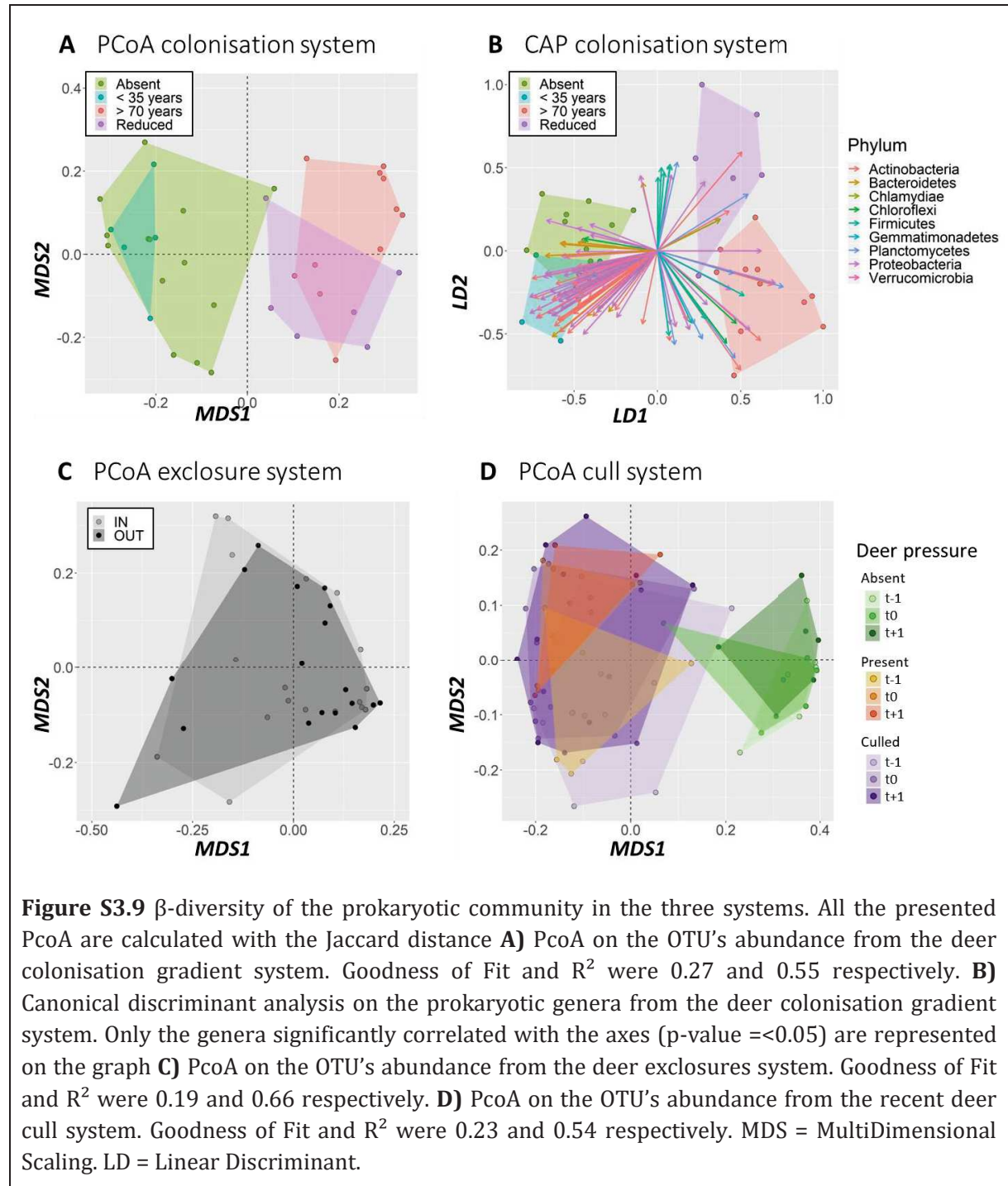
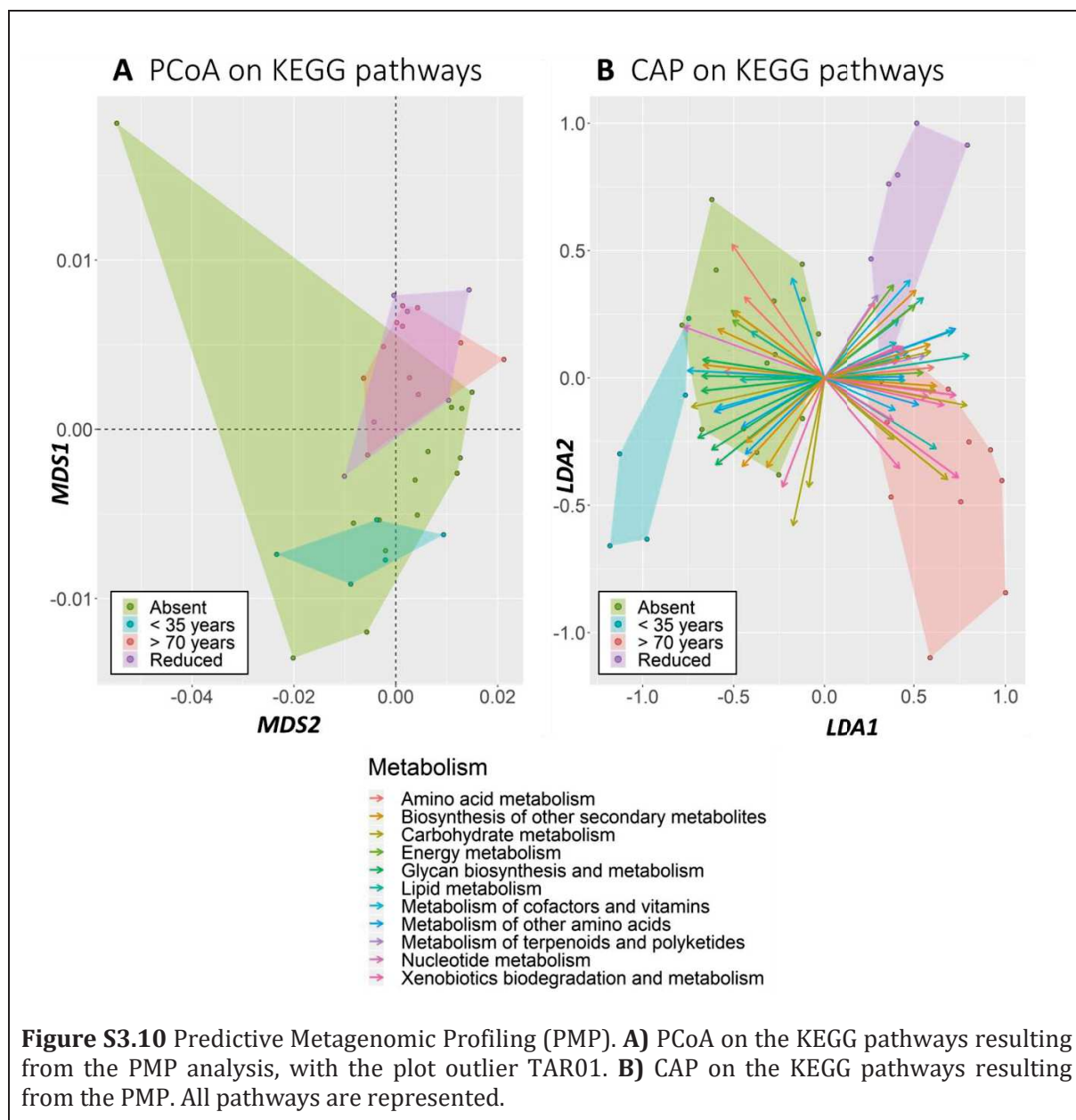


Figure S3.8 Heatmap representing the soil prokaryotic genus significantly correlated with the axis of the CAP analysis in the deer colonisation system. Colour of the genus names refer to the associated phylum.





Chapter 4: Abundant deer do not change nitrogen cycling processes in a temperate forest.

Maillard Morgane, Martin Jean-Louis, Christoph Müller, Chollet Simon, Anne Jansen-Willems & Grayston Sue

ABSTRACT

Nitrogen is a fundamental element required in the functioning of forest ecosystems. Recent overabundance of deer in North American and European forests has appeared as a potential factor influencing nitrogen concentrations and availability in soil. Deer may affect the nitrogen cycling directly through waste deposition and trampling, and indirectly through the reductions in litter quality and quantity. The effects of deer on nitrogen cycling in temperate forests have shown idiosyncratic patterns. Varying responses of nitrogen cycling to deer presence might be the result of a time-dependence of the soil response to deer pressure, with short-term changes being driven by direct interactions (i.e. waste deposition and trampling) and long-term changes being driven by indirect interactions (i.e. vegetation changes).

To disentangle the influence of deer on the gross rates of different nitrogen cycling processes in temperate forests, we used the ^{15}N -tracing method and the algorithm developed by Müller et al (2004). We compared three study systems contrasting in time since deer presence and removal. This chapter presents the results obtained for a subset of the plots used in the system, comparing an ecosystem without deer and an ecosystem colonised by deer for more than 70 years. We found that the presence of deer did not change gross nitrogen cycling rates and the net production of ammonium and nitrate. Instead, differences in nitrogen cycling rates reflected plot specificities in soil and vegetation properties.



Mesocosm in the ^{15}N -isotope tracing experiment

4.1- Introduction

Nitrogen (N) is a fundamental element required in the functioning of forests. As a constituent of proteins, it is essential to any organisms in order to sustain homeostasis and growth. Nitrogen is limiting to net primary production in many terrestrial ecosystems including temperate forests (Vitousek and Howarth, 1991). As a result, any changes to the availability of this element may have important consequences for the structure of these ecosystems (Nohrstedt, 2001; Strengbom and Nordin, 2008). Recent deer overabundance in North American and European forests has been shown to drastically change modify the structure of temperate forests through modification of plant abundance and diversity and simplification of animal communities, such as birds and insects (Cardinal et al., 2012; Côté et al., 2004; Martin et al., 2010; Stockton et al., 2005). Through direct and indirect pathways, deer may also affect soil properties and functioning including nutrient cycling, particularly nitrogen (Pastor et al., 2006).

The nitrogen cycle is complex and involves many steps and actors. In temperate forests, the main source of nitrogen reaching the soil is in organic form and is provided by aboveground litter and detritus. Two pools of organic nitrogen can be distinguished based on their dynamics in the soil. The labile pool is composed of simple organic molecules easily degradable, such as nitrogen monomers, and is mostly decomposed by prokaryotes (Müller et al., 2004). The recalcitrant pool is composed of more complex molecules, such as structural carbohydrates and plant defense compounds that require more energy to be degraded, and are mostly decomposed by fungi (Müller et al., 2004). Labile and recalcitrant organic nitrogen compounds are mineralised by soil microbes into ammonium (NH_4). Specific narrow functional guilds of bacteria and archaea can then oxidise ammonium into nitrite (NO_2), then nitrate (NO_3), to produce energy, a process called nitrification. Heterotrophic nitrification directly from recalcitrant organic nitrogen can also occur and is mostly conducted by specific species of fungi in acidic soils (Johnsrud, 1978; Stroo et al., 1986). The NH_4 and NO_3 produced are assimilated and used by plants as their preferential source of nitrogen. Under anaerobic conditions, chemoorganotrophic microbes can use and reduce

NO_3 into NH_4 by anaerobic dissimilatory reduction of NO_3 to NH_4 . When the concentration of nitrogen in the soil is low, microbes can retain NH_4 and NO_3 in a process called immobilisation. Microbial immobilisation restricts nitrogen availability to plants, initiating a competitive relationship between plants and microbes. In addition, soil NO_3 can be depleted through both denitrification, a microbial process transforming NO_3 into gaseous di-nitrogen, and leaching. Ammonium can also be made unavailable to both plants and microbes through abiotic immobilisation, which is due to the adsorption of these nutrients to the soil matrix, specifically to negatively charged clays and organic matter (Kowalenko and Cameron, 1978; Nommik and Vahtras, 1982).

Deer can alter nitrogen cycling through three main mechanisms. **1) Deer can change the quality and quantity of the litter entering the soil** through preferential consumption of more nutrient-rich (low C:N) palatable plant species, which are replaced by poor quality (high C:N) herbivore-resistant species. This reduction in nutrient-rich plant species can result in lower inputs of organic nitrogen to the soil that can slow down the microbial activity involved in nitrogen cycling (Pastor et al., 1993). In addition, less palatable plant species are associated with a higher content of structural carbohydrate and anti-herbivore compounds, such as phenols, which can complex with nitrogen and reduce its availability to micro-organisms (Palm and Sanchez, 1991; Schmidt-Rohr et al., 2004). Along with a decrease in litter inputs and belowground root exudation, due to reduced plant biomass with herbivory, these mechanisms may reduce decomposition processes (Chapter 2) and retard nitrogen cycling (Pastor et al., 1993). **2) Deer can induce a shortcut to nutrient cycling** through waste deposition, increasing cycling of organic nitrogen through inorganic pathways. By excreting urine, deer release the ingested organic ammonium directly into a mineral ammonium form. This by-passes the organic nitrogen mineralisation steps in the soil. Conversely to the first mechanism of alteration of N cycling processes by deer, this second mechanism could speed up decomposition and nitrogen cycling (Bardgett et al., 1998; Molvar et al., 1993). Similarly, dung is a digested form of plant litter containing easily decomposable nitrogen and that can stimulate nitrogen mineralisation (Molvar et al., 1993 and Chapter 2) **3) Deer can both accelerate and decelerate soil nitrogen cycling through soil physical modifications** (Schrama

et al., 2013b). Soil compaction due to trampling and increased soil exposure due to plant removal by deer can modify soil temperature, water content and oxygen levels (Cambi et al., 2015). Depending on the initial soil water and mineral content, such modifications can have positive, negative, or no effects on nitrogen mineralisation, with extreme soil moisture content (very wet or very dry) and high soil clay content favouring negative effect of deer on nitrogen mineralization and decomposition, and intermediate soil moisture and sandy soils favouring positive and neutral effect, respectively (Schrama et al., 2013b, 2013a). The multiplicity of interactions through which deer can alter nitrogen cycling makes it difficult to predict the net effect deer will have on nitrogen availability in temperate forests. Furthermore, these effects may act at different levels of the nitrogen cycle, hence compensating for, or strengthening each other. Understanding of the influence of each pathway mechanism on nitrogen cycling is required to predict the effect of deer on nitrogen availability in temperate forests.

To disentangle the influence of deer on gross rates of different nitrogen cycling processes in temperate forests, we used the ^{15}N -isotope tracing method and algorithm developed by Müller et al. (2004, 2007). This method allows measurement of the production and immobilisation of ammonium and nitrate through both labile and recalcitrant organic N pools, and under the regulation of both microbial and abiotic factors. We compared gross rates of soil nitrogen cycling between a temperate forest without deer and a temperate forest with an abundant deer population. For this, we used two islands located in the Canadian archipelago of Haida Gwaii. We predicted that the reduction in litter quality on islands with deer due to replacement of nutrient-rich with nutrient-poor plant species will result in a preferential cycling of nitrogen through the organic N recalcitrant pool. Reduction in nitrogen concentrations in litter should further result in higher microbial immobilisation rates. In addition, soil ammonium transformation rates through processes such as nitrification should be enhanced by the ammonium input due to waste deposition. Potential reductions in soil oxygen levels by trampling of deer should favour anaerobic processes such as DRNA. In forests, reduction in nitrogen availability has been shown to prevail over the positive effect of dung and urine deposition (Pastor et al., 2006). Therefore, we predict that

net production of bioavailable ammonium and nitrate should decrease in soils on islands with deer as a result of an enhanced competition between soil microbes and plants.

4.2- Material and Method

4.2.1 - Sites description

We conducted the sampling on the archipelago of Haida Gwaii, British Columbia, Canada. Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) were first introduced on these islands in 1878 by Europeans for hunting. In the absence of natural predators, deer populations increased rapidly, modifying the aboveground ecosystem (Allombert et al., 2005a, 2005b; Martin et al., 2010). The presence of islands varying in browsing histories offered a remarkable opportunity for the long-term accumulation of empirical and experimental data on the above-ground consequences of deer herbivory. This 30-year-long accumulation of data provided a situation of choice to study the impact of deer on soil processes. The climate of this archipelago is cool, temperate and oceanic. Haida Gwaii is covered with coastal temperate rainforest that is dominated by western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Sitka spruce (*Picea sitchensis*) at low elevations (< 600m). Soil bedrock geology is volcanic and sedimentary, together with intrusions of granitic rock (Sutherland Brown, 1968). Soil type is organic and is classified into the Folisol, Podzolic or Gleysolic orders (Banner, 2014; Pojar and Banner, 1984). We sampled soil from two islands varying in deer presence. We used a subset of plots previously used to characterise the effect of deer on vegetation and soil properties (Chapter 3). We set four plots on Low island, which had never been colonised by deer, and four plots on Louise island, which had been colonised by deer for more than 70 years (Figure S1.1). Each plot measured 20 m x 20 m.

4.2.2 - Vegetation characteristics

We surveyed the percent cover of the vascular plant species in each plot using a modified Braun-Blanket scale (Braun-Blanquet, 1932) (Table S4.1). We randomly placed a 20 x 20 cm

quadrat 20 times on the forest floor within each plot, and surveyed bryophyte species in each of these quadrats. Percent cover of each species was further calculated as the number of occurrences of the species divided by 20 and multiplied by the total bryophyte cover on the plot. We assigned a percent cover value of 0.01% to the bryophytes present on the plot, but that never occurred in the quadrat. We calculated the Shannon index of both the vascular and the bryophyte community as a measure of their α diversity.

4.2.3 - Soil physical properties

We determined soil moisture content after drying 2g of soil at 105°C during 24h, with three replicates per plot. We measured soil penetration resistance with a hand-held penetrometer and values were used as a proxy of soil compaction. We randomly measured and averaged 50 values within each plot to better represent spatial heterogeneity.

4.2.4 - Soil chemical properties

We sampled the soil F horizon using a soil probe 2.5 cm in diameter and 30 cm in depth. We sampled and pooled soil from at least 500 cores per plot, until we reached an approximate sample weight of 1kg. We then gently mixed and homogenized the soil samples by hand, and discarded debris larger than 5 mm. We kept samples at 4°C prior to chemical analyses. We measured soil pH in a 0.01 M CaCl₂ solution using a 1:10 (air dried soil:solution) ratio. We determined soil total carbon and nitrogen content from 3mg of freeze-dried and ground soil using an Elementar Vario El Cube Analyzer. We extracted soil nitrate (NO₃) and ammonium (NH₄) in a 2M KCl solution using a 1:10 (fresh soil:solution) ratio. We shook the solution on a mechanical shaker for one hour, and filtered it through a 12.5 cm fiberglass G6 microfilter. We determined NO₃ and NH₄ concentrations by colorimetry using the phenol-hypochlorite reaction method for NH₄ quantification (Weatherburn, 1967) and the VCl₃ reduction method for NO₃ quantification (Hood-Nowotny et al., 2010).

We performed all the chemical measurements in triplicate.

4.2.5 - ¹⁵N-isotope tracing experiment

We measured the gross rates of nitrogen transformation with a ¹⁵N-isotope tracing experiment following the procedure of Masse et al. (2016) and Müller et al. (2004). We performed this experiment on fresh soil stored at 4°C no longer than 1.5 months after sampling. For each plot, we filled six jars with 120g of fresh soil. We labelled the soil from three of the jars with 4mL of a ¹⁵NH₄-NO₃ solution at 61.5 atom % excess. We labelled the soil from the three remaining jars with 4mL of NH₄-¹⁵NO₃ at 61.5 atom % excess. Both labels were applied evenly in the jar using a needle and an application rate of 1.86 µmolN/g of fresh soil. We gently homogenized soil samples to ensure the equal repartition of the solution. We measured NH₄ and NO₃ concentrations for each jar 3 h, 24 h, 48 h and 288 h after labelling. ¹⁵N content in both the ammonium and nitrate pools was determined on filtrates obtained from NH₄ and NO₃ extraction. We placed 50 mL of each filtrate in a gas-tight container along with an acidified filter trapped in teflon tape. We then added 0.2 g of MgO to each container. We shook the containers for 72h using a mechanical shaker. We then removed the acid trap and air-dried the filter in a dessicator. We then added to each container a second acid trap and 0.2g of Devarda's alloy and shook the containers for 72h before removing the acid traps. Once dried, each filter was encapsulated into a tin cup and sent to the Stable Isotope Facilities at the University of Saskatchewan for ¹⁵N content measurement. We measured the excess of ¹⁵N atom % of each filter with a Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer.

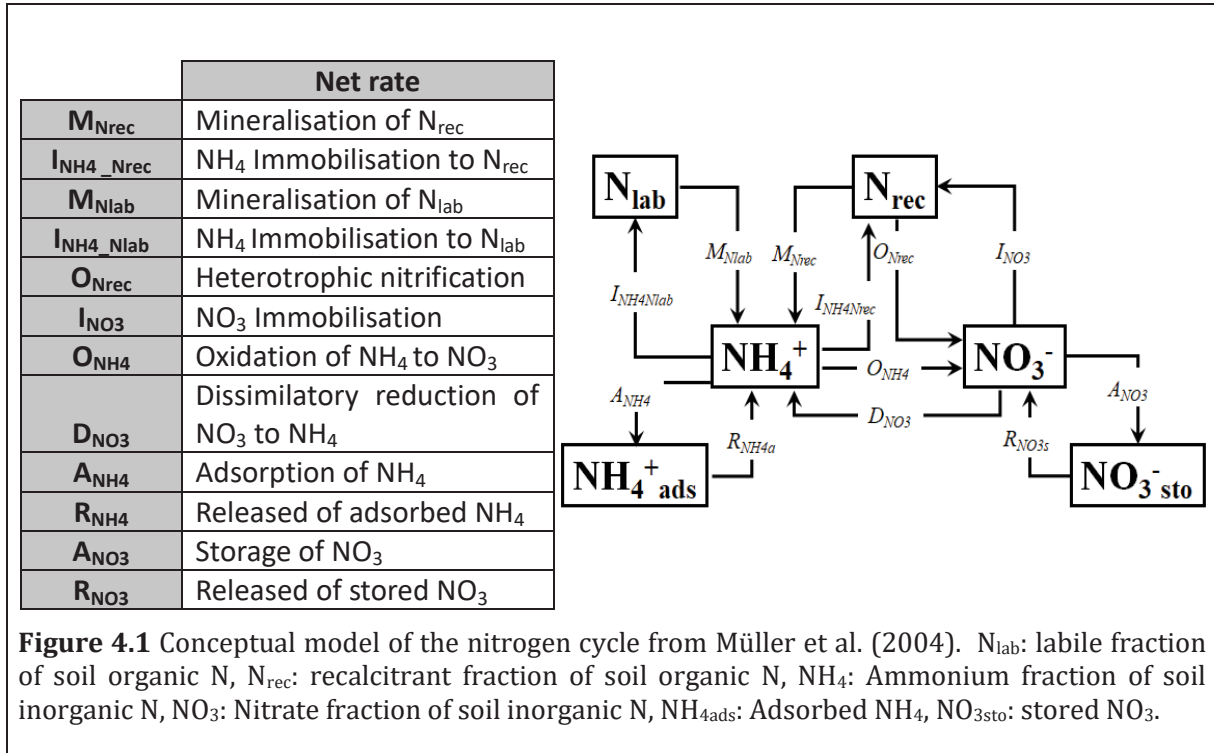
4.2.6 - Calculation and statistical analyses

We used a ¹⁵N-tracing model developed by Müller et al. (2007) to calculate the gross nitrogen transformation rates (Figure 4.1). Transformation rates presented in the results correspond to the average rates over the entire incubation period. We calculated the net production of NH₄ (Net_{NH4}) and NO₃ (Net_{NO3}) as:

$$Net_{NH4} = (M_{Nlab} + M_{Nrec} + D_{NO3} + R_{NH4}) - (I_{NH4-Nlab} + I_{NH4-Nrec} + O_{NH4} + A_{NH4})$$

$$Net_{NO_3} = (O_{NH_4} + O_{N_{rec}} + R_{NO_3}) - (I_{NO_3} + D_{NO_3} + A_{NO_3})$$

We assessed differences between treatments with a Wilcoxon test using the function `wilcox.test` from the package `stats` on R (R Core Team, 2018).



4.3 – Results

4.3.1 – Effect of deer on soil properties

Both vegetation and soil variables measured were fully discriminated between plots from islands with deer present and deer absent, as shown on the first axis of the PCA (Figure 4.2A and B). Aboveground, plots on the island without deer were characterised by a higher abundance of shrubs and pteridophytes, and a higher vascular plant diversity (Figure 4.2A). Conversely, plots on the island colonised by deer were characterised by a higher abundance of graminoids and conifers. The plots LOU03 and LOU05 were characterised by a higher abundance of bryophytes, whereas LOU01 and LOU02 were characterised by higher

bryophyte diversity. Belowground, plots on the island without deer were characterised by higher pH, organic horizon depth and phosphorus content (Figure 4.2B). The plot LOW05 was further characterised by high nitrogen and inorganic nitrogen content, a peculiarity that was not found on the other plots from the same island. Plots on the island colonised by deer were characterised by higher penetration resistance. Plots from the island with deer and plot LOW05 from the island without deer had a higher soil moisture content.

Assessment of soil nitrogen forms confirmed the peculiarity of the plot LOW05. Total soil nitrogen was not different between the island without deer and the island colonised by deer ($W = 11$ and $p\text{-value} = 0.49$). Soil on LOW05 had the highest nitrogen content. Soil ammonium was not different between the island without deer and the island colonised by deer (Figure 4.2, $W = 10$ and $p\text{-value} = 0.69$). Soil ammonium concentration on LOW05 was on average 5.5 times higher than on the other plots. Evidence of nitrate was only found on the island without deer on plots LOW04 and LOW05, with nitrate concentration being 11.8 times higher in LOW05 than in LOW04 (Figure 4.2).

4.3.2 – Effect of deer on gross N transformation rates

Ammonification – Ammonification rates from the labile organic nitrogen pool was not significantly different between island with or without deer (rate $M_{N\text{-lab}}$ on Figure 4.3, $W = 14$ and $p\text{-value} = 0.11$). The plot LOW05 had the highest $M_{N\text{-lab}}$ rate, being on average 4.8 times higher than the other plots. Ammonification rates from the recalcitrant organic nitrogen pool was not significantly different between island with or without deer (rate $M_{N\text{-rec}}$ on Figure 4.3, $W = 10$ and $p\text{-value} = 0.69$). The plot LOW01 had the highest $M_{N\text{-rec}}$ rate, being on average 2.7 times higher than the other plots. DNRA was detected on all the plots of the island colonised by deer, and on the plots LOW01 and LOW05 on the island without deer (rate D_{NO_3} on Figure 4.3). This rate was particularly high on the plot LOW05 where it reached an average value of $0.60\mu\text{g/g/day}$, which is 1.8 times higher than on the other plot where DNRA occurred. Overall, DNRA was not significantly different between island with or without deer ($W = 5$ and $p\text{-value} = 0.47$).

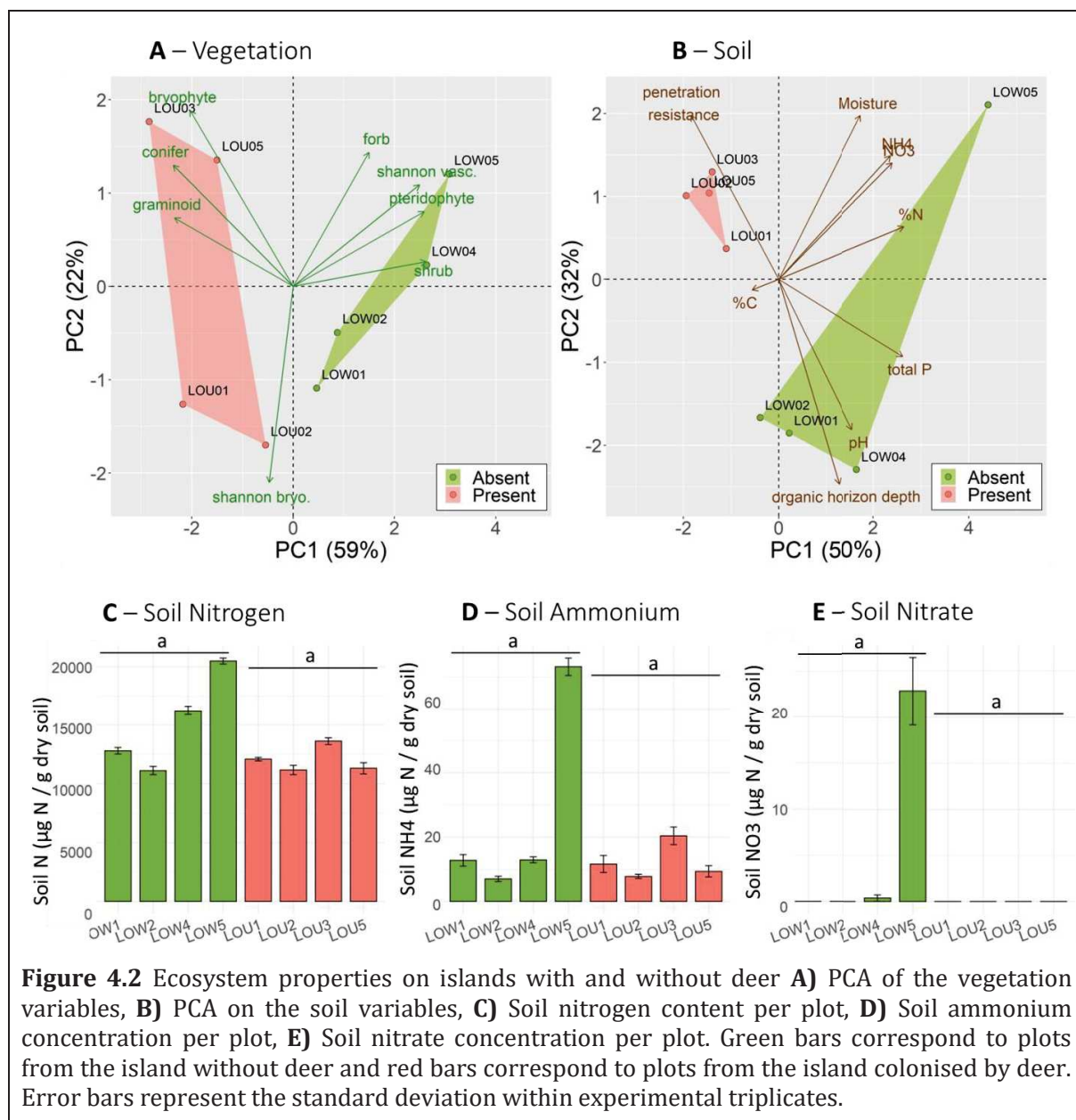


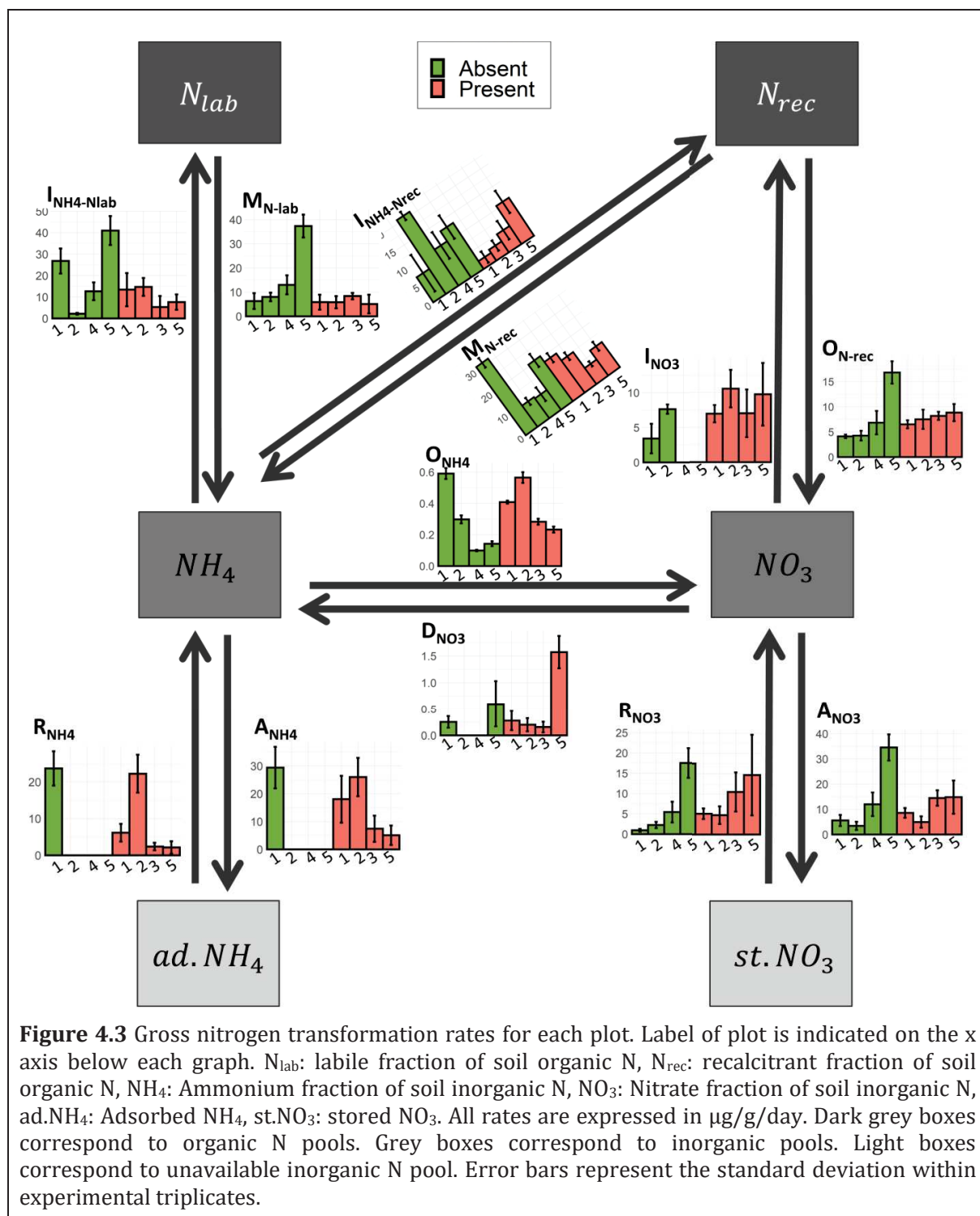
Figure 4.2 Ecosystem properties on islands with and without deer **A)** PCA of the vegetation variables, **B)** PCA on the soil variables, **C)** Soil nitrogen content per plot, **D)** Soil ammonium concentration per plot, **E)** Soil nitrate concentration per plot. Green bars correspond to plots from the island without deer and red bars correspond to plots from the island colonised by deer. Error bars represent the standard deviation within experimental triplicates.

Nitrification – Oxidation of NH_4 by autotrophic nitrification was not significantly different between island with or without deer, and had an average value of $0.33 \mu\text{g/g/day}$ across plots (rate O_{NH_4} , Figure 4.3, $W = 6$ and $p\text{-value} = 0.69$). Heterotrophic denitrification from recalcitrant organic nitrogen occurred at an average rate of $7.89 \mu\text{g/g/day}$ across plots, and was not significantly different between island with or without deer (rate O_{Nrec} , Figure 4.3, $W = 5$ and $p\text{-value} = 0.49$). The plot LOW05 had a particularly higher O_{Nrec} rate, which was on

average 2.8 times higher than on the other plots. On average, heterotrophic nitrification was nine times higher than autotrophic nitrification.

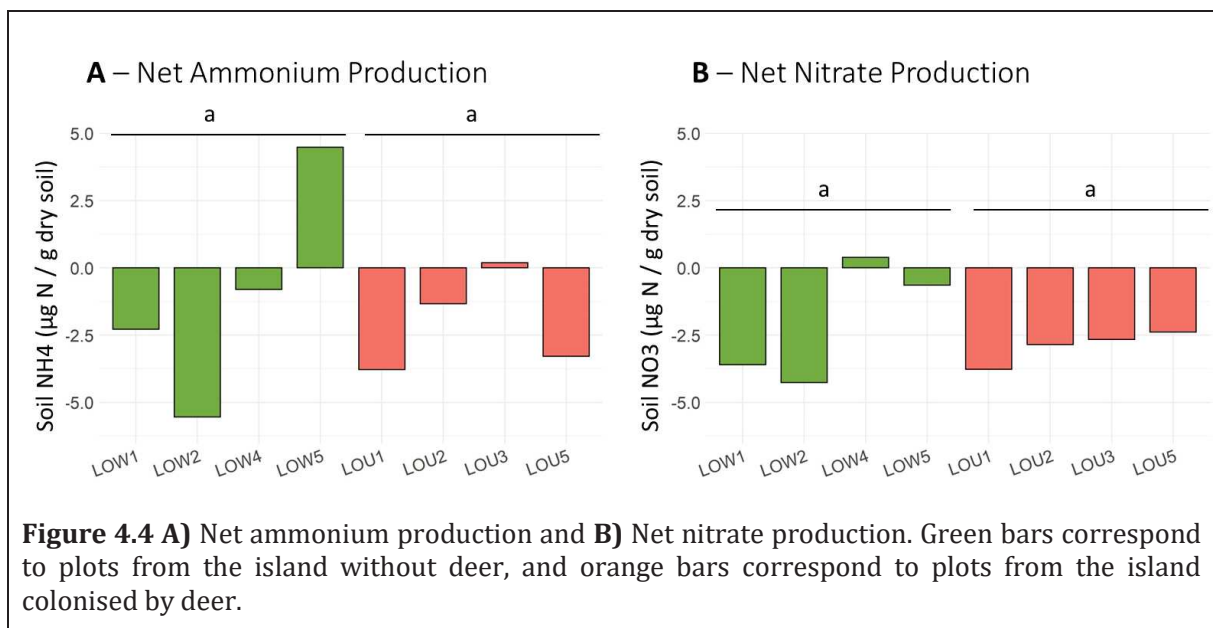
Immobilisation – Microbial immobilisation of ammonium into the labile organic nitrogen pool was not significantly different between island with or without deer (rate $I_{\text{NH}_4\text{-Nlab}}$, Figure 4.3, $W = 10$ and $p\text{-value} = 0.69$). This rate was particularly high on the plot LOW01 and LOW05, where it reached $26.82 \mu\text{g/g/day}$ and $41.03 \mu\text{g/g/day}$ respectively. Microbial immobilisation of ammonium into the recalcitrant organic nitrogen pool was not significantly different between island with or without deer (rate $I_{\text{NH}_4\text{-Nrec}}$, Figure 4.3, $W = 14$ and $p\text{-value} = 0.11$). This rate reached a maximum value of $20.81 \mu\text{g/g/day}$ on the plot LOW02. Microbial immobilisation of nitrate in the recalcitrant organic nitrogen pool was not significantly different between island with or without deer (rate I_{NO_3} , Figure 4.3, $W = 2$ and $p\text{-value} = 0.11$). There was no evidence of such immobilisation on the plots LOW02 and LOW04.

There was evidence of abiotic ammonium adsorption and release on all the plots from the island colonised by deer, and on the plot LOW01 from the island without deer (rates A_{NH_4} and R_{NH_4} , Figure 4.3). Abiotic ammonium adsorption was particularly high on plots LOW01 and LOW02, where it reached an average value of $29.40 \mu\text{g/g/day}$ and $26.01 \mu\text{g/g/day}$ respectively. These two rates were not significantly different between island with or without deer ($W = 4$, $p\text{-value} = 0.30$ and $W = 4$, $p\text{-value} = 0.34$ respectively). There was evidence of abiotic nitrate storage and release on all plots (rates A_{NO_3} and R_{NO_3} , Figure 4.3). Abiotic nitrate storage and release were not significantly different between island with or without deer ($W = 7$, $p\text{-value} = 0.89$ and $W = 6$, $p\text{-value} = 0.69$ respectively). For both ammonium and nitrate, release was lower than adsorption and storage.



4.3.3 – Effect of deer on net NH_4 and NO_3 production

Net ammonium production was not significantly different between the island without deer and the island colonised by deer (Figure 4.4A, $W = 9$ and $p\text{-value} = 0.89$). Net ammonium production was negative for most of the plots and showed an average of $-1.54 \mu\text{g/g/day}$. LOW05 and LOU03 had a positive net ammonium rates with a net production of $4.49 \mu\text{g/g/day}$ and $0.19 \mu\text{g/g/day}$ respectively. Net nitrate production was not significantly different between the island without deer and the island colonised by deer (Figure 4.4B, $W = 9$ and $p\text{-value} = 0.89$). Net nitrate production was negative for all the plots but LOW04 where it reached $0.39 \mu\text{g/g/day}$. Average net nitrate production across the plots was $-2.47 \mu\text{g/g/day}$.



4.4 – Discussion

We found that the effects of on deer aboveground plant communities and belowground soil properties were consistent with previous findings on these islands and in other parts of the world (Chapter 3 of this thesis, Chollet et al., 2013; Côté et al., 2004; Martin et al., 2010; Stockton et al., 2005). The plots used in this study correspond to a subset of the plots

previously used to decipher the impact of deer on soil properties and prokaryotic communities (Chapter 3). In this study we found that deer decreased shrub cover, pteridophyte cover and vascular plant diversity, and increased conifer and bryophyte cover, similarly to previous finding on the larger set of plots (Figure 4.2A, and Figure 3.1A). Belowground, we found that deer presence decreased soil phosphorus content, pH and organic horizon depth, and increased soil moisture content and penetration resistance, similarly the findings on the larger set of plots (Figure 4.2B, and Figure 3.1B). Our selection of four plots per island is, therefore, representative of the effect of deer on the ecosystem on these islands (Chapter 3). At a more global scale, this selection is also representative of the impact of deer on the vegetation in temperate forests (Côté et al., 2004).

We found soil nitrogen content and rates of nitrogen cycling processes that were consistent with those found previously in acidic organic soils and in wet coniferous temperate forests. Nitrification was mostly heterotrophic from the recalcitrant organic pool rather than autotrophic from the inorganic ammonium pool (rate O_{Nrec} and rate O_{NH_4} respectively, Figure 4.3). Nitrification in acidic coniferous forest soils has indeed been found to be constantly dominated by heterotrophic nitrifiers, and particularly fungi (Jordan et al., 2005; Schimel et al., 1984; Stein, 2011). We also found evidence of DNRA in most of our samples, a process that has been found to be significant in temperate and humid soils with high organic matter such as ours (Rütting et al., 2011). We found evidence of abiotic ammonium and nitrate fixation (A_{NH_4} and A_{NO_3}) in most of our plots. Abiotic ammonium adsorption can result from the binding of nitrogen to either the clay minerals or to the organic fraction of the soil and particularly to humic acids (Kowalenko and Cameron, 1978; Nommik and Vahtras, 1982). As our soil samples are organic in nature, the observed ammonium fixation must be the result of the high level of organic matter and, therefore, humic acids rather than mineral adsorption. Although high pH is believed to favour such fixation, abiotic adsorption of ammonium by organic matter has already been observed in low pH forest floors (Axelsson and Berg, 1988; Johnson et al., 2000; Nommik and Vahtras, 1982; Schimel and Firestone, 1989). Abiotic nitrate immobilisation has also been recorded previously in acidic forest floors (Dail et al., 2001; Perakis and Hedin, 2001), although the

mechanisms behind such fixation remain little known (Colman et al., 2007; Davidson et al., 2003). The overall negative net ammonification and nitrification rates were the result of a high microbial and abiotic immobilisation of both NH_4 and NO_3 , and explain the low or null soil ammonium and nitrate concentrations we found in our samples. This absence of net ammonium and nitrate production has been previously reported in Canadian coniferous and mixed natural forests in Canada (Masse et al., 2016; Ribbons et al., 2016).

Contrary to our hypotheses, we found that 70 years of colonisation by deer did not result in contrasting nitrogen cycling rates when compared to islands without deer. A decelerating effect of deer on nutrient cycling has been predicted in forest ecosystems (Bardgett et al., 1998; Bardgett and Wardle, 2003; Harrison and Bardgett, 2008). Different studies on the effect of ungulates on nitrogen cycling in temperate forests have actually found positive (Carline and Bardgett, 2005; Furusawa et al., 2016; Niwa et al., 2008), negative (Gass and Binkley, 2011; Harrison and Bardgett, 2004) or neutral effects (Niwa et al., 2011; Relva et al., 2014) of deer on net N mineralisation rates. Net nitrification has been less studied in temperate forest, but was found to be positively affected when investigated in a mixed forest of central Japan (Furusawa et al., 2016). In our study, we found that deer presence did not result in differences in net mineralisation and nitrification rates. Furthermore, we found that deer presence did not influence any of the gross N transformation rates, suggesting that waste deposition, trampling and vegetation shift by deer has no effect on the nitrogen cycle.

The absence of effect from dung and urine deposition might be due to the selective use of the landscape by deer for waste deposition and the patchy distribution of such inputs (Murray et al., 2013). Nitrogen loss in litter after one year of decomposition was enhanced by feces addition, but this effect was not found at the ecosystem level on the same islands (Chapter 2). High humic acid levels in organic soil may also explain the absence of any effect of feces and urine addition. Humic acids have indeed been shown to buffer the effects of urea addition into soil by inhibiting urease activity (Dong et al., 2009). In addition, the high bryophyte cover found on islands colonised by deer on the forest ground may further intercept any input of inorganic nitrogen from both urine and feces. Mosses have indeed

been shown as being efficient in absorbing nutrients from herbivore feces in a tundra ecosystem and from nitrogen addition in an Alaskan black spruce forest to promote their growth (Gornall et al., 2009; Weber and Cleve, 1984).

Soil compaction by deer trampling has been found to be a major factor modifying soil microbial communities on these islands (Chapter 3), confirming that deer interfere with soil properties through soil physical compaction. The influence of soil compaction on nitrogen cycle has been further highlighted in other studies (Schrama et al., 2013a). The absence of evidence of a trampling effect on soil N cycling in this study might be due to the *in vitro* design of this experiment. Sampling of the soil and its homogenisation by hand might have disturbed the soil physical structure and induced an artificial aeration of the sample. As a result this handling may have reduced any potential effect of compaction induced by deer on N-cycling processes.

The absence of evidence for an impact of litter quality change on N-cycling was more surprising. Previous work on these islands has indeed shown that deer significantly reduced litter nitrogen loss during decomposition due to a reduction of the litter quality (Chapter 2). Different possibilities may explain this discrepancy between the two experiments. A first explanation comes from the low sample size in this study, which may limit the statistical power in detecting significant differences. The addition of three new plots per treatments will allow to improve this limitation. Another hypothesis to explain the observed discrepancy between the two experiments could be a difference in nitrogen dynamics between the fresh litter horizon and the F horizon in the soil. In the surface litter layer of the soil (L layer), and hypothetically in the top of the F layer underneath, the most easily degradable nitrogen compounds are processed by soil microbes. In these layer of the soil, the reduction in litter quality by deer have a strong effect on decomposition (Chollet, Maillard et al., 2019). As the litter is decomposed and sinks into the F horizon, the difference in litter quality between deer islands and islands without deer may be diminished, because only the most recalcitrant part of the plant litter remains. As a result, the litter quality in the F horizon may be similar in soil from islands with and without deer, explaining the absence of effect observed on N-cycling processes.

The model we used in our study did not allow us to measure denitrification rate. Denitrification has important implications for N availability in ecosystems, as it contributes to N loss through the transformation of NO_3 into gaseous N. In a study realised in a French grassland, Patra et al. (2005) found a higher abundance of soil denitrifiers with higher sheep grazing intensity, suggesting that herbivory promoted denitrification in this ecosystem. In our system, the important soil compaction level caused by deer trampling may promote anaerobic processes through the reduction of soil oxygen (Hartmann et al., 2014), and could, therefore, enhance denitrification. In a theoretical study, de Mazancourt et al. (2000) showed that the net effects of herbivores on primary production depended on their effect on nitrogen loss via mechanisms such as denitrification. De Mazancourt et al. (2000) indeed found that increased nitrogen loss from the ecosystem led to a negative effect of herbivores on primary production, whereas increased nitrogen retention in the ecosystem by plants led to a positive effect of herbivores on primary production, or “grazing optimization”. Investigating the effect of deer on denitrification in our system is therefore an important next step to fully understand the effect of deer abundance on nitrogen cycle and the implications it can have for ecosystem functioning.

In our study, the observed differences in nitrogen cycling rates were due to plot specificities rather than reflecting an influence of deer. Soil from the plot LOW05 on the island without deer had particularly high inorganic nitrogen concentrations (Figures 4.2D and E). This plot was also the only plot where alders *Alnus crispa* were growing. Alder are known to live in symbiosis with diazotrophic bacteria, which have the ability of fixing atmospheric di-nitrogen N_2 to NH_4 (Dawson, 1983; Dinger, 1895; Tarrant and Trappe, 1971). Such association has a positive consequence for soil nitrogen, for example nitrogen content and availability was found to be higher in alder stands in a Northern Sweden forest and a Pacific Northwest forest in Oregon (Boyle et al., 2008; Myrold and Huss-danell, 2003). High gross N transformation rates found in the soil of LOW05 must, therefore, be the direct consequence of this extra input of nitrogen to the soil. We found no evidence of microbial immobilisation of nitrate (rate I_{NO_3} , Figure 4.3) on plots LOW04 and LOW05, where nitrate was initially present in the soil. This is coherent and highlights the absence of competition for nitrate

between plant and microbes when this compound is present in sufficient concentrations in the soil. The plot LOU05 showed a particularly high level of DNRA (rate D_{NO_3} , Figure 4.3) whereas such process was absent on the plots LOW02 and LOW04. DNRA has been shown to be maximal in wet soils, rich in high organic matter (Rütting et al., 2011). LOU05 had the highest carbon content and the second highest soil moisture content, which may explain the observed high DNRA rate (Figure S4.1). Conversely, LOW02 had the lowest soil moisture content and LOW04 had both a low soil moisture content and a low percent carbon, which might explain the absence of DNRA in these soil (Figure S4.1).

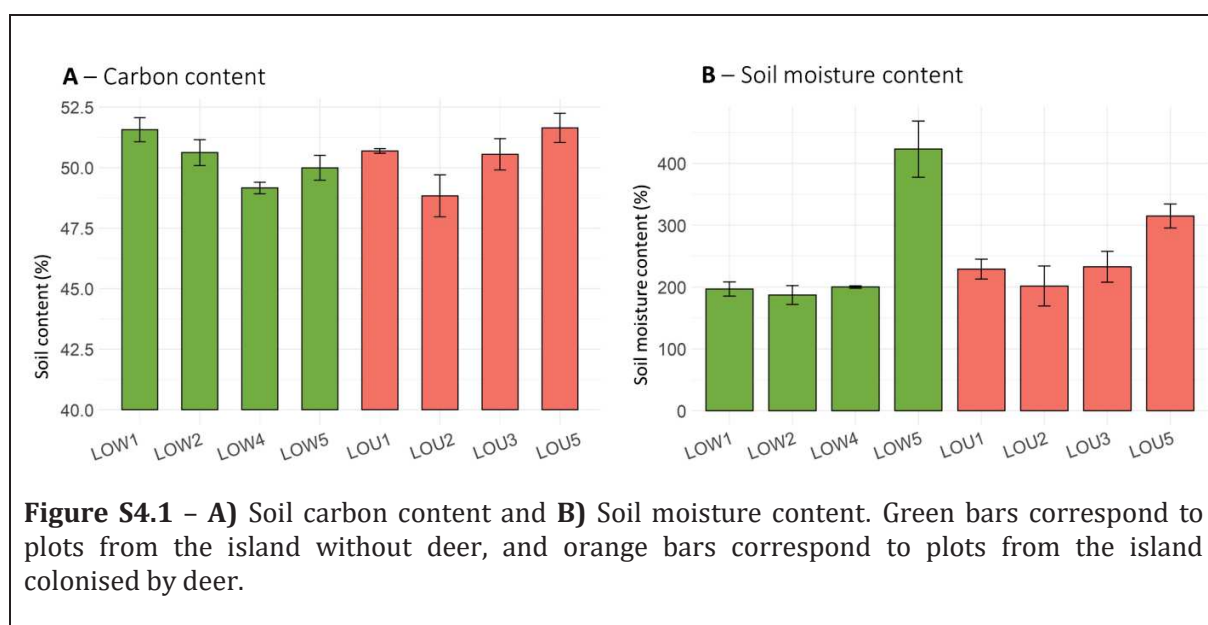
4.5 – Conclusion

We found that the presence of deer did not change gross nitrogen cycling rates and the net production of ammonium and nitrate in soil. Absence of evidence for a deer effect on the nitrogen cycle via waste deposition was consistent with previous finding on the same islands and in a coniferous forest (Chapitre 2, Chollet, Maillard et al., 2019; Pastor et al., 1993). Absence of evidence for a trampling effect by deer may be due to sample handling inherent to the *in vitro* design of this ^{15}N -isotope tracing experiment. Absence of evidence for a deer effect on the nitrogen cycle via a reduction in litter quality was surprising, considering the significant negative effect of deer on litter nitrogen loss highlighted in a previous study on the same islands (Chapitre 2, Chollet, Maillard et al., 2019). Possible explanations for this discrepancy include the low statistical power due to a low sample size, and a possible diminution of the effect of deer on nitrogen processes as we dig deeper in the forest floor. This study is a preliminary analysis of a larger study including more replicates per treatments. The analysis of the entire dataset will therefore allow to increase the precision of this study.

4.6 - Supplementary

Table S4.1 – 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 |



Chapter 5: Deer abundance and soil in a temperate forest: What's what and the way forward

In this thesis, I evaluated the effects of abundant deer on soil communities and their functioning in temperate forests. My goal was also to elucidate some of the idiosyncrasies found within and across past studies on the subject. To do so, I used the unique configuration of the Canadian archipelago of Haida Gwaii, which offers a quasi-experimental situation with the presence of islands without and with deer, the latter varying in deer presence history. An additional remarkable asset of these islands was the knowledge gathered over the past 30 years on the aboveground effects of deer on the islands they colonized. I first (Chapter 2; Chollet, Maillard et al., 2019) investigated how the presence or absence of deer affected litter decomposition using a year-long litter bag transplantation experiment. I measured the relative importance of changes in litter quality and in soil organisms' decomposition ability caused by deer on the amount of carbon and nitrogen lost in litter during decomposition. In the third chapter, I investigated how the aboveground effects of deer affected physical and chemical properties of the fermentation layer (F) of the forest floor, and the structure of its soil prokaryotic communities. For this, I compared three complementary systems to assess the long-term, intermediate and short-term response of the soil ecosystem to the history of deer presence. In the fourth chapter, I focused on determining the effect of deer on gross and net rates of nitrogen cycling processes using a ^{15}N -isotope tracing experiment. The results focus on a comparison of islands with different browsing histories. These results pertain to a subset of a larger study still in progress in which I will compare nitrogen cycling processes in the three systems: long-term deer presence, intermediate-term deer exclusion and short-term response to a recent deer cull. Together, these three experimental studies aimed at bringing new insights on the consequences of deer abundance and deer presence history on soil properties and functioning in a temperate forest, and on the mechanisms involved.

5.1- Deer modify soil in the forests of Haida Gwaii

5.1.1 – A decelerating effect of deer on soil nutrient cycling

(Bardgett and Wardle, 2003; Ritchie et al., 1998) proposed a conceptual framework to predict the effects of large herbivores on soil nutrient cycling, in which they suggested an accelerating effect of grazers in early successional ecosystems (i.e. grasslands) versus a decelerating effect of browsers in late successional ecosystems (i.e. forests). (Pastor et al., 2006) explain the threshold between these two opposing scenarios by the nitrogen content of the forage. For nitrogen-rich forage, an accelerating effect of herbivores on nitrogen cycling is predicted due to the increased concentration of nitrogen in dung and urine. Conversely, nitrogen-poor forage in nitrogen limited ecosystem is expected to result in a decelerating effect because herbivores retained nitrogen in their body to sustain their growth and homeostasis. In the forests of Haida Gwaii we should therefore expect a decelerating effect of deer on soil processes according to the hypothesis of Bardgett and Wardle (2003). Consistently with this hypothesis, I found that litter carbon and nitrogen losses from litter after one year of decomposition were slower in presence of an abundant deer population (Chapter 2; Chollet, Maillard et al., 2019). However, a focus on nitrogen cycling rates in the soil F horizon showed no effect of deer on the kinetics of this cycle (Chapter 4). The low sample size in this first sub-set of our ^{15}N -isotope tracing experiment may not allow to detect any difference in nitrogen cycling processes, but this issue will be improved with the addition of three new plots per treatment. In addition, diminution in the effect of deer on nitrogen processes between the fresh litter layer and the soil F layer may also explain this discrepancy between the two experiments (Chapter 4).

5.1.2 - Deer interact with soil through trampling and vegetation shift mainly

Deer can affect soil through several direct and indirect pathways. In the second chapter of this thesis, I found that decrease in litter quality due to changes in vegetation community structure in the presence of deer was the predominant pathway inducing changes in litter

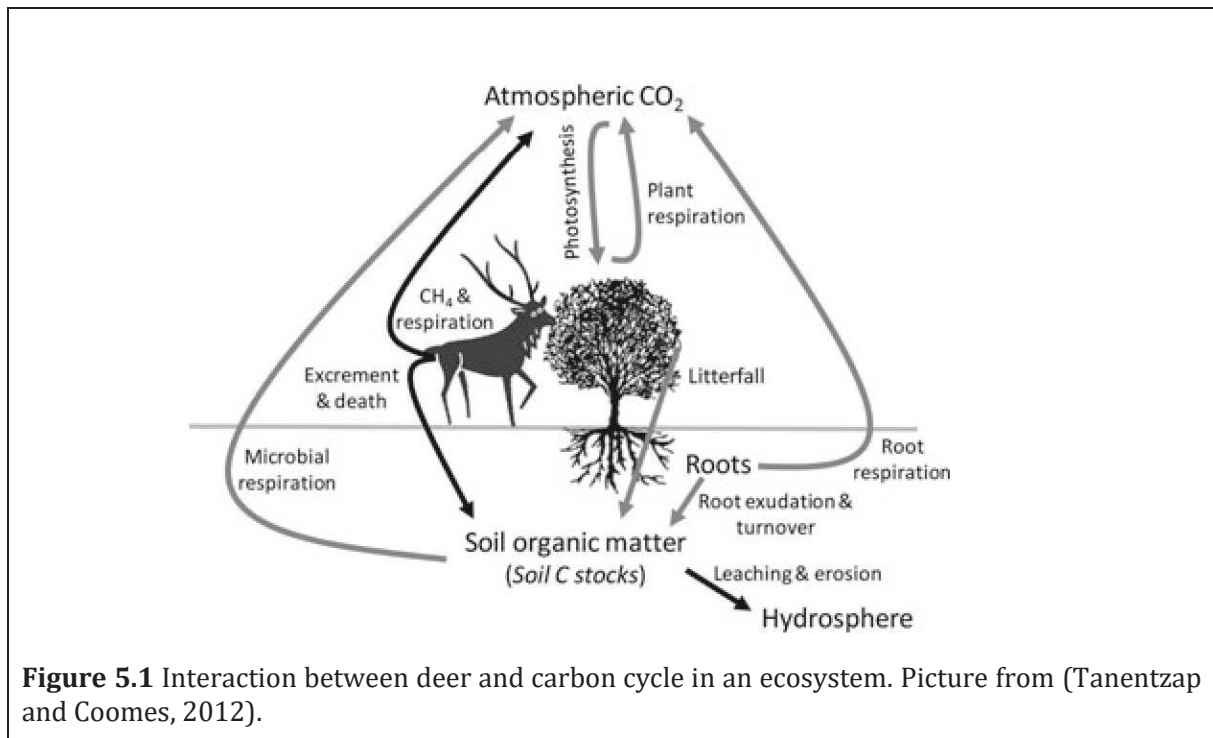
decomposition. The shift in plant community composition caused by deer browsing towards less palatable species such as conifers and bryophytes was indeed demonstrated in the two sets of plots (Chapter 2 and Chapter 3) and in all three study systems (Chapter 3). This vegetation shift was consistent with earlier findings on the same islands and confirmed results in other temperate forests of the world (Boulanger et al., 2018; Chollet et al., 2013b; Côté et al., 2004; Stockton et al., 2005). I also found that the ability of soil microbial communities to decompose carbon was enhanced by 5 % in the absence of deer (Chapter 2). The structure of soil prokaryotic communities was indeed significantly affected by the presence of deer, and these changes were driven by soil compaction resulting from deer trampling (Chapter 3). In all three experiments, I found that dung and urine deposition had little influence on soil properties and functioning. Dung significantly enhanced carbon and nitrogen decomposition when placed directly in contact with litter, but had no measurable effect at the ecosystem scale (Chapter 2). In chapter 3, I found evidence for a decrease in ammonium concentrations in soil the month following a deer cull, but this reduced ammonium did not lead to changes in soil prokaryotic community structure. Similarly, I found no evidence of enhanced nitrogen cycling in the F horizon through the labile inorganic nitrogen pool on islands where deer were present, which would have reflected waste input to the soil (Chapter 4). This result is consistent with the hypothesis of Bardgett and Wardle (2003) who suggested that, in late successional ecosystems such as forests, the slowing down effect of a reduction in litter quality by deer prevails over the positive effect of dung and urine deposition. The apparent negligible impact of waste deposition on soil organisms and processes might also be caused by the heterogeneity of this input across the landscape, and the relatively small concentrations of labile N contained in urine and dung. Deer tend to urinate and defecate in the same locations (Hoogendoorn et al., 2010). Deer are thought to, like sheep, urinate an average of 20 times per day with a mean urea N concentration of 6.4 g N in an urination event (Moen and Delgiudice, 1997), depending on the nitrogen concentration of forage consumed. Nitrogen may also have rapidly leached from the urine patches, being emitted as NH_3 , this occurs if the excreted nitrogen exceeds the ability of the surrounding vegetation to use it (Di and Cameron, 2007). Nitrogen losses through leaching

are greater where urine patches are aggregated, such as areas where the animals bed (Pleasants et al., 2007).

5.1.3 - Deer significantly reduce carbon and nitrogen stock in the forest floor

I did not directly consider the considerable impact deer have on litter quantity in the system studied. However, by dramatically reducing the aboveground biomass of understory vegetation, and thus the amount of litter produced, deer may decrease the source of carbon and nutrient stocks in the soil (Tanentzap and Coomes, 2012). In Europe, it is estimated that deer overabundance is responsible for the consumption of about 20 million tons of green vegetation every year (Apollonio et al., 2010a). In the forests of Haida Gwaii, Sitka black tailed deer removed over 85% of the understory cover, causing a dramatic reduction in litter quantity (Stockton et al., 2005). In this thesis, I found that long-term deer presence significantly reduced the carbon and nitrogen stored belowground (Figure 3.2D and E in Chapter 3). The reduction in these elements was parallel to the considerable reduction in depth of the soil organic horizon on islands colonised by deer (Figure 3.2B in Chapter 3). Although this shallower organic horizon can be partly explained by the greater soil compaction resulting from deer trampling (Figure 2.1C and 3.2C in Chapter 2 and 3), this cannot explain the lower soil carbon and nitrogen stock observed under deer presence. I interpreted this reduction as the result of a lower input of plant litter to the soil. Conversely, in presence of deer, part of the plant carbon and nitrogen accumulated in the plants consumed by deer will be retained in deer bodies in increasing quantity during the animal's growth. Contrary to plants that continue to accumulate C and N through growth during their entire lifetime, once fully grown, deer cease to accumulate nutrients. Elements not used in body maintenance will be released to the atmosphere via respiration and methane production or to the atmosphere and hydrosphere via urine deposition through evaporation and rapid leaching as mentioned above (Di and Cameron, 2007; Figure 5.1). A fraction will return to litter via feces deposition and ultimately after the animal's death (Figure 5.1). Finally, higher erosion due to the removal of protective understory by deer browsing may

also be an important mechanism behind the thinner soil organic layer observed on islands colonised by deer (Hartanto et al., 2003; Lei et al., 2010).



5.2- What does this research teach us on the response of belowground ecology to deer presence in temperate forests?

General laws in ecology can be defined as “widely observable tendencies” (Lawton 1999). An accumulation of evidence is, therefore, necessary to see emerging an ecological pattern that we can qualify as an ecological law (Lawton 1999). As such, defining the response of soil to the presence of large herbivores requires the generalisation of a number of independent observations. Effects of large herbivores belowground in grassland and in boreal forests have been well characterised and, despite intrinsic specificities in each situation, have allowed the elaboration of a conceptual *accelerating/decelerating* framework (Bardgett and Wardle, 2003; Ritchie et al., 1998). Results on deer effects in temperate forests, however, remain contrasted, making positioning them in this framework difficult (Wardle et al., 2001). The

increased abundance of deer in Europe and North America, and the consequences of their overabundance for aboveground forest structure suggest that such investigations on their role in belowground ecology are essential to improve predictions, and better management, of this phenomenon. In the current study, I showed that abundant deer in a coniferous temperate forest significantly slow-down decomposition processes via the promotion of unpalatable plant species, outperforming the potential decomposition-accelerating effect of waste deposition. Soil prokaryotic abundance also showed a tendency to be reduced by long-term deer presence. These findings are similar to the results of (Pastor et al., 1993), who found that 40 years of moose (*Alces alces*) exclusion significantly reduced litter decomposition via the promotion of nutrient-poor plant species, and significantly reduced soil microbial biomass in a boreal forest. Our work, therefore, suggests that the belowground effects of deer in coniferous temperate forests are similar to those found in coniferous boreal forests. This research also validates the hypotheses of Bardgett and Wardle (2003), who predicted a decelerating effect of large herbivores on soil processes in forests ecosystems. More importantly, we found that changes in soil properties and functioning by deer operated at different time scales, with short-term belowground changes driven by the direct interactions of deer with soil (i.e. waste deposition and trampling), and long-term belowground changes driven by both direct, and indirect, effects of deer with soil (i.e. trampling and vegetation shift). Analyses of additional samples in the ¹⁵N-isotope tracing experiment may strengthen this result. Previous work on a small grazer, the vole (*Microtus spp.*), also demonstrated such time dependence with short-term changes being driven by direct waste deposition and long-term changes being driven indirectly through vegetation shifts (Sirotnak and Huntly, 2000). In my study, the effect of deer on soil via vegetation shifts was a long-term process that took several decades. This temporal dimension may explain some of the idiosyncrasies found so far within, and across, studies on the effect of deer belowground. Indeed, many studies last only short periods of time which may show transitional states, in which modification of the vegetation has not yet fully reverberated on the soil. Long-term studies seem therefore necessary to better capture the belowground effects of deer in temperate forests. The results in this thesis also have implications for forest

restoration and management strategies of deer populations, indicating that, compared to aboveground resilience, reversibility of the long-term effects of deer belowground is an even longer process.

5.3- Limitations of this study

5.3.1 - Variation in the forest properties among islands: an island or deer effect?

The Island Biogeography theory predicts that species diversity increases with island size (MacArthur and Wilson, 1967). Different micro-climates could also affect islands differentially depending on island size and island distance from the coast. In my study, I compared islands presenting a gradient of deer colonisation. Un-colonised islands were the ones un-reachable by deer, because they were the most isolated islands on the archipelago and too small to harbor a significant deer population. As a result, these islands were significantly smaller when compared to the islands colonised by deer (Table 3.1 in Chapter 3). An island effect may, therefore, act as a confounding factor in our study, partly shaping the island communities and ecosystem properties. However, previous work showed that the observed differences among these islands in vegetation and bird communities were overwhelmingly the result of a deer effect rather than a direct island size or isolation effect (Gaston *et al.*, 2006; Martin *et al.*, 1995). Furthermore, the transplantation design used in the litter decomposition study outlined in chapter 2 allowed to partly overcome the issue of a potential island effect by separating the relative effect of litter quality and of decomposers ability on the loss of carbon and nitrogen in litter during decomposition. My work showed that decomposition was slower on the islands colonised by deer due to both a reduction in litter quality and a reduction in the ability of the soil microbial community in degrading carbon. Could this reduced litter quality on islands colonised by deer be the result of an island effect rather than a deer effect? As mentioned above, previous work of Martin *et al.* (1995) and Gaston *et al.* (2006) confirmed that the observed shift in vegetation on these islands is the result of deer browsing. Could the reduced decomposer ability in carbon

degradation be the result of an island effect? In chapter 3, I found that changes in the soil prokaryotic community were linked to changes in soil penetration resistance, a proxy for soil compaction in the presence of deer. Predictive Metagenomic Profiling (PMP) further suggested that this shift in soil prokaryotic community could have implications for carbon cycling (Figure 3.6B). These results, therefore, suggest that the difference in soil prokaryotic community structure and their ability to decompose litter carbon between islands is the result of a deer effect through soil trampling, rather than reflective of an island effect.

5.3.2 - Small enclosure area: belowground communication as a potential homogenisation factor

I used a set of 19 deer exclosures to study the resilience of the ecosystem to medium-term length of deer removal. These exclosures had a small area of 5 m². Although soil is a dense matrix of solid materials that prevent easy displacement of organisms and nutrients, belowground communication over significant distances has been highlighted previously. Roots from the plants located outside the exclosures are unlikely to stop at the level of the fence. Instead, roots may grow under the fence and, through exudation and nutrient assimilation, may maintain a flow of nutrients and small organic compounds in the soil outside and inside deer exclosures. Similarly, fungal hyphae are likely to connect the exterior to the interior of deer exclosures. In particular, mycorrhizal fungi have been shown to constitute important belowground communication networks linking plants. Resources such as carbon, nitrogen and phosphorus can flow from plant to plant through this mycorrhizal network (Eason et al., 1991; Simard et al., 1997; Teste et al., 2009). Furthermore, bacteria have also been shown to be able to migrate via fungal hyphae (Warmink et al., 2011; Warmink and van Elsas, 2009). Belowground communication from outside of the exclosures may, therefore, interfere with soil response to deer exclusion. As a result, the small area of our deer exclosures may not be representative of an ecosystem after twenty years of deer exclusion. In Chapter 3, I found that islands colonised by deer for less than 35 years had similar soil chemical and biological properties than islands without deer. Similarly, I found that soil properties after twenty years of controlled deer populations were similar to soil properties on islands colonised by deer for more than 70 years. This inertia, therefore, seems

to support our conclusions in the exclosure system, i.e. that the shift in the soil prokaryotic community that follows deer introduction or removal is a long process taking longer than 20 years.

5.4- Moving forward

5.4.1 - Many questions to answer

As it is often the case in research, this study opens new questions, while some older questions remain un-answered. What are the consequences of the observed changes in soil properties and processes for the ecosystem aboveground? Would we observe a restoration of the soil properties after a longer period of deer exclusion and over the years in the exclosures and in the recent deer cull systems? How long does it take to observe a complete restoration of soil properties and organisms after deer removal? This also prompts the question of soil properties in situations where deer populations are under the control of their predators? In this thesis, I chose to focus on the effect of deer on the nitrogen cycle, an essential element limiting primary production in temperate forests (Vitousek and Howarth, 1991). But I also found that the content of phosphorus in soils, another essential element that can also limit plant growth in forests (Vitousek et al., 2010), was significantly lower in the presence of deer (Chapter 3). Is the phosphorus cycle affected in the presence of deer? Preliminary work on a subset of these islands suggested that phosphorus availability in the soil was significantly higher in the presence of deer (Mendenhall, 2018), but further investigations are required to answer this question. In our study, we chose to focus on the case of a coniferous temperate forest. In broad-leaved temperate forests, where plant litter shows higher nutrient content and where soil is characterised by a mull humus, the effects of deer on soil might tell a very different story. What is the relative importance of the characteristics of the initial vegetation in the response of soil to abundant deer?

My thesis focused on the properties and dynamics of the upper layers of the forest floor: the fresh litter and the F horizon. This choice was driven by the close interaction between deer and these layers and the greater biological activity within these layers than within deeper mineral layers of the soil. I found that the effect of deer on nitrogen dynamics was different

between the decomposition study on fresh litter (Chapter 2), where deer significantly slowed-down litter N loss during decomposition, and the isotope tracing study on the soil F horizon (Chapter 3), where I found no evidence of changes in nitrogen cycling processes with deer presence. These differences might be exacerbated in mineral soil, where mineral particles such as clay can interact and bind with nutrients (Kowalenko and Cameron, 1978; Nommik and Vahtras, 1982). Schrama *et al.* (2013a) indeed found that soil characteristics played an important role in the response of soil processes to trampling. Soil horizon and intrinsic soil properties may, therefore, significantly influence the belowground response to deer pressure.

What is the relative importance of the initial soil properties in the response of soil to abundant deer in temperate forests? Deer density is an additional confounding factor that may explain part of the observed idiosyncrasies across studies. In a semi-quantitative review, Ramirez *et al.* (2018) indeed found that impact of deer on forest functioning, characterised as soil nutrient cycling, tree growth and wild forest food provision, increased with deer density (Ramirez *et al.*, 2018). My thesis, through the use of an island with regular control of the deer population, only partly addressed the influence of deer density. What is the relative importance of initial deer density in the response of soil to abundant deer in temperate forests? Are initial vegetation characteristics, initial soil properties and deer density modulating the kinetic of the soil response to deer pressure? Answering these questions is fundamental if we want to fully understand the effect of abundant deer in temperate forests, and be able to predict and manage ecosystem changes due to their over-abundance. One possibility to address these questions would be the realisation of an experiment integrating the different soil horizons, soil types and forest vegetation types along a gradient of both deer density and study length. Such an experimental design would however require important logistic and financial means that may be unrealistic.

5.4.2 - Modelling as a powerful tool to dig deeper into these questions

Modelling is a powerful tool that offers great opportunities to integrate and test all these factors. Ecological models allow, through the simplification and formalisation of a system, to gain a better understanding of the interactions among the different actors in a system. Several models have been previously developed to represent the interactions between large herbivores and their ecosystem, including the soil subsystem (Cherif and Loreau, 2013; de Mazancourt et al., 2000). Cherif and Loreau (2013) described a stoichiometric model linking plant communities, litter, decomposers and inorganic nutrients. For each organic compartment (*i.e.*, plant community, litter and decomposers), two subunits were considered: the carbon and a nutrient (Figure 5.2). Such a model allows the dynamics of both carbon and a nutrient to be followed throughout the ecosystem under herbivory pressure. The pertinence of this stoichiometric approach is supported by the fact that carbon and nutrient dynamics have been shown to be decoupled under herbivory pressure (Chapter 2, Stark et al., 2003). In their study, Cherif and Loreau (2013) investigated the effect of plant nutrient contents on the interaction between herbivores and decomposition. They found that high nutrient content in litter enhanced soil nutrient availability, consistently with the prediction of the *decelerating/accelerating* conceptual framework (Ritchie et al., 1998). This model offers a good starting point to dig deeper into the role that initial ecosystem properties have in modulating the effect of deer on carbon and nutrient cycling. A few adjustments would need to be made to completely adapt this model to our study questions. Although this model considers the alteration of quantity and quality of resources returned to the soil through the reduction in litter quantity and waste deposition, it does not include the alteration of the plant community composition. As a result, the reduction in litter quality due to the preferential browsing of palatable species is not represented. de Mazancourt et al. (2000) theoretically studied changes in litter quality with herbivory, and their work could be used to adapt the model of Cherif and Loreau (2013). In their simulations, Cherif and Loreau (2013) kept the parameters representing the initial activity of soil decomposers and the herbivore biomass at a constant value. Comparison of numerical simulations using a broad range of values for these parameters (or “bifurcation analysis”) can be run to study their

effect on the behaviour of the system. By doing so, we can investigate the role of each initial parameters (i.e. vegetation characteristic, soil properties via the initial activity of decomposers, herbivore density ...) on the carbon and nutrient cycling, as well as on the kinetics of the system to reach an equilibrium state. Parameter values can also be chosen to reflect the dynamics of a specific nutrient such as the nitrogen or the phosphorus. Not only would such models allow a better understanding of the interactions between deer, the forest and soil organisms, it would also provide a valuable tool for the management of deer population in temperate forests. Studying the effect of herbivore density on the system can indeed allow determination of the critical deer density that maintains forest biodiversity and functioning. Whether the system is resilient to deer removal, or reduction, and how long it takes to return to a state similar to what is found in absence of deer can be further investigated by studying the behaviour of the system after suppression of the herbivore pressure. Furthermore, an additional compartment representing deer predators could be added to such a model in order to follow the behaviour of the system to potential re-introduction or restoration of natural predators.

Modelling, therefore, offers interesting perspectives to decipher the influence of ecosystem properties and deer density on the effect of abundant deer in forest functioning. Modelling also gives suggestions for conservation strategies and environmental management.

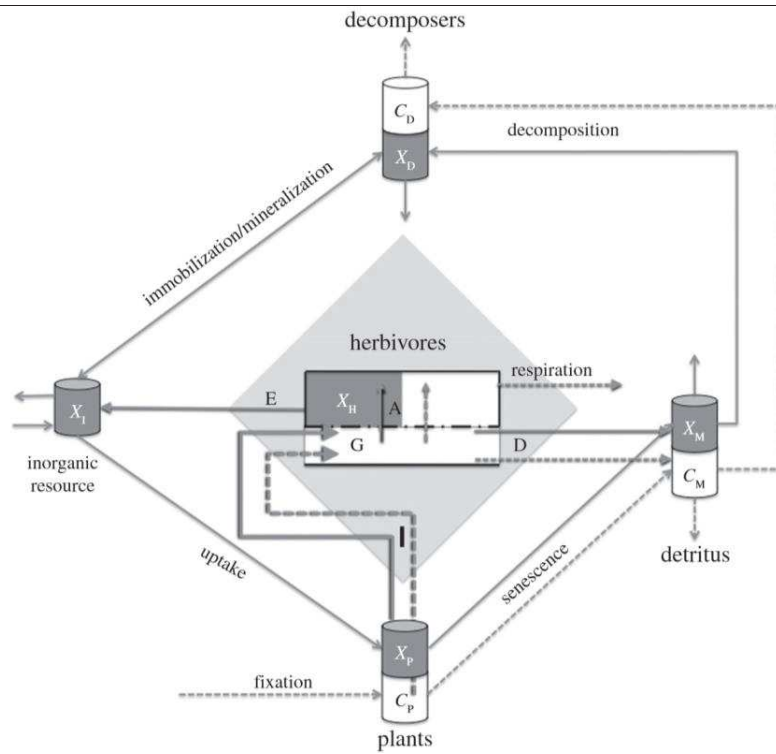


Figure 5.2 Stoichiometric model developed by Cherif and Loreau (2013). X = a nutrient; C = carbon, I = ingestion of plant material by herbivores, A = carbon and nutrient assimilation by herbivores, G = digestion by herbivores, D = defecation, E = nutrient excretion. Picture from Cherif and Loreau (2013).

De l'abondance des cerfs aux propriétés du sol : Une étude de cas dans les forêts d'Haïda Gwaii

L'augmentation récente et spectaculaire de l'abondance des cerfs à l'échelle continentale en Amérique du Nord et en Europe occidentale a entraîné de profonds changements dans la structure des forêts tempérées (Côté et al., 2004; Ramirez et al., 2018). Cette forte abondance s'est révélée être un facteur prévenant la régénération de la forêt dû à une forte pression d'abrouissement et au piétinement des jeunes arbres (Gill, 1992). Elle a de plus été corrélée à une diminution drastique du couvert et de la diversité du sous-bois (Horsley et al., 2003; Stockton et al., 2005). Cette restructuration de la forêt sous la pression d'herbivorie a également eu des conséquences négatives sur l'abondance et la diversité d'autres niveaux trophiques tels que les oiseaux et les insectes, dont la survie dépend du sous-bois (Cardinal et al., 2012; Martin et al., 2010; Nettle et al., 2011; Takada et al., 2008). L'augmentation de la densité des cerfs en forêts tempérée a donc été responsable de la simplification de cet écosystème forestier à différents niveaux trophiques (Martin et al., 2010).

Si les effets des cerfs sur la partie aérienne sont aujourd'hui bien caractérisés, les interactions avec le compartiment 'sol' restent cependant encore largement méconnues. Elles sont pourtant fondamentales au vu du rôle des sols dans le stockage du carbone et les cycles des nutriments, et des rétroactions attendues sur la nutrition et la croissance des plantes. Dans un écosystème forestier, les cerfs interagissent avec le sol et les cycles de nutriments à travers plusieurs mécanismes (Schrama et al., 2013a; Wardle and Bardgett, 2004):

- 1) En rejetant de nouvelles litières – urine et fèces – possédant des caractéristiques chimiques propres,
- 2) En compactant le sol par leur piétinement,
- 3) En réduisant la quantité de litière retournant au sol par consommation d'une partie de la matière organique végétale,

- 4) En modifiant la qualité des litières par broutage préférentiel des plantes les plus riches en nutriments qui, à long terme, disparaissent au profit des espèces moins riches (ces dernières étant plus difficilement décomposables).

Ces mécanismes peuvent avoir des effets très différents sur le fonctionnement des écosystèmes, puisqu'ils peuvent à la fois ralentir les cycles des nutriments (via la réduction de la quantité et de la qualité de litière végétale), ou les accélérer (via l'ajout de matière plus facilement décomposable telle que l'urine ou les fèces) (Wardle and Bardgett, 2004). Ceci induit de fortes incertitudes sur les conséquences potentielles de l'activité des grands herbivores et on observe effectivement dans la littérature des effets idiosyncratiques sur le sol (Wardle et al., 2001).

Cette thèse vise donc à mieux comprendre les mécanismes sous-jacents à l'influence des cerfs sur le cycle des nutriments des sols en forêt tempérées. De plus, ce projet cherche également à expliquer une partie des idiosyncrasies observées dans la littérature actuelle sur le sujet. Pour cela, nous avons profité d'une 'expérience naturelle' provoquée par l'introduction du cerf à queue noire en 1878 sur l'archipel d'Haïda Gwaii (Canada). En effet, suite à son introduction et à sa prolifération sur l'archipel, le cerf à queue noire a profondément modifié ces forêts, réduisant significativement la biodiversité végétale et animale (Allombert et al., 2005a, 2005b; Chollet, 2012). Ce projet de thèse s'insère donc à la suite d'un projet à long terme qui s'est intéressé à l'étude de la pression d'herbivorie sur la végétation et la faune de ces écosystèmes forestiers.

L'archipel d'Haïda Gwaii est une zone d'étude sans équivalent pour comprendre les effets de l'introduction et de la densité élevée d'un herbivore. Le cerf à queue noire a en effet été introduit sur certaines îles de l'archipel alors qu'il n'existait aucun grand herbivore par le passé. En comparant des îles colonisées par les cerfs à des îles de référence non colonisées, il est donc possible d'évaluer les effets de l'introduction d'un herbivore sur différentes composantes de l'écosystème. A compter de 1997 ce système a été complété par une expérience de forte réduction de l'abondance des cerfs par la chasse sur une des îles du dispositif permettant un suivi de la réponse de la végétation et de la faune sur près de deux décennies, tout en procurant une situation permettant d'étudier les conséquences de cette

réduction des densités sur les caractéristiques du sol (Chollet et al., 2016). A ceci s'ajoute la construction en 1997 de 20 exclos par le groupe de recherche sur les espèces introduites (RGIS). Ces exclos, d'une surface de 25m², sont délimités par un grillage d'une hauteur de 2,4m prévenant l'entrée du cerf. Ce système permet ainsi d'étudier la résilience de la végétation et du sol après 20 ans d'exclusion du cerf. Enfin l'agence gouvernementale de Parks Canada, qui gère la protection de la faune et la flore sur l'archipel, a organisé pendant l'été 2017 une session d'éradication des cerfs sur certaines îles. La comparaison de l'écosystème l'année précédent, le mois suivant et l'année suivant cette éradication permet de mesurer la résilience de l'écosystème à court terme.

Cette thèse s'organise autour de trois expériences dont les résultats sont décrits et discutés dans les trois chapitres suivants :

Chapitre 2 : Les cerfs ralentissent la décomposition de la litière en réduisant la qualité de la litière dans une forêt tempérée

Dans ce chapitre, nous nous sommes intéressés à l'effet des cerfs sur le processus de décomposition par le biais d'une modification de la composition de la litière, des modifications de la communauté de décomposeurs et des propriétés du sol, ainsi que de l'addition d'excréments. Nous avons comparé le processus de décomposition de trois traitements représentés par trois îles: un écosystème sans cerf, un écosystème colonisé depuis plus de 70 ans, et un écosystème partiellement rétabli après la réduction de la population de cerf. Nous avons mesuré la décomposition de la litière sur une année avec une expérience de sac à litière ou, en anglais, de « litterbags ». Cette technique consiste à remplir de litière végétale des petits sacs possédant une taille de maille suffisamment grande pour laisser passer les organismes détritivores du sol. Ces sacs sont ensuite disposés sur le terrain à la surface du sol puis recueillis après un certain nombre de mois ou d'années, afin de mesurer le stade de décomposition de la litière. Pour ce faire, nous avons recueilli les litières fraîches et sénescents sur les trois îles et comparé leur décomposition au sein de ces trois traitements après un an. Nous avons également transféré la litière entre les îles pour étudier le rôle des propriétés du sol sur la décomposition, indépendamment de la qualité de la

litière. Enfin, nous avons également comparé la décomposition de la litière de l'épinette de Sitka (*Picea sitchensis*) avec et sans les excréments de cerfs sur les trois types d'îles. Pour ces deux expériences, nous avons utilisé des sacs de litières avec deux types de mailles afin d'étudier séparément la décomposition par les microbes de la décomposition par la méso et la macro-faune. Nous avons trouvé que la présence des cerfs ralentissait considérablement la perte de masse pendant la décomposition de la litière, et que ce ralentissement était majoritairement dû à une réduction de la qualité de la litière par les cerfs. La perte de masse observée correspondait principalement à une perte de carbone (C) et d'azote (N), réduite de 21 et 38% respectivement en présence de cerfs. La présence des herbivores a également entraîné une diminution de la capacité des détritivores à décomposer le carbone, mais pas de l'azote. Le niveau de perte de carbone après un an était en effet 5% plus élevé pour la litière en décomposition sur une île sans cerfs. Les effets supplémentaires des cerfs sur le processus de décomposition par le rejet d'excréments étaient significatifs mais mineurs. Ces résultats remettent en question le rôle que la forte augmentation des populations de cerfs observée dans les forêts tempérées peut jouer dans les modèles à grande échelle du cycle du carbone et de l'azote.

Chapitre 3 : La forte abondance des cerfs modifie les propriétés et les communautés de procaryote du sol dans une forêt tempérée

Nous avons étudié l'évolution des propriétés édaphiques et de la structure de la communauté procaryote du sol après la présence et l'exclusion du cerf. Pour cela, nous avons comparé trois systèmes d'études complémentaires, qui représentent les effets des cerfs à court, moyen et long termes. Nous avons tout d'abord comparé des îles non colonisées (îles de Low, Lost et Tar) à des îles colonisées par les cerfs depuis plus de 70 ans (îles de Louise et Lyell) (Vila et al., 2004b). A ce système nous avons intégré deux îles colonisées par les cerfs depuis moins de 35 ans (île de South et West Skedans), ainsi qu'une île colonisée depuis plus de 70 ans mais sur laquelle la densité de cerfs a été régulée par la chasse (île de Reef) (Chollet et al., 2016). Dans un deuxième système, nous avons comparé le sol à l'intérieur et à l'extérieur des 19 exclos installé par RGIS. Enfin, nous avons suivi

l'évolution du sol un an avant et un an après la campagne d'éradication organisé par Parks Canada sur l'île de Ramsay. Dans ce dernier système, nous avons ajouté deux îles témoins : L'île de Tar, qui n'a jamais été colonisée par le cerf, et l'île de Lyell, colonisée depuis plus de 70 ans.

Nous avons mesuré les propriétés physiques (résistance à la pénétration, teneur en eau du sol) et chimiques (pH, teneur en carbone, teneur en azote, teneur en phosphore, concentrations d'ammonium et de nitrate) du sol pour chaque traitement et pour les trois systèmes décrits ci-dessus. Nous nous sommes concentrés sur les procaryotes du sol, qui sont des acteurs importants des cycles de nutriments. Nous avons utilisé la technique de qPCR et de séquençage Illumina du gène de l'ARNr 16S afin de mesurer l'abondance, la diversité et la composition potentielles de la communauté procaryote du sol. Nous avons trouvé que la réponse du sol aux pressions exercées par les cerfs dépend de la durée de présence ou d'exclusion de ces herbivores. Pour des durées inférieures à 35 ans, cette réponse dépend des interactions directes que les cerfs ont avec le sol, à savoir le rejet d'excrément et d'urine ainsi que le piétinement. Pour des durées supérieures à 70 ans, cette réponse dépend à la fois d'interactions directes dues au piétinement et d'interactions indirectes dues à la modification de la végétation. La structure de la communauté procaryote du sol était significativement impactée par la présence prolongée des cerfs, et notamment par la compaction du sol dû au piétinement. La prédiction du profil métagénomique des communautés procaryotes du sol suggère que cette restructuration a des implications pour le cycle du carbone et des nutriments.

Chapitre 4 : La forte abondance des cerfs ne modifie pas le cycle de l'azote dans une forêt tempérée

Dans ce chapitre, nous avons étudié l'impact des cerfs sur les taux de transformation de l'azote en utilisant une méthode de traçage isotopique de l'atome ¹⁵N (Masse et al., 2016; Müller et al., 2004). Cette méthode permet de mesurer les taux bruts et nets du cycle de l'azote. Nous avons comparé l'effet du cerf sur le cycle de l'azote entre trois systèmes d'étude complémentaires. Nous avons d'abord comparé l'effet de la colonisation des cerfs à

long terme sur le cycle de l'azote en utilisant sept parcelles situées sur deux îles sans cerfs (îles Tar et Low) et sept parcelles situées sur deux îles colonisées depuis plus de 70 ans (îles Louise et Lyell). Nous avons ensuite étudié la résilience à moyen terme du cycle de l'azote face à l'exclusion des cerfs dans un sous-ensemble de 4 exclos sur l'île de Graham. Troisièmement, nous avons étudié la résilience à court terme du cycle de l'azote face à l'élimination des cerfs. Pour cela, nous avons suivi le cycle de l'azote un mois après et un an après une campagne d'éradication des cerfs sur trois parcelles situées sur une île sans cerfs, trois parcelles sur une île colonisée depuis plus de 70 ans et trois parcelles avant et après une récente éradication sur une île colonisée depuis plus de 70 ans (Ramsay) à Juan Perez Sound. Les résultats présentés dans ce chapitre sont des résultats préliminaires correspondant à un sous échantillon de cette expérience, et constitué des quatre parcelles d'une île sans cerfs (île Low) et des quatre parcelles d'une île colonisée depuis plus de 70 ans (île Louise). Les sols des autres parcelles ont été analysés expérimentalement, et les données résultant de ces analyses sont actuellement en cours de traitement. Les résultats préliminaires de cette expérience suggèrent une absence d'effet des cerfs sur la cinétique des taux d'azote dans la première couche du sol.

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From deer abundance to soil properties: A case study in the forests of Haida Gwaii

The past century witnessed a dramatic increase in deer abundance in North America and Western Europe that triggered profound changes in the structure of temperate forests. If these changes are today well characterised, the effects of abundant deer belowground in these forests remain unclear. Deer can interfere with the soil through waste deposition, trampling, and reduction of litter quantity and quality by preferential browsing of palatable plants. What are the consequences of these interactions for the soil? To answer this question, we studied the soil response to the colonisation and removal of Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) in the forests of Haida Gwaii. We found that deer slowed-down litter decomposition by reducing litter quality. They also modified microbial community structure and ability in decomposing carbon via soil trampling. Most of these effects became only apparent in the long term, hence questioning the results obtained through short term studies.

Keywords: Ungulate herbivores, Decomposition, Soil prokaryotic communities, Nitrogen cycle

De l'abondance des cerfs aux propriétés du sol: Une étude de cas dans les forêts d'Haïda Gwaii

L'augmentation récente et spectaculaire de l'abondance des cerfs en Amérique du Nord et en Europe occidentale a entraîné de profonds changements dans la structure des forêts tempérées. Si ces changements sont aujourd'hui bien caractérisés, les effets de cette forte abondance sur le sol restent cependant mal compris. Les cerfs peuvent interagir avec le sol par le rejet de fèces et d'urine, le piétinement et la réduction de la quantité et de la qualité de la litière par le broutage préférentiel des plantes appétantes. Quelles sont les conséquences de ces interactions pour le sol ? Pour répondre à cette question, nous avons étudié la réponse des sols à la colonisation et à l'élimination du cerf de Sitka (*Odocoileus hemionus sitkensis*) dans les forêts d'Haïda Gwaii. Nous avons constaté que les cerfs ralentissaient la décomposition en réduisant la qualité de la litière. La structure de la communauté microbienne et sa capacité à décomposer le carbone était impactée par la compaction du sol dû au piétinement. Nous avons également constaté que les effets des cerfs à court et moyen termes n'avaient que peu ou pas d'effet sur le sol, remettant en question les conclusions des études actuelles basées sur de plus court terme.

Mots clefs : Herbivores ongulés, Décomposition, Communautés procaryotes du sol, Cycle de l'Azote.