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Testing for bottom–up effects in an overbrowsed boreal landscape

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On Anticosti Island (Quebec, Canada), overbrowsing by white-tailed deer *Odocoileus virginianus* has substantially modified plant communities and reduced the recruitment of balsam fir *Abies balsamea* seedlings over most of the territory. An exception to this phenomenon has been observed in localised patches occurring on a single geological deposit named Chicotte, where the natural recruitment of balsam fir is occurring even in the presence of a large white-tailed deer population. We hypothesized that edaphic properties within the Chicotte deposit could result in lower forage quality, which in turn could reduce browsing pressure and allow fir regeneration to occur (i.e. bottom–up effects). To test this hypothesis, we measured soil properties and foliage chemistry of four forage species (balsam fir, white spruce *Picea glauca*, Canada mayflower *Maianthemum canadense* and Canada bunchberry *Cornus canadensis*) collected on each of three geological deposits on Anticosti Island: Chicotte, Becscie and Jupiter (the latter two considered as controls). Contrary to expectation, results from principal component analysis suggested that Chicotte was the most fertile, whereas Becscie was the least fertile, of the three deposits. Furthermore, balsam fir foliage chemistry did not respond to geological deposit. Conversely, Mantel et Procrustes tests revealed a significant correlation between soil properties and forage quality for white spruce, consistent with the carbon–nutrient balance hypothesis. Univariate tests confirmed that neutral detergent fiber concentrations in white spruce were higher on the Becscie than on the Chicotte deposit. Likewise, in vitro true digestibility of both white spruce and Canada bunchberry foliage were lower on the Becscie than on the Chicotte deposit. Although we failed to demonstrate why balsam fir recruitment occurs on the Chicotte deposit, our data demonstrate that edaphic properties may affect the quality of some forage types, which potentially affect foraging patterns in overbrowsed boreal landscapes.

Forest diversity and structure may be influenced by both top–down and bottom–up trophic interactions (Hunter and Price 1992, Vucetich and Peterson 2004). For instance, high population densities of herbivores may exert a top–down pressure on plant communities by limiting the recruitment, growth and survival of forage plants while encouraging the expansion of less palatable species (Kielland and Bryant 1998). The resulting plant community structure may, in turn, affect soil properties by controlling rhizosphere interactions, nutrient uptake and plant litter quality (Wardle et al. 2004, Dufresne et al. 2009). Conversely, bottom–up interactions may result from low soil fertility promoting plant chemical defenses against herbivores (Bryant et al. 1983, Coley et al. 1985, Dufresne et al. 2011), which in turn can limit the amount of energy transferred to higher trophic levels. While both top–down and bottom–up processes may occur concomitantly, most studies in forest landscapes characterized by overbrowsing have focused on top–down control (Chollet et al. 2013).

On Anticosti Island, heavy browsing from a large population of introduced white-tailed deer prevents the natural recruitment of native balsam fir *Abies balsamea* (Potvin et al. 2003), the preferred winter forage species. This, in turn, has favoured the recruitment of white spruce *Picea glauca* seedlings (Hidding et al. 2013, Barrette et al. 2014), a less palatable winter forage species for white-tailed deer. Although

these top–down effects seem to control forest succession on most of Anticosti Island, natural regeneration of balsam fir stands can commonly be observed within localized patches over one specific geological deposit named Chicotte (Chouinard and Filion 2005), which covers approximately 700 km² (Supplementary material Appendix 1). These patches of balsam fir regeneration correspond to an area that had previously been dominated by mature balsam fir stands, but was subsequently ravaged in 1971–1972 by an eastern hemlock looper *Lambdina fiscellaria fiscellaria* epidemic. The epidemic covered a total of 1165 km² (15% of the island) and extended far beyond the Chicotte formation, into areas where balsam fir regeneration is uncommon. We thus hypothesized that balsam fir regeneration on the Chicotte formation is driven by low soil fertility, compared to other geological deposits with infrequent occurrence of balsam fir regeneration. Low soil fertility is expected to stimulate carbon-based plant defenses and reduce their digestibility and palatability to herbivores (Bryant et al. 1983).

Overbrowsing on Anticosti Island by white-tailed deer has also reduced the diversity and abundance of preferred summer forage species. For example, many deciduous trees such as paper birch *Betula papyrifera*, as well as a large number of palatable understory plants, have been eradicated on most of the island. Two notable exceptions are Canada mayflower

Maianthemum canadense and Canada bunchberry *Cornus canadensis*, two herbaceous forage species preferred by deer (Crawford 1982, Rooney 1997) that remain abundant on Anticosti Island. To date, there is no assessment of how edaphic properties may affect the nutritional quality of these two summer forage species.

We report on a study performed on Anticosti Island, where we tested for bottom-up controls on forage quality of four forage species. We related the chemical quality and in vitro digestibility of forages to soil properties measured within the Chicotte geological region, as well as within two other geological deposits (i.e. controls) named Becscie and Jupiter. Our data provide presumptive evidence that bottom-up effects may influence foraging patterns in over-browsed boreal landscapes.

Material and methods

Study area

Anticosti Island (7943 km²) is located in the Gulf of St-Lawrence, Canada (49°28'N, 63°00'W). It is part of the eastern balsam fir–white birch bioclimatic zone (Grondin et al. 1996). The sub-boreal maritime climate brings cool summers (mean July temperature of 16°C) and cold winters (mean January temperature of –11°C). The average annual precipitation is 917 mm (Environment Canada 2006), of which approximately one third falls as snow. About 200 white-tailed deer were introduced on the island in 1896, and the population increased rapidly in the absence of natural predators. Accordingly, white-tailed deer herbivory pressure has been drastically transforming plant communities since the late 1920s (Marie-Victorin and Rolland-Germain 1969). Heavy browsing has resulted in a loss of the most palatable plant species at the ground level, across the entire island.

Our study was conducted on three distinct geological deposits. The Chicotte deposit originates from the upper Llandovery (Telychian) and is characterized paleontologically as a speciose and crinoid-rich sand-shoal complex (Desrochers 2006).

The older Jupiter deposit dates back to the late Llandoveryan and is composed primarily of highly fossiliferous limestone (Duffield 1985). The oldest Becscie deposit is predominantly limestone with calcareous shale and siliciclastics, and is characterized by a distinct earliest Silurian (early Rhuddanian) brachiopod fauna (Sami and Desrochers 1992). A map of these three geological deposits is provided in the Supplementary material Appendix 1.

Sampling

On each geological deposit, we established seven sampling plots (700 m²) that each contained the four forage species of interest (balsam fir, white spruce, Canada mayflower and Canada bunchberry). The minimal distance between plots within each deposit was 1 km, which far exceeds the spatial dependence of forest soil chemical properties (Qian and Klinka 1995). Foliar samples of the two summer forage species (Canada mayflower and Canada bunchberry) were collected between 10–14 July, whereas winter forage species (balsam fir and white spruce) were sampled between 1–7

September. For the winter forage species, only mature trees were sampled given the low recruitment of this species on the Jupiter and Becscie deposits. The 84 plant samples (i.e. 7 plots × 3 geological deposits × 4 species) were dried in an air-draft oven at 45°C and ground in a centrifugal mill to pass a 2 mm mesh. For the Becscie deposit, we removed one sample of Canada bunchberry and Canada mayflower from the subsequent analyses due to insufficient plant material.

In the centre of each plot, we established a nine-point soil sampling grid (10 × 10 m) from which we noted the average thickness of the organic forest floor. We then collected one mineral soil core (0–10 cm depth) at each sampling point, and bulked these nine cores into one composite sample per plot.

Forage and soil analyses

To evaluate forage quality, we measured both chemical properties and in vitro true digestibility (IVTD) of each forage type. Proximate C fractions (neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignin (ADL)) were determined based on the protocol of Goering and Van Soest (1970), using a fiber analyzer. Hemicellulose concentration in forage was estimated as the difference between NDF and ADF concentrations, while cellulose concentration was estimated as the difference between ADF and ADL concentrations. Foliar N was measured by high temperature combustion followed by gas analysis, using a CN-analyzer. Total P was determined using a magnesium acetate dry ashing method to avoid volatilisation of organic P (Schulte et al. 1987); ashes were then dissolved into nitric acid, evaporated to dryness, redissolved into a Mehlich III matrix and measured by an ascorbic acid-molybdate colorimetric method (Murphy and Riley 1962).

We determined IVTD of each forage sample based on the protocol described by Goering and Van Soest (1970), using filter bags and an incubator. To do so, we accompanied hunters on Anticosti Island and collected the rumen of seven freshly killed deer. Each rumen was ligatured to maintain anaerobiosis and conserved in a 40°C water-filled insulated chamber for transport, which never exceeded 2 h. The rumen content was filtered through cheese cloths and IVTD (i.e. weight loss) was measured following a 48 h incubation period at 39.5°C.

Fresh soil subsamples were extracted in aqueous 1.0 N KCl and analysed colorimetrically for NH₄⁺ (salicylate–nitroprusside–hypochlorite) and NO₃[–] (cadmium reduction) concentrations. The pH of air-dried and sieved (2 mm mesh) mineral soil was measured in 1:2 (w:w) mixtures with deionized water. Air-dried samples were extracted in Mehlich III solution to which 2% lanthanum oxide had been added (Tran and Simard 1993), and the extracts were analyzed for base cations (Ca²⁺, Mg²⁺, K⁺) using an atomic absorption spectrometer. Total C and N were analyzed by high temperature combustion followed by gas analysis, using a CN-analyzer. Mineral soil texture was determined by the hydrometer method (Bouyoucos 1936) after removing the organic matter by combustion in a muffle furnace (400°C for 24 h).

Statistical analyses

We first performed principal component analysis (PCA) in order to discriminate sampling plots based either on soil

properties or on forage quality. For each PCA, we retained the seven variables that explained the maximum amount of variance in the first two principal components, in order to keep a 3:1 ratio between sites and variables (Grossman et al. 1991, Williams and Titus 1988). From each PCA, we extracted a distance matrix based on the scores along the first two principal components. We then tested for the correlation between soil properties and forage quality among sites by correlating the two score sets using both a Mantel test (Mantel 1967) and a Procrustean approach (Jackson 1995). PCAs, Mantel tests and Procrustes tests were performed using the R *vegan* package (Oksanen et al. 2014). Finally, for each plant species, we tested for statistically significant differences in forage quality between geological deposits, using one-way analyses of variance and Tukey's tests (<www.r-project.org>).

Results

Results of PCA based on soil properties suggest higher soil nutrient concentrations, higher soil pH, and thinner forest floors on the Chicotte than on the Becscie deposit, while soil properties on the Jupiter deposit are intermediate between Chicotte and Becscie (Fig. 1a, Supplementary material Appendix 2). Both Mantel ($r = 0.21$, $p = 0.04$) and Procrustes tests ($r = 0.52$, $p = 0.002$) revealed significant

correlations between PCA scores of soil properties and those of forage quality variables for white spruce. Based on these results, we present PCA biplots for white spruce (Fig. 1b), which show positive correlations between fiber concentrations (NDF, ADF, ADL, hemicellulose) and the Becscie deposit, and a positive correlation between IVTD values and the Chicotte deposit. The results of both Procrustes and Mantel tests are summarized in Supplementary material Appendix 3. One-way analyses of variance showed significant correlations between geological deposits and digestibility for white spruce ($F_{2,18} = 5.03$, $p = 0.02$) and Canada bunchberry ($F_{2,17} = 3.89$, $p = 0.04$), and between geological deposits and NDF concentration for white spruce ($F_{2,18} = 7.3$, $p = 0.005$). Subsequent Tukey's tests confirmed that white spruce foliage collected on the Becscie deposit had significantly higher concentrations of NDF than foliage collected on both Jupiter ($p = 0.04$) and Chicotte ($p = 0.004$) deposits (Fig. 2a), as well as significantly lower IVTD values ($p = 0.02$) than foliage collected on the Jupiter deposit (Fig. 2b). Canada bunchberry also showed significantly higher IVTD values ($p = 0.02$) on the Chicotte than on the Becscie deposit (Fig. 2c). The detailed results of univariate tests are provided in Supplementary material Appendix 4.

Discussion

Our results do not provide evidence that recruitment of balsam fir on the Chicotte deposit resulted from bottom-up controls limiting deer herbivory, as previously hypothesized by Dufresne et al. (2011). On the contrary, balsam fir forage quality did not respond to geological deposits. Furthermore, our data suggest higher soil fertility, and a potential for higher forage quality and higher IVTD on the Chicotte than on the Becscie deposit. Thus, balsam fir regeneration on the Chicotte deposit may have resulted from historical factors. For example, the current balsam fir stands on Chicotte originated from a 1971–1972 eastern hemlock looper *Lambdina fiscellaria fiscellaria* epidemic, which produced large quantities of coarse woody debris (Harmon et al. 1986). Casabon and Pothier (2007) found that coarse woody debris on Anticosti Island could provide provisional safe sites against deer herbivory and increase the recruitment of seedlings by 650%. A higher number of safe sites, combined with higher vertical growth rates resulting from higher soil fertility, may have allowed regenerating balsam fir on the Chicotte formation to escape deer browsing (Coley 1988, Skarpe and Hester 2008). A study from McLaren (1996) supports this hypothesis, as it showed that balsam fir was more resilient to moose herbivory in forest gaps where light availability and growth rates were higher.

In spite of our unexpected results regarding soil fertility on Chicotte relative to the other two deposits, our data did provide presumptive evidence that bottom-up pressures could control forage quality on Anticosti Island, consistent with the carbon–nutrient balance hypothesis (Bryant et al. 1983). This hypothesis stipulates that plants growing under low nutrient availability are more prone to exhibit carbon-based defenses that can reduce their digestibility and palatability to herbivores. The Chicotte and Becscie deposits represent opposite ends in a soil fertility gradient, with Chicotte plots

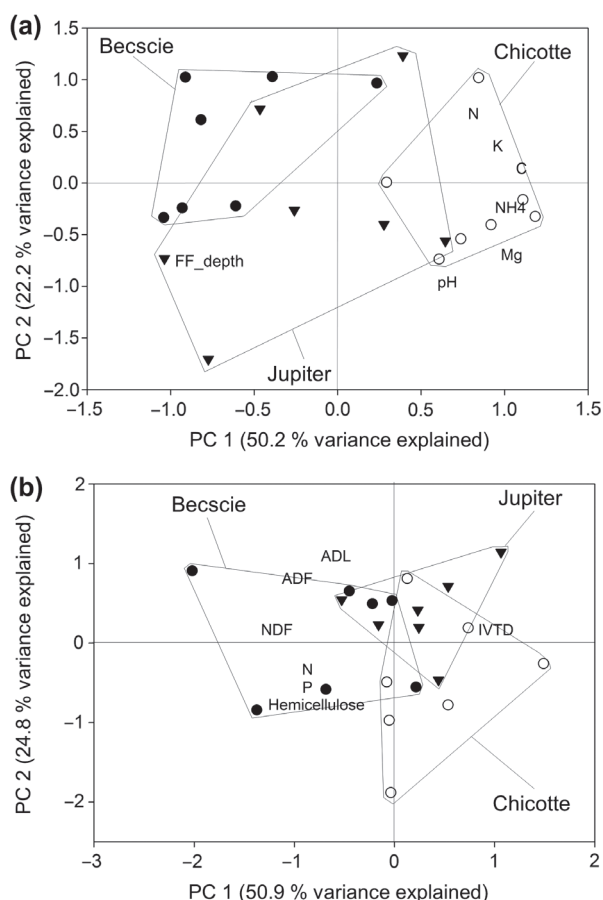


Figure 1. Ordination biplots generated from principal component analyses discriminating sampling plots according to (a) soil chemical properties, and (b) white spruce foliar chemistry. Polygons circumscribe plots found within each of the 3 geological deposits.

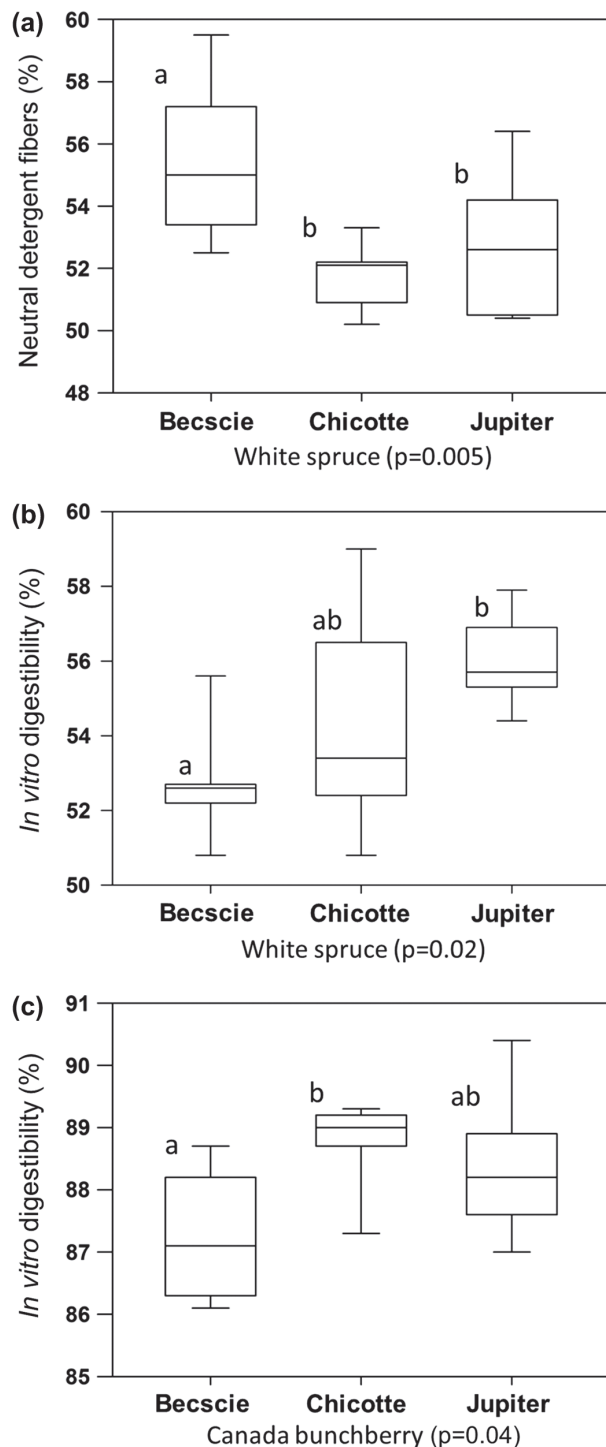


Figure 2. Results of one-way analyses and Tukey's tests describing the effects of geological deposit on (a) in vitro true digestibility of white spruce foliage, (b) neutral detergent fiber concentration of white spruce foliage, and (c) in vitro true digestibility of Canada bunchberry foliage. Statistically significant differences in forage quality are denoted by different lower-case letters.

being positively correlated with soil nutrient concentrations and Becscie plots being positively correlated with forest floor depth (i.e. amount of sequestered nutrients). Results from multivariate Mantel and Procrustes tests suggest that these differences in soil properties affected forage quality of white spruce. Univariate Tukey's tests confirmed significantly higher

NDF content in white spruce foliage that was collected within the Becscie deposit. NDF are structural carbon-based compounds and their concentration in forages is generally negatively correlated with digestibility and food intake (Van Soest 1994). Furthermore, univariate tests confirmed a lower IVTD for white spruce and Canada bunchberry foliage from the Becscie than from the Chicotte deposit. The question remains as to why such an effect was not observed for the other two forage species in our study (i.e. Canada mayflower and balsam fir). Notwithstanding this conundrum, our data illustrate that soil properties may affect forage quality and digestibility, which in turn could affect foraging patterns in overbrowsed boreal landscapes.

Further research is needed to explain how localized events of balsam fir regeneration on Anticosti Island are occurring. For example, it would be useful to correlate geological deposits with plant secondary metabolites that affect forage quality for herbivores, such as tannins (Robbins et al. 1987) and other phenolics (Stolter et al. 2010). Such knowledge could help forest managers limit the loss of native balsam fir forests, as well as help ecologists better understand plant-herbivore interactions.

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Supplementary material (available online as Appendix wlb.00135 at <www.wildlifebiology.org/readers/appendix>). Appendix 1–4.