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The cost of deer to trees: changes in resource allocation from growth-related traits and phenolic content to structural defence

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Background and aims – Plants may use various defence mechanisms to protect their tissues against deer browsing and the allocation of resources to defence may trade-off with plants’ growth. In a context of increasing deer populations in European forests, understanding the resource allocation strategies of trees is critical to better assess their ability to face an increasing browsing pressure. The aim of this study was to determine how deer removal affects the resource allocation to both defensive and growth-related traits in field conditions for three tree species (*Abies alba*, *Picea abies* and *Fagus sylvatica*).

Methods – We compared eight pairs of fenced-undefenced plots to contrast plots with and without browsing pressure. The pairs were set up in 2005 and 2014 to compare different fencing duration. We measured leaf and shoot traits related to the defence against herbivores (phenolic content, structural resistance, C:N ratio) and to the investment in plants’ growth and productivity (specific leaf area and nutrient content).

Key results – For the three species, the structural resistance of leaves and shoots was negatively correlated with SLA, nutrient content and phenolic content. For *Abies alba*, exclusion of deer decreased shoot structural resistance in favour of higher nutrient content, SLA and phenolic content. The fencing duration had no effect on the different measured traits.

Conclusions – Our results support the assumption of a trade-off between structural defence and growth-related traits at the intraspecific scale for the three studied species. We also confirmed the hypothesis that exposure to deer browsing is involved in the resource allocation of woody species. For *Abies alba*, fencing led to a change in resource allocation from structural defence to growth-related traits and chemical defence.

Keywords – Deer browsing; functional traits; mixed forests; resource allocation; chemical defence; structural defence; fencing experiment.

INTRODUCTION

The modification of the chemical composition and/or structural properties of leaves and shoots is a well-known defence mechanism used by plants against herbivores (Keefover-Ring et al. 2016; Ohse et al. 2016). Plants chemical defence corresponds to the production of secondary metabolites – e.g. phenolics, terpenes – that reduces the digestibility and nutritional value of plant tissues and can even have toxic effects for herbivores (Iason 2005; Bergvall & Leimar 2017). Structural defence can be described as the production of spines, hairs or cuticles, or as an increase in leaf and shoot thickness, toughness (resistance to fracture) or stiffness (resistance to deformation), or a combination of these three properties (Carmona et al. 2011; Pérez-Harguindeguy et al. 2013). Former theories attempting to explain the allocation of resources to chemical and structural defence were based on the availability of resources (Bryant et al. 1983; Ayres 1993; Wise & Abrahamson 2005). These ideas have notably been questioned by the optimal defence theory, which assumes that the allocation of resources to defence can be predicted based on three identified factors: the value of the plant tissue to the plant, the cost of defence and the likelihood of being attacked by herbivores. Under this theory, the acclimation to environmental factors such as temperature, drought or herbivory may influence a plant's resource allocation (Hamilton et al. 2001). The optimal defence theory is supported by several observations on woody species showing increasing leaves' phenolic content or structural resistance in response to browsing by wild ungulates not only in controlled environments (Keefover-Ring et al. 2016; Nosko & Embury 2018) but also in field conditions (Ohse et al. 2016).

The growth of tissues, the synthesis of defensive compounds and cellular differentiation processes (such as the lignification of cell walls or the thickening of the leaf cuticle) all compete for the same pool of carbohydrates (Ayres 1993), implying a trade-off between the allocation of resources to defence and growth (Bryant et al. 1983; Herms & Mattson 1992). This trade-off in resource allocation has been well described at the interspecific scale through the comparison of selected leaf traits – namely leaf nitrogen (N) and phosphorus (P) content, photosynthetic activity, leaf lifespan, dark respiration rate and leaf mass per area – across a wide range of plant species (Wright et al. 2004). Plant species investing in defence against herbivores are characterized by long leaf lifespans, high level of chemical and/or structural defence and thus by an effective conservation of resources (Wright et al. 2004). On the contrary, plant species investing in high growth rates are characterized by a short leaf lifespan, and a high N and P content and specific leaf area (SLA), allowing for a higher carbon gain per unit area that can be invested in the production of leaves (Westoby et al. 2000). Although inter-specific differences are well established, this pattern has rarely been described at the intraspecific scale, as most trait-based studies on herbivory focus on defensive traits without addressing the potential cost of an investment in chemical and/or structural defence.

European mixed forests have been facing major increases in deer populations, and current population levels alter the diversity of forest tree species and potentially decrease these

forests' ecological and economical value (Hegland & Rydgren 2016; Boulanger et al. 2018; Ramirez et al. 2019). A better understanding of seedlings and saplings resource allocation strategy is thus essential to assess woody species ability to face an increasing browsing pressure. In this study, using experimental paired fenced and unfenced plots set in 2005 and 2014, we investigated how deer removal affects the resource allocation to both defensive and growth-related traits in field conditions for three emblematic species of mixed forests in European mountains: silver fir (*Abies alba*), Norway spruce (*Picea abies*) and common beech (*Fagus sylvatica*). We hypothesized that (i) the three species invest in structural and/or chemical defence at the expense of nutrient content and SLA (i.e. growth-related traits) and that (ii) deer removal induces a lower investment in defensive traits and a higher investment in growth-related traits.

MATERIALS AND METHODS

Study area and experiment design

The experiment was conducted in La Petite Pierre National Hunting and Wildlife Reserve (NHWR), a 2700 ha unfenced forest area located in the Vosges mountain range, in north-eastern France (48.5°N, 07.0°E, 200–400m a.s.l.). The two large herbivore species present in the area are red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). Figure 1 presents the yearly abundance dynamics of these two species in La Petite Pierre NWHR, estimated as the number of individuals observed per kilometre using the methodologies developed by Garel et al. (2010) for red deer and Vincent et al. (1991) for roe deer. See Storms et al. (2008) for a more detailed description of the study site.

We compared eight pairs of fenced-unfenced plots of 0.36 ha each to contrast plots with (unfenced) and without (fenced) browsing pressure. Pairs of fenced-unfenced plots are hereinafter referred to as experimental units. Three experimental units were set in 2005 – i.e. 12 years of fencing duration (FD) – including two in mixed oak-beech stands and one in a pure silver fir stand. Five experimental units were set in 2012 – i.e. three years of FD – including three in mixed oak-beech stands and two in pure silver fir stands. The fences were 2 m high with a 10 × 10 cm mesh size. The fences were subject to careful maintenance and no marks of browsing were observed in the fenced plots since their establishment.

Data collection

Among each experimental unit located in mixed oak-beech stands, we sampled 6 beech individuals (3 fenced and 3 unfenced). Among each experimental unit located in pure silver fir stands, we sampled 10 silver fir and 10 Norway spruce individuals (5 fenced, 5 unfenced). In the unfenced plots, we only sampled individuals on which we could observe evidence of browsing damage on the current-year shoots. As the sampling occurred two months after budburst, the unfenced individuals were thus browsed in the last two months.

We sampled the shoots for fir and spruce and leaves for beech since leaves of beech are more frequently found in red deer and roe deer rumen than the whole shoots, whereas for

fir and spruce, the shoots are more frequently found (S. Said, unpublished data). For all the species sampled, we collected three current year's shoots (conifers) or leaves (beech) per individual. We collected leaves and shoots on the highest parts of the plant – i.e. leaves and shoots located above 75% of the plants' height – as these parts are targeted in priority by both deer species. The height of the sampled individuals ranged from 10 cm to 160 cm.

Global radiation above each individual tree was recorded using a light meter with a quantum sensor (LiCor LI-250A, Lincoln, NE). Once a plot was sampled, the total incident light was also measured in a clearing close to the plot. The available light for each individual tree was computed as the ratio of these two measurements. All the light measurements were performed in summer, between 10 AM and 3 PM and during clear weather.

Plant structural defence

Young's modulus and maximum tensile stress were measured as these two traits are correlated with the plant's carbon investment in the structural protection of photosynthetic tissues, notably against herbivores (Cornelissen et al. 2003). Young's modulus in tension is the initial linear slope of the curve representing the variation of tensile stress (ratio of the force applied to the section area) with elongation. Maximum tensile stress is the ratio of the maximum stress to the section area. These two traits are expressed in MPa. Tensile tests were chosen because they represent a convenient way of measuring stiffness and maximum resistance and because they are geometrically similar to the biting/gripping then pulling-to-failure gesture used by deer when browsing.

All mechanical tests were performed within 48 h following the collection of all samples, all kept in humid conditions. Needles of fir and spruce were cut away from the stem prior to tensile tests and kept for SLA and chemical measurements. The Young's modulus and maximum stress characteristics of leaves and shoots were measured using an electronic mechanical testing device equipped with a load cell of 125N (In-Spec 2200, Instron Corporation, Norwood, MA, USA). Tensile tests were performed on leaves of common beech and on the current-year, leafy shoots of silver fir and Norway spruce, following the basic protocol of Vincent (1990, 1992). For each parameter, the mean value for an individual was computed as the mean of the two closest values out of the three plant fragments sampled per individual. Higher values of these two traits indicate a higher level of structural defence.

Chemical and morphological analysis

The three samples of each individual were pooled so that each chemical analysis was performed at an individual scale. Beech leaves and fir and spruce needles were scanned to compute the foliar surface (mm^2) using ImageJ version 1.51 software (Rasband 1997). The samples were then oven-dried and weighed to obtain their dry mass. SLA of each individual was calculated as the ratio of the foliar surface and the dry mass, expressed in $\text{mm}^2 \cdot \text{mg}^{-1}$ of dry weight.

The leaves and needles of each individual were then ground to a fine, homogenous powder – particle size of approximately $10 \mu\text{m}$ – with a ball mill (Retsch MM2-type, Haan, Germany). To measure the P content, the leaf extract of each individual was mineralized using a mixture of nitric acid and oxygenated water. The solution was analysed by col-

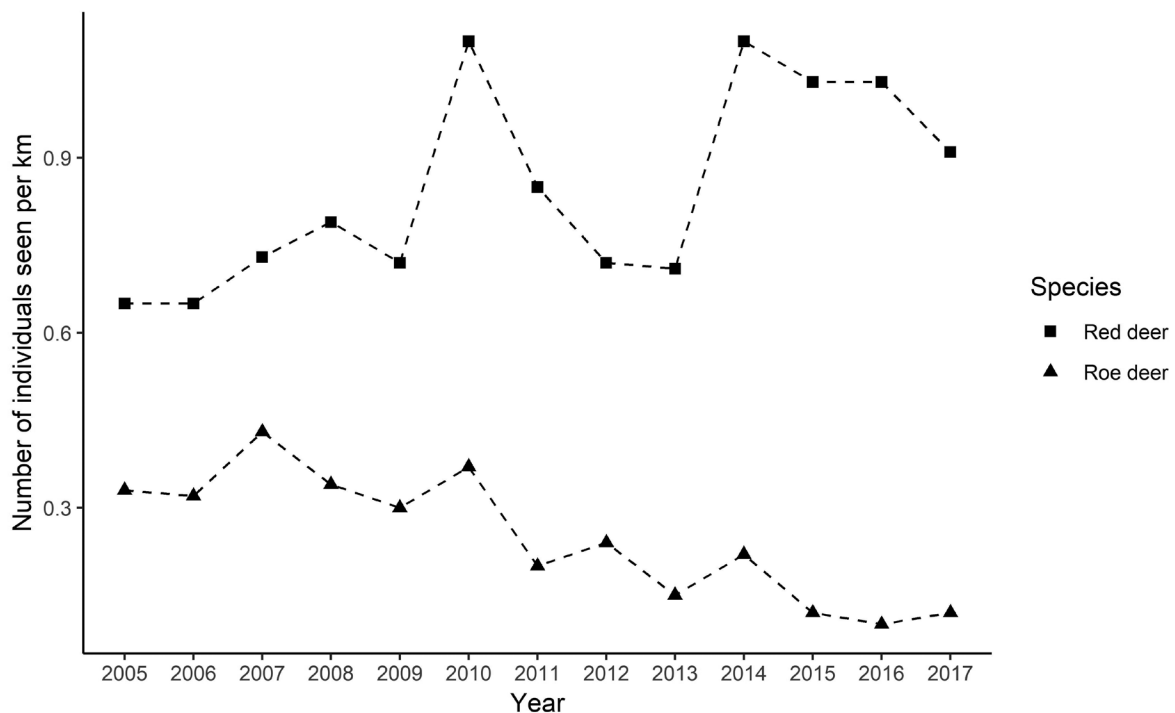


Figure 1 – Yearly dynamics of the mean number of roe deer (pedestrian kilometric index according to Vincent et al. 1991) and red deer (spotlight counts according to Garel et al. 2010) observed per kilometer in La Petite Pierre NHWR from 2005 to 2017.

ometry with a VICTOR3 Multilabel Plate Reader (Wallace 1420 Software) using the ammonium molybdate ascorbic acid method (Kuo 1996). The optic density was measured at 880 nm and compared to a calibration curve to obtain the P content. The total phenolic concentration was determined with a colorimeter (DR/890 – HACH Company, Colorado, USA) using the Hach Tanniver method (method no. 8193, Hach, Loveland, CO, USA). N and C content were measured with an elemental analyser (Thermo Finnigan, Flash EA 1112, Thermo Fisher Scientific, Waltham, MA, USA). N, P and C content were expressed in percentage of dry weight and the content of total phenolics was expressed in mg.g⁻¹ of dry weight.

Data analyses

As the budburst dates of the individuals sampled varied among stands and individuals, the measured structural properties could be influenced by the age of the leaves or shoots. To overcome this bias, we used the length of the samples as an estimator of its age in relation to budburst. We modelled the development of the two structural properties (maximum stress and Young's modulus) with the length of the samples using a simple linear regression model for each species. The statistical analyses were then performed on the residuals of this model. For the model of silver fir only, we log-transformed the data prior to analysis to correct the non-normality of the residuals.

We studied the correlations between defensive and growth-related traits with a principal component analysis (PCA) on the seven studied traits – i.e. SLA, C:N ratio, N and P content, phenolic content, Young's modulus and maximum stress – using the vegan package (Oksanen et al. 2019).

To assess the effect of browsing on SLA, C:N ratio, N and P content, phenolic content, Young's modulus and maximum stress, we conducted for each species linear mixed models with the lmer function of the lme4 package (Bates et al. 2014), followed by a Type-II Anova using the car package (Fox & Weisberg 2011). We included fencing (three levels ordered factor: “12-years fencing”, “3-years fencing” and “unfenced”) as fixed effect and experimental unit as random effect [$Y \sim \text{fencing} + (1|\text{experimental unit})$]. For significant models, the differences between the fencing levels were tested with Tukey's post-hoc test.

In order not to be limited to a simple trait-by-trait comparison and to take into account the correlations between defensive and growth-related traits, we studied for each species the multivariate response of the seven studied traits to fencing by means of partial redundancy analyses (pRDA) conditioned for the experimental unit [$Y \sim \text{fencing} + \text{Condition}(\text{experimental unit})$]. Significance of the model and of the ordination axes was tested with Monte Carlo test with 999 permutations. We performed one-way ANOVA followed by Tukey's post-hoc test on the coordinates of the records on each significant axis to investigate the overall fencing effect. Partial RDA were conducted using the vegan package (Oksanen et al. 2019). All the analyses were performed with R statistical framework (R Core Team 2017).

RESULTS AND DISCUSSION

Correlations among traits

For the three species, on the first PCA axis, traits related to structural defence – i.e. Young's modulus and maximum stress – are opposed to growth-related traits – i.e. SLA and nutrient content – and to phenolic content (fig. 2). The negative correlation between structural defence and SLA, N and P content is consistent with our initial hypothesis that tree species tend to invest in structural defence at the expense of growth-related traits. This result confirms, at least for the three studied species, that the trade-off between growth-related and defensive traits, found by Wright et al. (2004) at the interspecific scale, is also valid at the intraspecific scale.

However, we also found that for the three studied species, phenolic content is positively correlated with SLA and nutrient content and negatively correlated to structural defence (fig. 2). This result is inconsistent with the literature and with our initial hypothesis as most studies reports that chemical defence trade-offs with growth (Bryant et al. 1983; Herms & Mattson 1992). Hanley & Lamont (2002) found a similar result with species of the genus *Hakea* that had either high SLA and phenolic content, either high structural resistance. This result might be explained by a trade-off between chemical and structural defence. Because plant species have access to a limited pool of resources and can produce different types of defence against herbivores, several authors have discussed the potential trade-off between chemical and structural defence across plant species (Steward & Keeler 1988; Twigg & Socha 1996; Moles et al. 2013). Although Twigg & Socha (1996) observed a negative correlation between chemical and structural defence among several herbaceous species of the genus *Gastrolobium*, such correlation was to our knowledge never found among woody species (Steward & Keeler 1988; Moles et al. 2013). Our results suggest the existence of such a trade-off at the intraspecific scale with individuals investing either in growth and chemical defence, either in structural defence. A possible interpretation for this strategy is that according to Koricheva (2002), the production of phenolics is less costly for plants growth than other defensive compounds such as alkaloids or terpenes. It is thus possible that the studied species privileged either growth while maintaining a relatively low-cost type of defence – i.e. phenolics – either a costly but possibly more efficient type of defence – i.e. structural resistance – at the expense of growth.

It is interesting to note that the C:N ratio is positively correlated to structural resistance for silver fir (fig. 2A) and to chemical defence for spruce and beech (fig. 2B, C). Low values of leaf C:N ratio usually reflect a high investment in growth and in resource acquisition whereas high values indicate an investment in the conservation of resources and in the defence against herbivores (Mondolot et al. 2008). However, the types of defence that are associated with a high C:N ratio are still debated : depending on the studied species, it was found that C:N ratio may be positively correlated to chemical defence only (Royer et al. 2013), to structural defence only (Agrawal & Fishbein 2006) or to both types of defence (Mondolot et al. 2008). Our results support the assumption that a high C:N ratio indicates either an investment in structural defence (for silver fir here), either in chemical defence

Table 1 – Foliar trait differences between individuals exposed and unexposed to deer (mean ± standard error).

The effect of fencing on each functional trait was tested with mixed models [$Y \sim \text{fencing} + (1|\text{experimental unit})$]. Bold values indicate a significant fencing effect ($\alpha = 0.05$). Groups with different letters are significantly different (Tukey's post-hoc test).

	Unfenced (mean ± se)	3-years fencing (mean ± se)	12-years fencing (mean ± se)	$\chi^2_{df=2}$	p-value
Silver fir					
SLA	8.8 ± 0.8 a	9.4 ± 0.6 ab	11.0 ± 0.2 b	7.4	0.025
C:N	49 ± 4 a	43 ± 3 b	36 ± 3 ab	8.8	0.012
N	0.010 ± 0.000 a	0.011 ± 0.000 a	0.013 ± 0.000 a	8.0	0.018
P	0.066 ± 0.009	0.110 ± 0.029	0.110 ± 0.018	5.8	0.055
Phenol	0.017 ± 0.001	0.019 ± 0.002	0.023 ± 0.003	3.5	0.180
Young mod.	-0.013 ± 0.280	0.210 ± 0.191	-0.650 ± 0.180	0.16	0.920
Max. stress	0.074 ± 0.199	0.230 ± 0.119	-0.700 ± 0.171	2.2	0.340
Norway spruce					
SLA	9.8 ± 1.0 a	8.2 ± 0.8 a	19.0 ± 7.6 b	14	< 0.001
C:N	39 ± 2	40 ± 4	29 ± 7	2.5	0.280
N	0.013 ± 0.001	0.013 ± 0.001	0.017 ± 0.004	3.0	0.220
P	0.13 ± 0.02	0.11 ± 0.02	0.05 ± 0.00	4.0	0.140
Phenol	0.022 ± 0.002	0.018 ± 0.002	0.027 ± 0.008	3.5	0.180
Young mod.	-11 ± 13	11 ± 14	-7 ± 1	2.5	0.280
Max. stress	-0.29 ± 0.27	0.64 ± 0.38	-0.15 ± 0.04	4.7	0.097
Beech					
SLA	24 ± 1	24 ± 1	28 ± 3	3.5	0.170
C:N	21 ± 1	20 ± 2	22 ± 1	1.7	0.420
N	0.023 ± 0.001	0.026 ± 0.002	0.022 ± 0.001	2.5	0.280
P	0.054 ± 0.006	0.050 ± 0.005	0.076 ± 0.012	4.5	0.110
Phenol	0.043 ± 0.003	0.042 ± 0.008	0.043 ± 0.005	0.80	0.670
Young mod.	560 ± 32	620 ± 37	480 ± 33	2.0	0.360
Max. stress	21 ± 1	20 ± 1	21 ± 1	1.9	0.390

(for beech and spruce) and that there is a high interspecific variability in these correlations.

Effect of browsing on resource allocation – univariate approach

We found no variation of the leaf phenolic content with the fencing treatment for the three species (table 1), although phenolics are widely known as one of the main defence mechanisms used by plants against herbivores (Iason 2005; Nosko & Embury 2018). This result may be linked to the kinetics of inducible defence: depending on the plant and the herbivore species, there is a high variability in the persistence of herbivore-induced chemical changes. Changes can occur within two minutes (Furstenburg & van Hoven 1994) or a few hours (Schultz et al. 2013) after the herbivore attack and may persist for several months (Tarald et al. 2017). For instance, Furstenburg & van Hoven (1994) reported a return to normal of the levels of condensed tannins of *Acacia* spp. only two hours after being browsed by giraffes. As we are not able to precisely estimate the day when the sampled sap-

lings had been browsed, we cannot exclude the possibility that phenolic concentration had already returned to its pre-disruption level before the time of sample collection. Moreover, our study focuses on total phenolics which include a vast array of molecules with diverse functions that are not strictly limited to anti-herbivore defence (Close & McArthur 2002). It is thus possible that browsing only affected a specific molecule or a small set of molecules among total phenolics: in that perspective, the effect of browsing may have been diluted by the other phenolic compounds that were not affected by herbivory. Similarly, Väisänen et al. (2013) found that deer browsing could induce variations in the relative proportions of different phenolic compounds without changing the total foliar phenolic content. Finally, it cannot be excluded that the studied species responded with other secondary metabolites – e.g. terpenes or alkaloids – as it has been reported among several woody species (Langenheim 1994; Iason 2005).

For the three species, the fencing treatment has no effect on seedlings and saplings Youngs modulus and maximum stress (table 1). However, fenced silver fir individuals have higher SLA and nutrient content and lower C:N ratio than

unfenced individuals (table 1). This result indicates that the investment in growth and in resource acquisition is higher for individuals protected from deer browsing for this species. Considering the traits correlations revealed by the PCA analysis for this species (fig. 2A), these observations also suggest a lower investment in structural defence and a higher investment in chemical defence but multivariate testing of the fencing effect is necessary to confirm this trend.

Effect of browsing on resource allocation – multivariate approach

For silver fir, the RDA performed with the seven studied traits – i.e. SLA, C:N ratio, N and P content, phenolic content, Young’s modulus and maximum stress – revealed that shoots are significantly affected by the fencing treatment (ta-

ble 2). The first axis of the RDA, which accounts for 17.7% of the variance explained, contrasts fenced individuals characterized by a high SLA, nutrient content and phenolic content with unfenced individuals characterized by a high C:N ratio and maximum stress (fig. 3). Overall, the PCA analysis (fig. 2A), the univariate models (table 1), and the RDA (fig. 3) are consistent and converge on the idea that for silver fir, fencing leads to a switch in resource allocation from structural defence to growth and chemical defence.

Most of the saplings we sampled were older than three years, and thus individuals from the 2014-fenced-plots likely experienced browsing before fence protection, unlike the saplings from the 2005-fenced-plots. However, for silver fir, the post-hoc analysis on the 1st RDA axis revealed no significant differences between individuals fenced for 3 years and for 12 years (fig. 3). The trait differences between fenced and

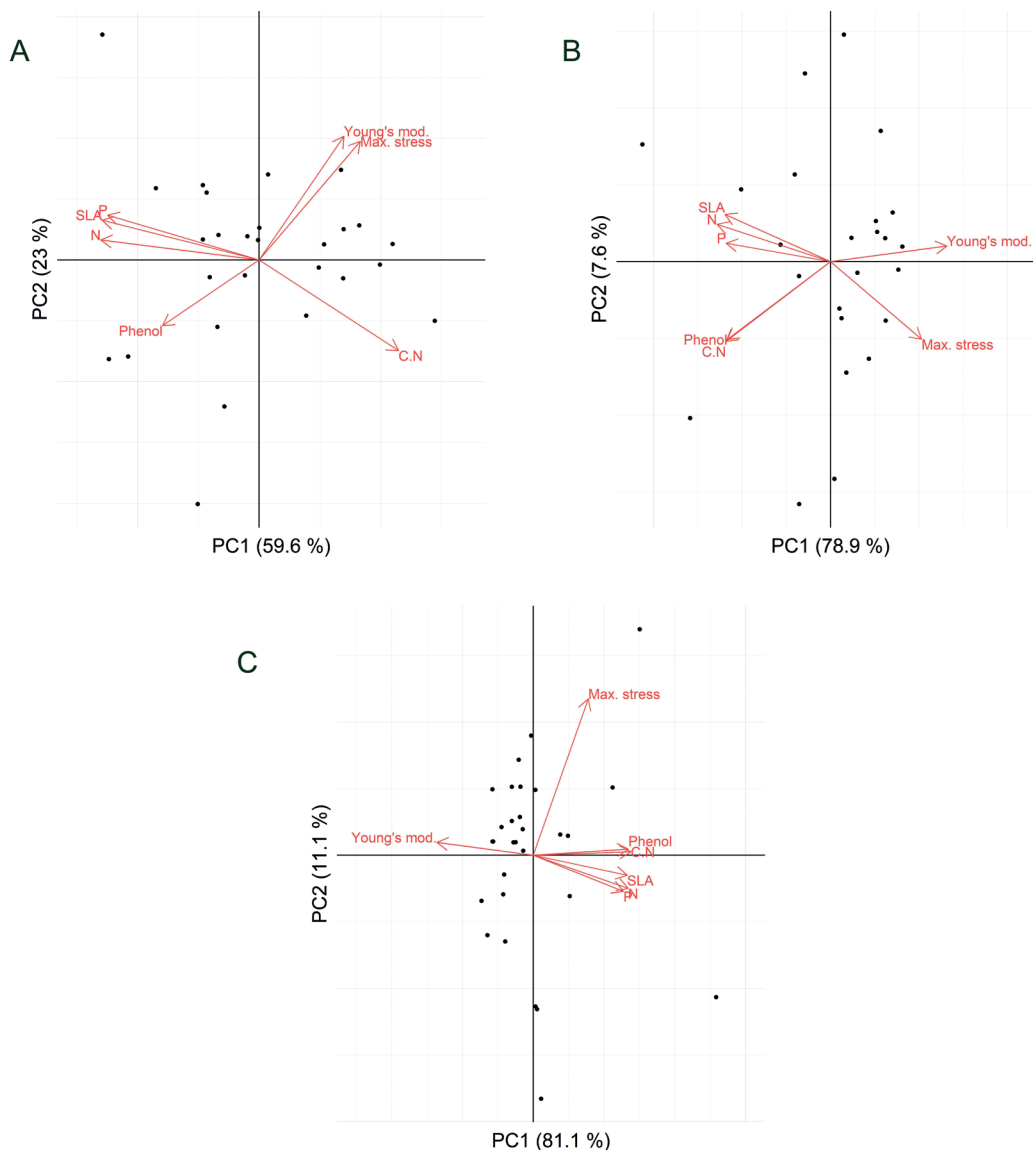


Figure 2 – Principal Component Analysis performed with silver fir (A), Norway spruce (B) and beech (C) data. Variables tested are SLA, C:N ratio, N and P content, phenolic content, Young’s modulus and maximum stress. PC1 and PC2 respectively stand for 1st and 2nd principal component.

Table 2 – Adjusted R², F-values and p-value obtained from the RDA testing the effect of the fencing treatment on the three studied species functional traits [traits ~ Fencing + Condition(experimental unit)].

RDA was performed for each species with SLA, C:N ratio, N, P, phenolic content, maximum stress and Young's modulus as response variables. F-values and p-values were obtained with the Monte Carlo permutation test (n = 999, $\alpha = 0.05$). Bold value indicates a significant fencing effect.

	Adjusted R ²	F-value	p-value
Silver fir	0.07	2.7	0.012
Norway spruce	0.00	0.94	0.424
Beech	0.00	0.42	0.770

unfenced plots are thus not affected by the duration of deer removal. Although this might be the consequence of a relatively low number of replicates, this result also suggests that the observed variability is mainly explained by phenotypic plasticity rather than by evolutionary mechanisms. Under this assumption, the resource allocation of silver fir saplings would mainly depend on the recent occurrences of browsing – the unfenced individuals were browsed in the last two months before the sampling – rather than on the genetic differences between individuals or on previous occurrences of browsing (Holeski et al. 2012).

For Norway spruce and beech, mixed models show that only a very limited number of traits – i.e. SLA for spruce – are affected by fencing (table 1). The RDA permutation test confirms this trend and reveals no significant effect of fencing for Norway spruce and beech (table 2). It is interesting to note that among the three studied species, only silver fir

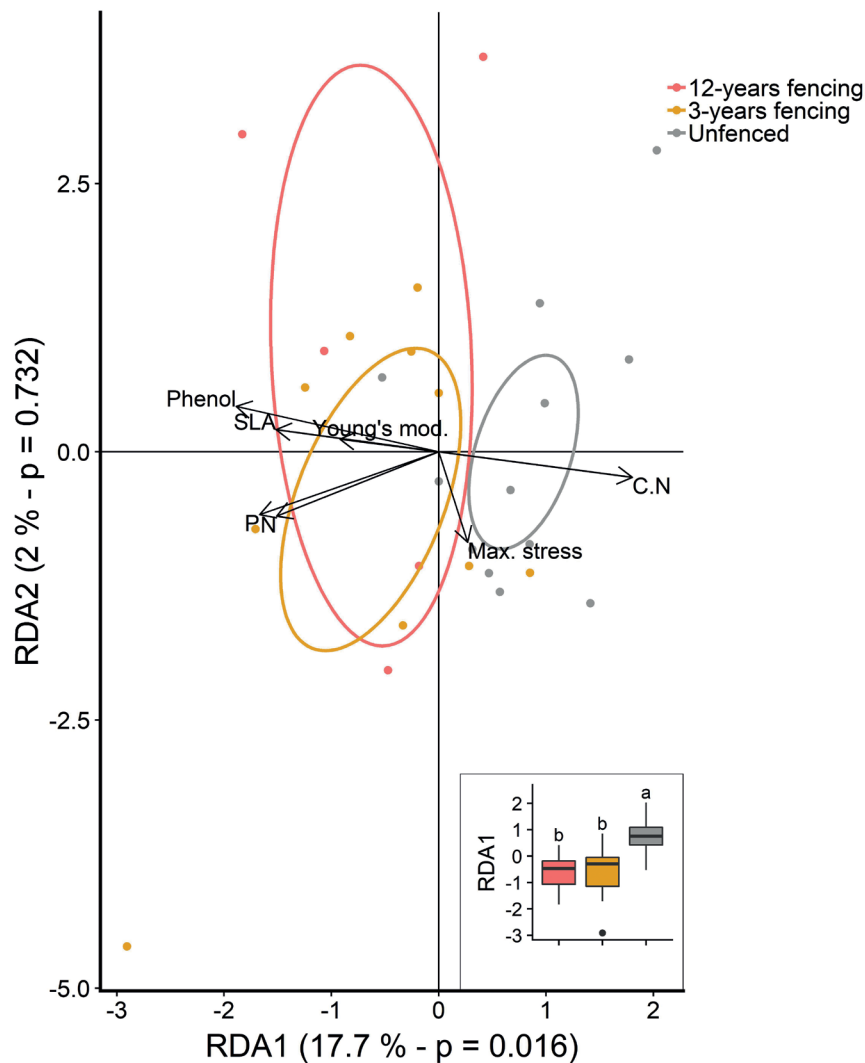


Figure 3 – Scatter diagram of the two first axes of the partial RDA testing the effect of the fencing treatment on silver fir functional traits [traits ~ Fencing + Condition(experimental unit)]. The traits included in the RDA are SLA, C:N ratio, N and P content, phenolic content, Young's modulus and maximum stress. RDA 1 and RDA 2 respectively stand for 1st and 2nd axis of the RDA. The fencing factor is represented by standard-error ellipses around group centroids with a confidence level of 0.95. The inset shows the differences in RDA1 coordinates between the three levels of the fencing treatment, tested by a one-way ANOVA followed by Tukey's post-hoc test.

is affected by the fencing treatment. This could be explained by the fact that this species has been reported to be more frequently browsed by ungulates than spruce and beech (Storms et al. 2008). We may thus assume that silver fir is more likely to develop defence mechanisms against herbivores because it faces greater browsing pressure than the two other species.

Previous work on plant defences have underlined the importance of multi-trait approaches that also detect the cost of resource allocation to defence (Harvell 1990). Our results show that the effect of deer browsing is not limited to defensive traits and that the growth capacity of plants may be affected as well. In the case of woody species in managed forests, this indirect effect merits further consideration as it may have important consequences in the long run for forest growth and productivity.

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