Simulated winter browsing may lead to induced susceptibility of willows to beavers in spring

Annelies J. Veraart, Bart A. Nolet, Frank Rosell, and Peter P. de Vries

Abstract: Browsing may lead to an induced resistance or susceptibility of the plant to the herbivore. We tested the effect of winter browsing by Eurasian beavers (*Castor fiber* L., 1758) on food quality of holme willows (*Salix dasyclados* Wimm.) in and after the following growth season. Shrubs were pruned in February, and new shoots from these (cut) shrubs were compared with those of untreated (uncut) ones in May and November. The shoots were analysed for dry matter, nitrogen, acid detergent fibre, and total phenolics. In May, the leaves from the cut treatment had a better food quality (more water, more nitrogen, and less phenolics) than those from the uncut one. There was in part also a systemic response, with lower total phenolics in both the cut and untreated parts of pruned shrubs (uncut–cut) than in the uncut shrubs. In November, we did not find significant differences in biochemistry of bark among cut, uncut, or uncut–cut treatments. These results are in accordance with a cafeteria experiment in the field: in May the beavers preferred shoots from the cut treatment, but in November they showed no preference. The results suggest that willows invest in compensatory growth rather than a defence response early in the regrowing phase.

Résumé : Le broutement peut provoquer chez la plante soit une résistance soit une vulnérabilité à l'herbivore. Nous avons vérifié l'effet du broutement en hiver par les castors d'Europe (*Castor fiber* L., 1758) sur la qualité de la nourriture fournie par le saule à rameaux velus (*Salix dasyclados* Wimm.) durant la saison suivante de croissance et par la suite. Nous avons émondé les buissons en février et comparé en mai et en novembre les nouvelles pousses de ces buissons « taillés » à celles de buissons « non taillés ». Nous avons soumis les pousses à des analyses de matière sèche, d'azote, de fibres au détergent acide et de substances phénoliques totales. En mai, les feuilles des buissons taillés représentent une nourriture de meilleure qualité (plus d'eau, plus d'azote, moins de substances phénoliques) que celles des buissons non taillés. Il y a aussi une réaction systématique partielle, puisque les concentrations de substances phénoliques totales, tant dans les parties émondées que non émondées (« non taillés – taillés ») des buissons ébranchés, sont moins importantes que dans les buissons non traités. En novembre, il n'existe pas de différence biochimique significative entre les écorces des buissons taillés, non taillés et non taillés – taillés. Ces données s'accordent avec les résultats d'une expérience de type cafétéria faite en nature : en mai, les castors préfèrent les pousses provenant de buissons taillés, mais en novembre ils ne montrent aucune préférence. Ces observations indiquent que les saules investissent en une croissance compensatoire plutôt qu'en une réaction de défense durant les premières phases de la régénération.

[Traduit par la Rédaction]

Introduction

In a reaction to herbivory, a plant faces a trade-off between regrowth and defence (Freeland et al. 1985; Van der Meijden et al. 1988; Tallamy and Raup 1991). If the plant invests in compensatory growth (McNaughton 1983), browsing may lead to increased susceptibility to herbivory, not only because the plant is still undefended but also because the newly grown parts tend to be rich in nutrients (Baker et al. 2005). For example, several studies on birch (genus *Betula* L.) and willow (genus *Salix* L.) showed that winter browsing led to a higher water content and higher nutrient levels of the leaves in the next growing season, probably because of an increase in the root/shoot ratio (Bryant et al. 1985; Danell et al. 1994; Bryant 2003; Riipi et al. 2005; Stolter et al. 2005). In contrast, if the plant invests in defence (the defence response), full compensation of herbivore losses may not be achieved (Peinetti et al. 2001). These plant responses to herbivore damage may in turn be important to the herbivores, affecting their growth and survival (Nykänen and Koricheva 2004) or reproduction (Bryant et al. 1985; Bryant 2003).

There is some controversy as to which plant response is strongest after herbivore damage (Nykänen and Koricheva 2004). Some plant species show both an increase in nutrients in leaves or twigs and at the same time produce some defence compounds (Riipi et al. 2005; Stolter et al. 2005). Alternatively, plants may pursue the best of both worlds by, for instance, showing a rapid compensatory growth response shortly after the attack and a delayed defence response at a later stage when partially or fully regrown. The effect of past herbivory on future nutritional

A.J. Veraart,[1] B.A. Nolet,[2] and P.P. de Vries. Department of Plant–Animal Interactions, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 1299, NL-3600 BG Maarssen, the Netherlands.
F. Rosell. Faculty of Arts and Sciences, Telemark University College, N-3800 Bø, Norway.

[1]Present address: Department of Biology, University of Amsterdam, Kruislaan 320, NL-1098 SM, Amsterdam, the Netherlands.
[2]Corresponding author (e-mail: b.nolet@nioo.knaw.nl).

value for the herbivore may then change at different growth stages. This may hold in particular when the herbivore feeds on different plant parts at different time of the year, which is often the case in seasonal environments.

Members of the Salicaceae family (poplars, genus *Populus* L., and willows, *Salix* spp.) are rapidly growing trees and shrubs (Kindschy 1989). Their leaves and bark are a favourite food of beavers (*Castor* spp.) in the summer and winter season, respectively, probably since they are highly digestible (Fryxell et al. 1994). Nevertheless, they are also rich in secondary compounds. On the basis of these, they can roughly be divided into two groups: a tannin-containing group and a phenolic glycoside-containing group (van Genderen et al. 1997). Both tannins and phenolic glycosides have a defence function in plants (Thieme 1971; Palo 1984; Tahvanainen et al. 1985*a*; Robbins et al. 1987; Forkner et al. 2004) and have been identified as a repellent to beavers (Basey et al. 1990; Bailey et al. 2004). As fast-growing species, one would expect defences to be inducible rather than constitutive (Bryant et al. 1983; Coley et al. 1985). In quaking aspen (*Populus tremuloides* Michx.), an induced response was found with high concentrations of an apparently beaver-repellent phenolic glycoside compound in juvenile-form sprouts (Basey et al. 1990).

In this study, we distinguished compensatory growth and defence plant responses and investigated which response dominated (from a beaver's perspective) in the course of the growing season. We simulated Eurasian beaver (*Castor fiber* L., 1758, hereafter referred to as beaver) browse on willows in winter and tested in a factorial design its effects on the food quality in subsequent spring leaves and autumn twigs. Previous research has shown that beavers generally prefer food rich in nitrogen (N) but poor in indigestible fibres and secondary compounds (Champ et al. 1981; Basey et al. 1990; Doucet and Fryxell 1993; Fryxell and Doucet 1993; Ganzhorn and Harthun 2000; Bailey et al. 2004), factors correlated with a low dry matter content (Elger and Willby 2003). The biochemical analyses, therefore, consisted of these four elements to characterize plant responses and food quality. Additionally, the preference of beavers for untreated or browse-simulated shoots was tested in a cafeteria-style experiment in the field where beavers were given a choice between shoots of different treatments. We also included the unbrowsed part of the pruned willows to test for differences in local and systemic responses in the willows.

Materials and methods

Study area

The study area was located in the National Park the Biesbosch (90 km^2), situated in the Rhine–Meuse estuary (51°45′N, 4°50′E), the Netherlands. The vegetation largely consists of reed beds and former willow coppices (Saris 1987). Forty-two beavers were reintroduced between 1988 and 1991 (Nolet 1995; Nolet and Baveco 1996). Beavers are territorial and live in family groups (Wilsson 1971; Campbell et al. 2005). They are usually generalist herbivores, but in our study area, their diet mainly consisted of willows (Nolet et al. 1994). At the time of the study, the area contained more than 100 beavers (Spitzen-van der Sluijs 2005).

Fig. 1. Beaver (*Castor fiber*) feeding on a cafeteria rack during a pilot experiment in an enclosed area. The picture was captured from a video sequence, contrast was enhanced, and edge details were accentuated by unsharp mask (Adobe Photoshop version 6.0; Adobe Systems Incorporated, San Jose, California).

Pruning experiment and collection of samples

We simulated beaver browsing by pruning holme willow (*Salix dasyclodos* Wimm.) shrubs on 4 February 2004 at a moment that catkins had just begun to form. Holme willow belongs to the phenolic glycoside-containing willows (Tahvanainen et al. 1985*b*; Julkunen-Tiitto 1989; Denno et al. 1990). It is regarded a sterile triple-hybrid of *Salix caprea* L. × *Salix cinerea* L. × *Salix viminalis* L. (Weeda et al. 1985). We used holme willows (hereafter referred to as willow) because all the individuals in the Biesbosch are females and therefore considered one clone (Weeda et al. 1985), thereby avoiding any confounding effects of sexual or genetic differences on our results.

Willows were pruned on 10 sites; the average distance between neighbouring sites was 560 ± 470 m SD. The willows at the sites stood >10 m from the water's edge to avoid any beaver browsing during the experiment (in the Biesbosch, beavers hardly forage on willows more than 6 m from the water's edge (Nolet et al. 1994)). At each site, four neighbouring holme willow shrubs were marked with ribbon. Two randomly chosen shrubs of these four willows were pruned by removing 20–30 branches of 1.5–2.0 cm in diameter; the two other shrubs were untreated. At each pruning site, three treatment types were distinguished for sampling: shoots from the untreated shrub (uncut), new shoots from a cut branch of the pruned shrub (cut), and shoots from the uncut part of the pruned shrub (uncut–cut).

In May and November of the same year, samples were taken from each site for the biochemical analyses as well as a cafeteria experiment. Because beavers eat mainly leaves of the willows in May and mainly bark in November (Nolet et al. 1995), we collected leaves in May and twigs in November. On 10 May at one site (site 7), all shrubs appeared to

Table 1. Mixed model analysis of variance (random factor: site; fixed factors: month and treatment) results for the interaction between month and treatment, and the interaction between site and treatment for dry matter (DM), acid detergent fibre (ADF), nitrogen (N), and total phenolics (TP) concentrations in leaves in May and twig bark in November.

| | Month × treatment | | Site × treatment | | | |
| | | | May | | November | |
	df	F	df	F	df	F
DM	2, 131	28.062***	16, 80	3.606***	14, 66	4.474***
ADF	2, 132	3.723**	16, 81	2.055*	14, 66	3.554***
N	2, 131	4.822**	16, 80	4.822***	14, 65	0.742
TP	2, 132	8.362***	16, 81	4.974***	14, 66	4.205***

Note: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

have been heavily attacked by aphids. At the remaining nine sites, one untreated and one pruned willow was randomly chosen from the four shrubs for sampling. The two remaining shrubs were sampled on 8 November. At two sites (site 3 and 5), two of the four shrubs did not have enough regrowth for sampling in May because of shading by surrounding trees. Therefore, the other two shrubs were sampled in May, and these sites had to be excluded from the experiment in November.

In May, we clipped five large branches of 2.0 cm in diameter from each treatment at each site. From one of these five branches we took nine twigs with leaves (one for each rack in the cafeteria test). From each of the other four branches we took a sample consisting of 20 randomly picked leaves. Samples were stored in sealed bags and kept cool (on ice) until processing in the laboratory for the biochemical analysis. In November, we collected 11 twigs from each treatment (one for each rack in the cafeteria test) at each site. Per site, another four twigs from each treatment were collected for the biochemical analysis. At each site, twigs of approximately the same diameter (total range 8–12 mm) were collected.

Biochemical analyses

In May, leaves were weighed in the afternoon of the day of collection. In November, bark was stripped from the twigs the following morning and weighed. Directly thereafter, all willow samples were freeze-dried for 72 h to preserve the material, and the samples were reweighed to determine the proportion of dry matter (DM). After drying, the material was electrically shredded into 1 mm fractions.

We measured N concentration in duplicate using a HEKAtech Euro EA3000 elemental analyser (HEKAtech GmbH, Wegberg, Germany). The proportion of acid detergent fibre (ADF) (Van Soest 1982) was analysed using an ANKOM[220] fibre analyser with filter bag technology (ANKOM Technology, Macedon, New York, USA).

Part of the samples from a pilot study in 2003 were analysed by high performance liquid chromatography. Compounds were separated on 100 mm × 4.6 mm Spherisorb ODS 2 column with 3-μm particle size (Waters Corp., Milford, Massachusetts, USA). For solvents, we used 10%–80% methanol and water with 1.8% tetrahydrofuran and 0.5% orthophosphoric acid. Compounds were detected with a 1040A diode array detector (Hewlett-Packard, Waldbronn, Ger-

many). Samples were compared with the standards D-salicin, tremulacin, and tremuloidin, but no clear pattern with treatment was apparent, apart from possible differences in phenolic glycoside concentrations in general (observed averages in integration units per 100 mg dry mass/mL extract: salicin, 20.2 ± 36.5 (mean ± SD); salicortin, 134.5 ± 65.1; hyperoside, 727.7 ± 431.2). Therefore, we further focussed on the concentration of total phenolics (TP). TP was derived from the absorption at 725 nm in 50% methanol extracts of the samples on a Helios Delta photospectrometer (Unicam, Cambridge, UK) (Folin and Ciocalteu 1927; Lundborg and Levin 1953; Waterman and Mole 1994). For estimating TP of leaf material from May, we used this Folin–Ciocalteu method (24 samples) in combination with near-infrared reflectance spectroscopy (93 samples) on an MPA FT-NIR (Bruker Optics BV, Wormer, the Netherlands). By comparing the spectra from these latter samples with the spectra of the samples with a measured TP, their phenolic content was interpolated (calibration $R^2 = 0.954$, root-mean-square error of estimation = 0.013 mg chlorogenic acid equivalents; cross-validation $R^2 = 0.606$, root-mean-square error of cross-validation = 0.031 mg chlorogenic acid equivalents, $n = 24$ (Foley et al. 1998)).

Cafeteria-style food preference experiment

We built a cafeteria rack on 12 beaver landings with signs of recent activity. Racks were placed 1790 ± 850 m (mean ± SD) apart in May and 1500 ± 630 m apart in November to avoid pseudoreplication by positioning them in different beaver territories, as judged from the locations of scent mounds and former radio-tracking of beavers in the Biesbosch (Campbell et al. 2005). The racks were constructed within 2 m from the water's edge. They were made of two bamboo poles, connected by steel wire at a height of ~1 m. The shoot bundles were attached to the steel wire by tie wraps, leaving them 20–30 cm above the ground. During a pilot in August 2003, we offered a similar setup to a pair of beavers in a large-scale enclosure and observed the beavers browsing the shoots from the rack (Fig. 1).

Before the start of the experiments, the beavers were familiarized to the racks by attaching three bundles of six shoots of their favourite food, European aspen (*Populus tremula* L.) (cf. Fryxell and Doucet 1993), to the racks for 1 week. Thereafter, on each rack three shoot bundles were attached: one bundle of each treatment. Each bundle was a collection

Fig. 2. Nutritional value of holme willow (*Salix dasyclados*) under three different treatments (uncut = untreated shrub (solid bars); cut = new shoots from cut part of pruned shrub (light gray bars); uncut–cut = untreated part of pruned shrub (dark gray bars) on all sites. Graphs are shown for nutritional parameters that showed a significant ($P < 0.05$) interaction between site and treatment in May (leaves): DM, dry matter (%); ADF, acid detergent fiber (%); arcsine-transformed nitrogen (N) (°); TP, total phenolics (in chlorogenic acid equivalents). Error bars denote 95% confidence intervals.


of shoots, one shoot from each pruning site (May: nine shoots per bundle; November: eight shoots per bundle). The bundles of the three treatments were made of roughly equal weight per rack and put in random order on the racks to control for size and side preferences. In May the bundles consisted of twigs with many leaves, whereas in November the bundles consisted of twigs with senescent or no leaves. The shoot bundles were weighed daily with a spring scale (Pesola A.G., Baar, Switzerland) on 3 subsequent days after the start of the experiment and after 1 week to determine the total amount of beaver browse. We also recorded the beaver cuttings of the shoots, but as we had clipped some of the shoots to obtain roughly equal weights of the bundles at a given rack, false positive recordings could not be completely excluded. A control rack was put up in the harbour, where no beavers could reach it, to correct for weight losses due to desiccation. In May, the beavers ate much less from the cafeteria racks than in November; therefore, the food preference experiment in May was repeated once, 1 week after the first collection.

Data treatment and statistical analyses

In November, six samples were outliers (ADF > 70%) and were also divergent in color and structure, suggesting the twigs consisted of only dead tissue; these were therefore removed from the analysis. This resulted in an unequal sample size per (pruning) site.

Duplicates of N were averaged and arcsine-transformed. Effects of treatment on the amount of DM, ADF, N, and TP were analysed using a mixed model analysis of variance with site as a random factor and treatment and month as fixed factors (Bennington and Thayne 1994; Paterson and Lello 2003). Interactions were allowed up to the second degree, as higher degree interactions are difficult to interpret. When significant ($P < 0.05$) effects were found, post hoc analyses (unequal sample size honest significant difference test) were used to test for differences between the treatments. A principal component analysis (PCA) was performed to examine whether the different chemical variables (DM, ADF, arcsine N, and TP) were interrelated.

The weights of the shoot bundles of the preference experiment were corrected for the weight loss by desiccation of the control shoots. We only counted a corrected weight loss as evidence for beaver browse when it was greater than a certain threshold value. We determined this threshold by looking at which weight loss yielded the maximum agreement between directly recorded beaver cuttings (yes or no) and our indirect measure based on weight loss (achieved agreement 91.7% and 94.4% during the first and second trial in May, respectively; in November agreement 84.8%). With
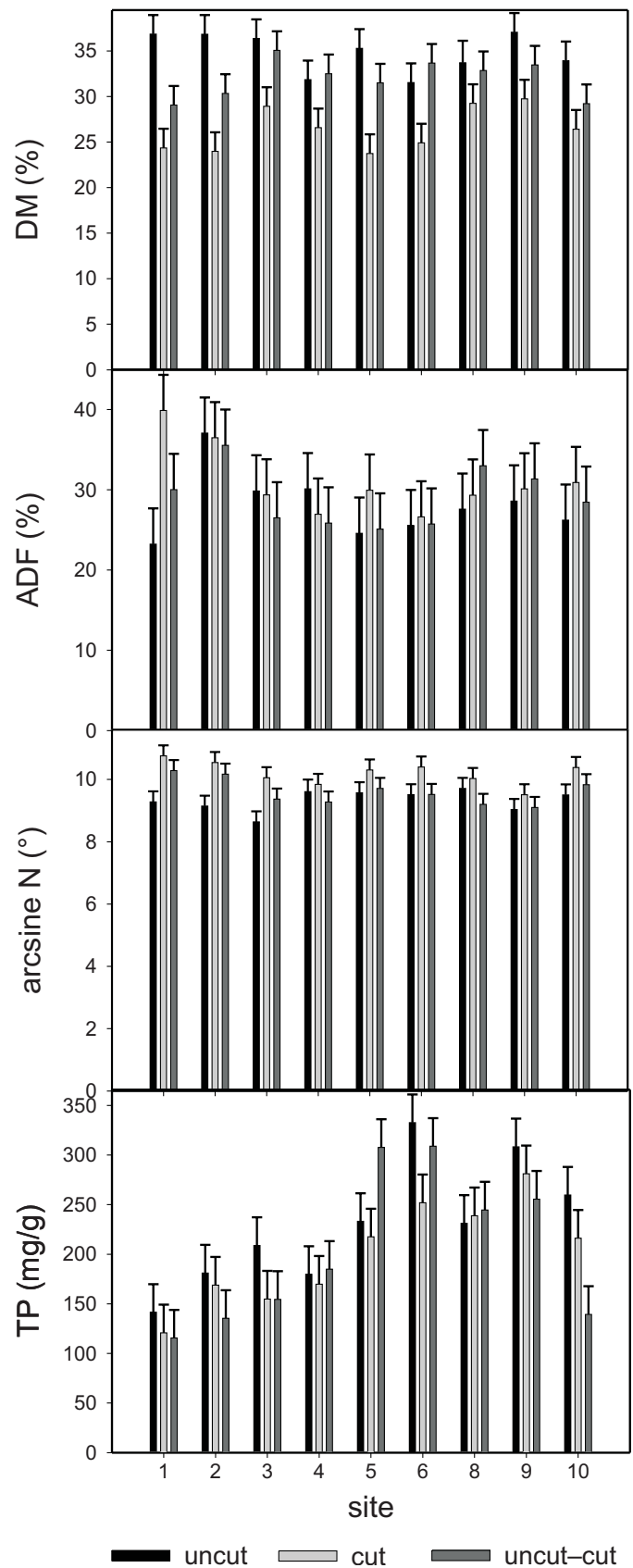
this threshold weight loss (in May 0.22 g, in November 0.13 g), we determined for each day and for each shoot bundle whether it was browsed or unbrowsed. Thereafter, we

determined the probability of being browsed of the individual shoot bundles at the end of the experiment.

Only the racks of which one or more of the shoot bundles were browsed (and therefore visited by beavers) were used for the statistical analysis. Data were analysed with a generalized linear model for a binomial distribution with a logit link function, using the probability on beaver browsing on the end of the experiment as dependent variable and treatment as categorical predictor. We tested for significance at the $P = 0.05$ level. When a significant effect was found, a post hoc analysis (Tukey's honest significant difference test) was used to test for differences between the treatments. Analyses were carried out using STATISTICA® version 6 (StatSoft Inc. 2004).

Results

Biochemical analysis

We found significant interactions between month and treatment for all biochemical factors. Thus, the effect of the treatment differed in the May leaves from that in the November twigs (Table 1). In both months, we also found significant interactions between pruning site and treatment for all biochemical factors except for N in November (Table 1; Figs. 2, 3). To investigate the most common type of response further, aberrant sites were excluded from the analyses on the basis of a post hoc analysis. Thereafter, as expected, the significant interactions between site and treatment disappeared (removed sites, May, DM: 1, 2, 5, 6; N: 1, 2, 3; ADF: 1; November, DM: 4, 6, 10). For the TP in both months and the ADF in November, the significant interaction between site and treatment was caused by almost all sites, and obviously, aberrant sites could therefore not be identified nor excluded.

In May, simulated winter browsing generally led to an increased nutritional value of the willows. Leaves from the cut parts of pruned shrubs contained significantly less DM, more N, and less TP than the untreated shrubs (Fig. 4, Table 2). With respect to DM and N, this effect was even stronger in the removed sites (see sites 1, 2, 5, 6 for DM and sites 1, 2, 3 for N in Fig. 2). The uncut part of the pruned shrubs (uncut–cut) showed a diverse response; DM was intermediate between the uncut and cut treatments, and N did not differ from that in the uncut shrubs, whereas TP had similarly low levels as in the cut part. ADF concentrations were not affected by the treatments.

Not surprisingly, twigs from November were overall of lower nutritional value for the beavers than the leaves in May. In contrast with the results for the leaves in May, we found no differences in biochemical quality between the three treatments in the twigs in November.

In May, factor one of the PCA described 57.9% of the variation and factor two 24.5%. In November, factor one described 52.0% of the variation and factor two 26.5%. In May, DM and N on the one hand and ADF and TP on the other hand were negatively associated; leaves rich in N tended to be also moist, and leaves high in TP tended to be low in ADF (Fig. 5). Leaves from the cut parts of pruned shrubs (cut) were at the high N, low DM side, whereas leaves from untreated willows (uncut) were at the low N, high DM side, with leaves from uncut parts of
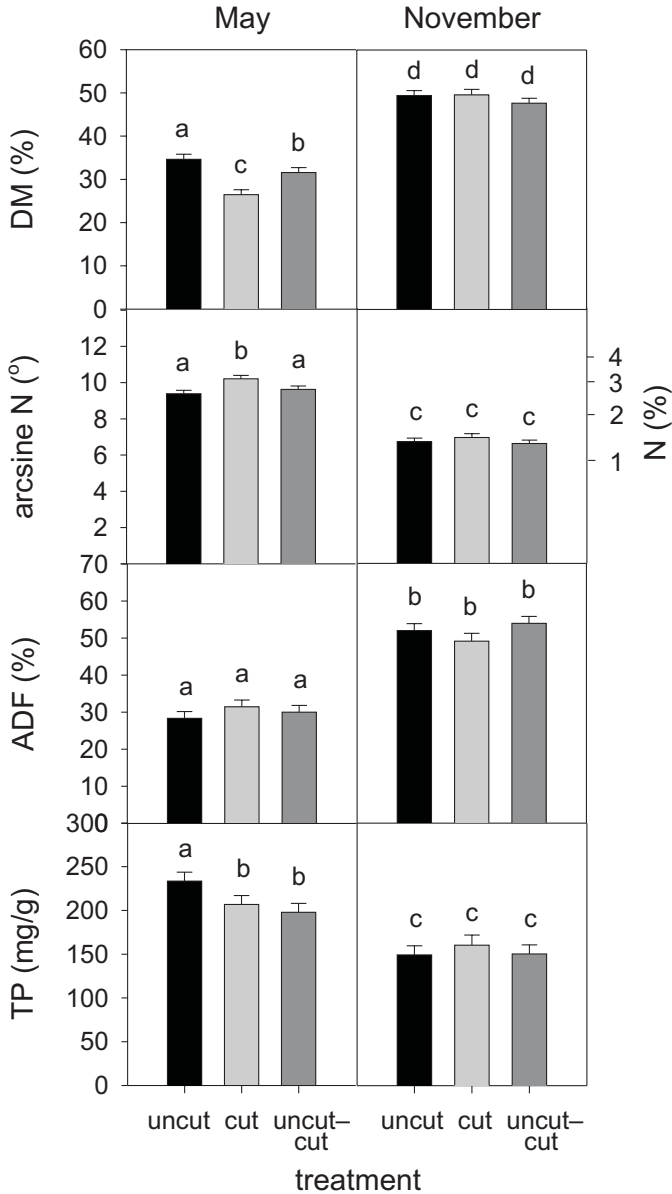
Fig. 3. Nutritional value of holme willow under three different treatments (see legend Fig. 2) on all sites. Graphs are shown for nutritional parameters that showed a significant ($P < 0.05$) interaction between site and treatment in November (bark): DM, dry matter (%); ADF, acid detergent fiber (%); TP, total phenolics (in chlorogenic acid equivalents). Error bars denote 95% confidence intervals.
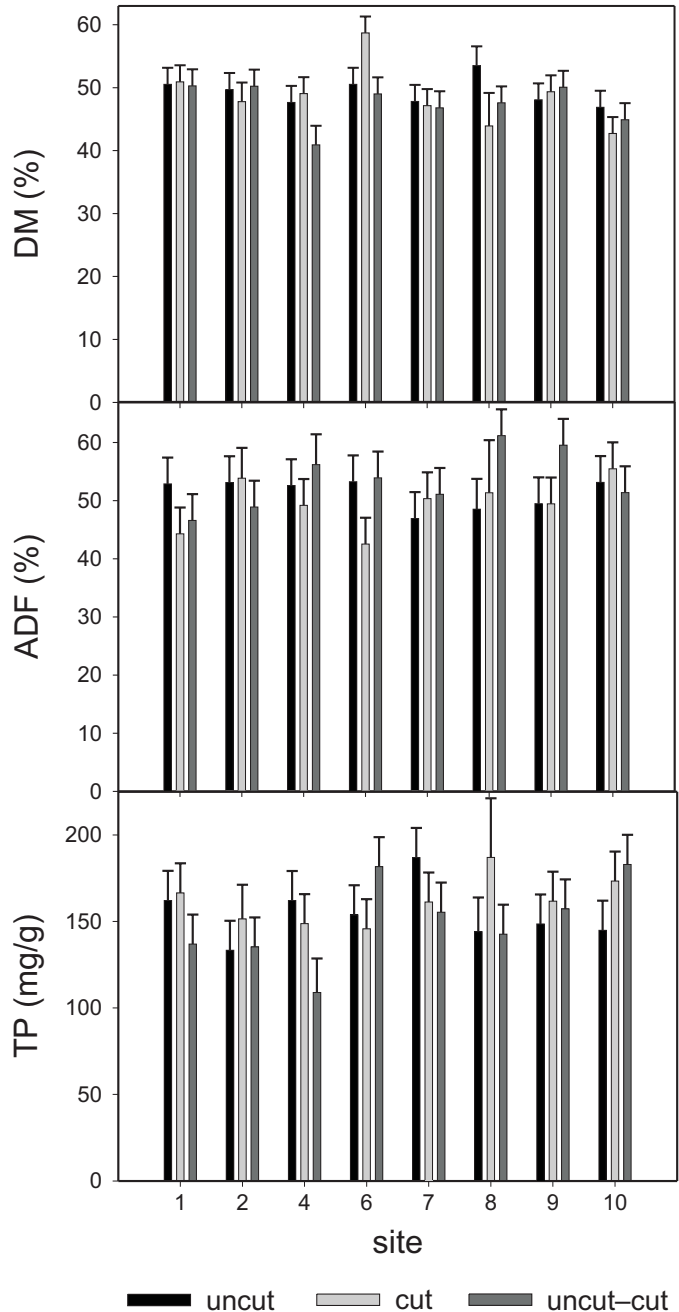
pruned shrubs (uncut–cut) intermediate. Similarly, the cut treatment shrubs were at the low TP, high ADF side, whereas uncut treatment leaves were at the high TP, low ADF side, with again the uncut–cut treatment leaves being intermediate.

In November, the opposite was found with regard to the DM and N; twigs high in N tended to be high in DM also. No clear separation of the three treatments was apparent in these twigs (Fig. 5).

Fig. 4. Nutritional value of holme willow under three different treatments (see legend Fig. 2) in May (leaves) and November (bark). Proxies of nutritional value are dry matter (DM, %), acid detergent fibre (ADF, %), arcsine-transformed nitrogen (N, °), and total phenolics (TP, in chlorogenic acid equivalents). Different letters above bars indicate significant differences (post hoc, unequal HSD; $P < 0.05$). Error bars denote 95% confidence intervals.

Table 2. Mixed model analysis of variance (see Table 1) results after removing aberrant sites (i.e., no significant ($P > 0.05$) interactions with site remaining; see text for details).

	Treatment			
	May		November	
	df	F	df	F
DM	2, 52	49.568***	2, 48	0.775
ADF	2, 86	0.791	2, 14.5	1.573
N	2, 53	29.090***	2, 86	1.209
TP	2, 97	6.556**	2, 80	0.900

Note: **, $P < 0.01$; ***, $P < 0.001$.

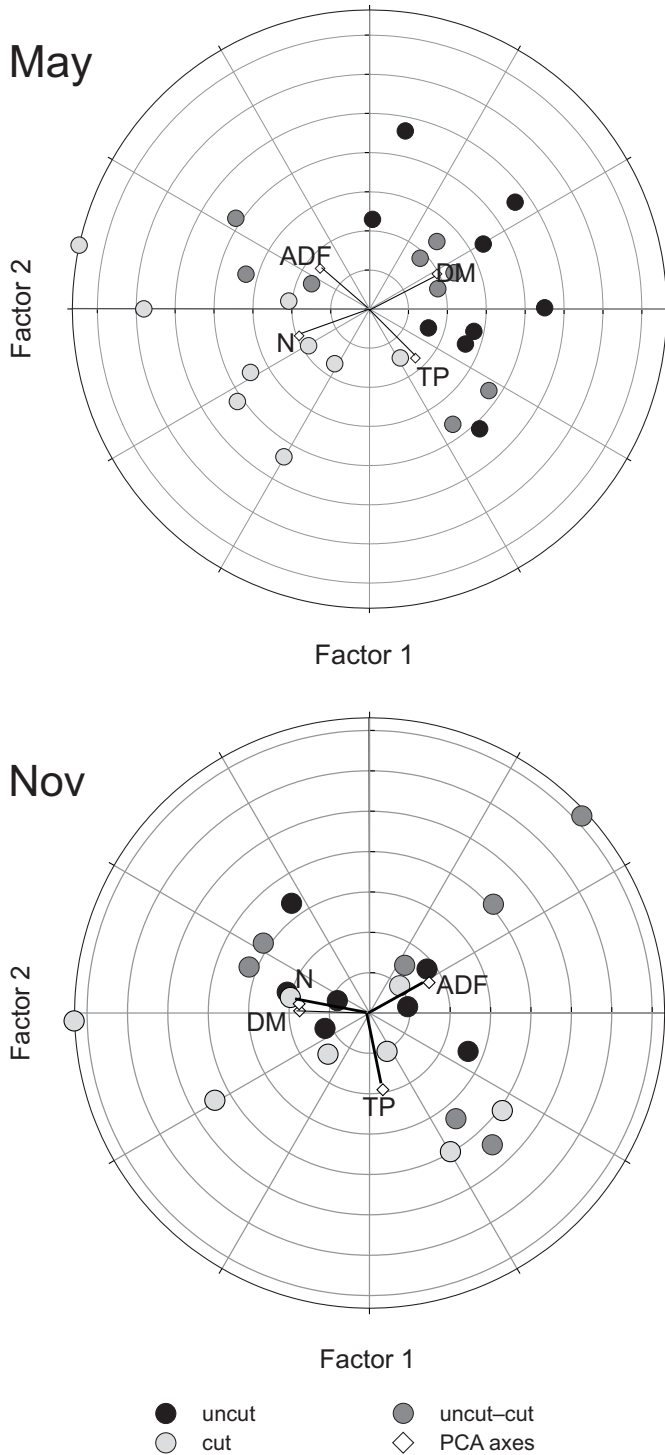probabilities to be browsed ($\nu = 2$, $\chi^2 = 2.404$, $P = 0.301$) (Fig. 6C).

Discussion

At most pruning sites, simulated winter browsing improved the food quality (richer in water and N, lower in TP) of willows in leaves in May but not in twigs in November. These results are supported by the cafeteria-style experiment in the field. Beavers preferred the shoots from the cut part of the pruned shrub over the other two treatments in May, whereas in November they showed no preference for a certain treatment.

The most striking results of our experiment are the low TP concentration and the high N content in the willow leaves in May from the shrubs with simulated browse. There are some differences in how the concentrations of these compounds differ within the shrub: N was only increased locally in the cut part of the pruned shrub, whereas TP showed a systemic response, with TP in the whole pruned shrub, both cut and uncut parts, being lower than the TP concentration in the untreated plants. The low concentration of TP in the whole shrub despite partial pruning is in line with the carbon–nutrient balance hypothesis (Bryant et al. 1983). Pruning reduces the carbon delivery capacity of the plant, but hardly affects the root uptake of nutrients such as N, so the C/N ratio falls. It seems that the browsed plants invest in compensatory growth to restore competitive ability and allocate their carbon in regrowth rather than the production of carbon-based defence compounds like phenolic glycosides (Bryant et al. 1983).

The increase in N levels of the leaves after browsing, as we observed in May, occurs frequently in woody plants (Hobbs 1996; Baker et al. 2005). Moderate pruning reduces the competition for nutrients among twigs, resulting in higher N concentrations in the leafs but less available carbohydrates for the production of phenolic glycosides (Moorby and Wareing 1963; Bryant et al. 1991). Hence, simulated browsing reverses ageing in the pruned part of the shrub (Kozlowski 1971). This is in contrast with severe pruning, which may cause a reversion to the juvenile phase. In the latter case, as opposed to reversed ageing, the new shoots have a reduced palatability for mammals (Bryant et al. 1991; Swihart and Bryant 2001; Bryant 2003), including american beavers (*Castor canadensis* Kuhl, 1820) (Basey et al. 1990).

Cafeteria experiment

In both May and November, nine racks were visited by beavers. In May, the beavers generally ate less from the shoot bundles than in November. The first experiment in May showed a significant interaction between territory and treatment ($\nu = 7$, $\chi^2 = 431.409$, $P < 0.001$). Post hoc analysis (Tukey's HSD, $P < 0.05$) showed that the beavers preferred the cut treatment over the uncut–cut treatment (Fig. 6A). Including the second experiment, the beavers preferred the cut treatment over the other two treatments in May ($\nu = 2$, $\chi^2 = 9.528$, $P < 0.01$) (Fig. 6B). In November, however, the twig bundles of all three treatments had equal

Fig. 5. Projection of the variables and cases of the different treatments of holme willow shrubs (uncut = untreated shrub; cut = new shoots from cut part of pruned shrub; uncut–cut = untreated part of pruned shrub) on the factor-pane factor (1 × 2) of a principal component analysis for the month May and November.

Fig. 6. The probability of being browsed by beaver in the cafeteria experiments for the holme willow shoot bundles of three different treatments (uncut = untreated shrub; cut = new shoots from cut part of pruned shrub; uncut–cut = untreated part of pruned shrub): (A) first trial in May, (B) first and second trials in May combined, (C) November. Different letters above bars indicate significant differences (post hoc, Tukey's HSD, $P < 0.05$). Error bars denote 95% confidence intervals.
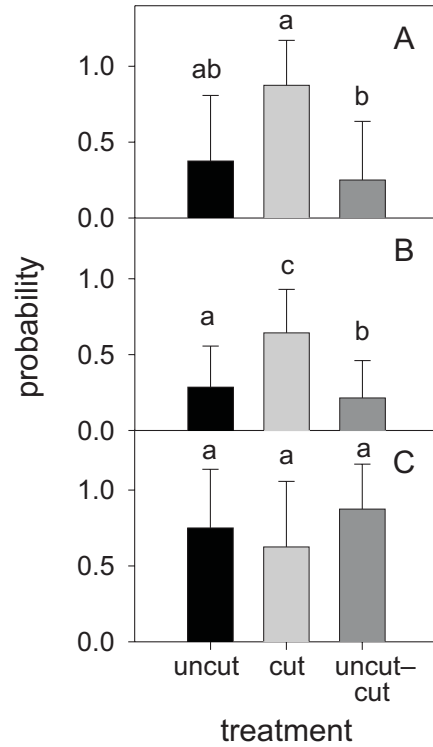
part, since the nutrient levels in willow leaves are highest shortly after budburst and then decline rapidly (Nolet et al. 2005).

In the cut treatment, N concentration was higher, but DM was lower. As leaf protein content and leaf water content are generally linked (Wilson et al. 1999), the question arises whether beavers can really benefit from regrowth of winter-browsed willows in spring. This would depend on what limits the beavers' daily intake, or more specifically what determines their digestive constraint. Some authors have assumed that bulkiness (wet mass/dry mass) determines the filling effect of beaver food (Belovsky 1984; Doucet and Fryxell 1993). In that situation, despite the difference in N concentration, the N intake of the beavers would not differ among the treatments, because the differences in DM would cancel out the differences in N among the treatments. However, other research indicates that fibres like ADF constrain daily intake (Fryxell et al. 1994). In that case, differences in concentrations in N would be reflected in similar differences in daily N intake, because ADF concentrations did not differ among treatments. We consider this latter scenario more likely because gut passage rates of fluids and solids are known to be different (Hobbs 1990), and we previously found a negative relation instead of no correlation between fresh food intake and DM content of the food in beavers (Nolet et al. 1995). In line with this, snowshoe hares (*Lepus*

Also, in our study the leaves from the cut part of the pruned shrub were fresher than the leaves from the uncut part of that shrub; the cutting postponed budburst date (B.A. Nolet and P.P. de Vries, personal observation). This causes a higher nutrient level of the leaves from the cut

americanus Erxleben, 1777) fed on leaves from winter-browsed willows consumed, digested, and retained more N than hares fed on unbrowsed willow leaves (Bryant 2003).

For the beavers in our study area, the increased N concentrations in spring due to winter browsing might be of importance. These beavers have been translocated from a region with moderately cold winters to one with a milder climate, at a time of rapid local change into an even milder climate. Currently, the date of willow budburst in the Biesbosch is almost 1 month earlier than in the middle Elbe region from where the beavers originated, but in both areas most beavers give birth around mid-May (Nolet et al. 2005). As willow leaves contain most N directly after budburst, this probably caused a time lag between nutrient need and nutrient availability and contributed to a relatively low reproductive success (Nolet et al. 2005). Hence, it seems profitable for beavers in the Biesbosch to boost N concentrations in willow leaves by winter browsing, so that a high nutrient availability coincides with their peak nutrient demand during late gestation and early lactation. However, because Stolter et al. (2005) found a different reaction in induced responses after winter browsing between two willow species (*Salix myrsinifolia* Salisb. and *Salix phylicifolia* L.), it remains to be seen whether similar changes occur in the other willow species in our study area and in different clonal lines of holme willows in other areas.

Site effects

We found significant interactions between pruning site and treatment for all biochemical indicators except N in November. Apparently, the shrubs, although belonging to the same clone, react differently on the treatments at different sites. This is probably due to variation among sites in soil fertility, shade, and insect damage. A meta-analysis looking at the interaction effects of resource levels and herbivory on plants showed that woody plants grew more after herbivory at, counterintuitively, low resources than at high resources (Hawkes and Sullivan 2001). Previous research also showed that the combined effects of soil fertility, shading, and clipping can cause different concentrations of phenolic compounds (Bryant 1987). Unclipped feltleaf willow was more sensitive to fertilization, whereas clipped willows were more sensitive to shade, but both shading and fertilization reduced the total amount of phenolics (Bryant 1987). At the sampling date in May, we also noted that herbivore insect densities varied with site (data not presented), while it is known that insect herbivory may affect the biochemical variables that we measured (Rieske and Raffa 1998).

In conclusion, simulated browsing in winter has a positive effect on the food quality of willows for beavers in May, when they eat leaves, but not in November, when they eat twigs. It seems that the willows invest in regrowth rather than defence early in the growing season. Since beavers have an increased nutrient requirement in spring owing to gestation and lactation, the increased food quality after winter browsing of willows might have a positive effect on the reproduction of beavers, but whether beavers can benefit from these higher N concentrations depends on what limits their daily food intake.

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