

Effects of litters with different concentrations of phenolics on the competition between *Calluna vulgaris* and *Deschampsia flexuosa*

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Abstract We hypothesized that the outcome of competition between ericaceous plants and grasses is strongly affected by the concentrations of phenolics in the litter that they produce. To test the effect of phenolic-rich litter on soluble soil nitrogen concentrations, plant nitrogen uptake and inter-specific competition, we conducted a greenhouse experiment with the shrub *Calluna vulgaris* and the grass *Deschampsia flexuosa* and their leaf litters. Two litters of *C. vulgaris* were used, with equal nitrogen concentration but different (high and low) concentrations of total phenolics. The *D. flexuosa* leaf litter contained lower concentrations of phenolics, but higher concentrations of nitrogen than the *C. vulgaris* litters. The plants were grown in monocultures and in mixed cultures. Inorganic and dissolved organic nitrogen were measured monthly during the experiment. After four months, we measured above- and belowground biomass and the nutrient concentrations in above- and belowground plant parts. In monocultures, *C. vulgaris* produced more shoot and root biomass on its own litter than with no litter. Growth of *Calluna* was reduced on grass litter. *D. flexuosa* plants produced most biomass on their own litter type,

whether in monocultures or in mixed cultures. Addition of *Calluna* litter stimulated the growth of *D. flexuosa* both in monoculture and in mixtures. The grass plants outcompeted *Calluna* both on shrub litter and on grass litter but not when grown without litter. The two *C. vulgaris* litter types that differed in their concentration of phenolics did not differ in their effects on the competition between the two species or on the production of inorganic and dissolved organic nitrogen. We conclude that the nitrogen content of the litter is more important as a plant feature driving competition between shrubs and grasses than the concentrations of phenolics.

Keywords *Calluna vulgaris* · Competition · *Deschampsia flexuosa* · Dissolved organic nitrogen · Phenolics

Abbreviations

dw dry weight
DON dissolved organic nitrogen
ITS internal transcribed spacer

Introduction

Dutch heathlands used to be dominated by the evergreen dwarf shrubs *Calluna vulgaris* (L.) Hull and *Erica tetralix* L., but during the past few decades these species have been increasingly replaced by the

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perennial grasses *Deschampsia flexuosa* (L.) Trin. and *Molinia caerulea* (L.) Moench. High levels of nitrogen deposition have strongly accelerated the accumulation of soil organic matter and the successional change from heathlands into grass-dominated vegetation and woodland (Berendse and Aerts 1984; Berendse and Elberse 1990; Berendse 1994). Previous experiments investigating competition between dwarf shrubs and dominant grasses have shown that the dominant grasses benefit more rapidly from an increased nutrient supply than the dwarf shrubs due to their faster growth rate at higher nutrient levels (Berendse and Aerts 1984; Heil and Bruggink 1987). At lower nutrient levels ericaceous plant species are more competitive, as they are better able to conserve the absorbed nutrients (Berendse et al. 1987; Berendse and Elberse 1990).

As a consequence of features that increase leaf life spans and reduce nutrient losses from the plant (Berendse 1994), ericaceous plants can suppress nitrogen mineralisation by producing slowly decomposable litters rich in lignin and phenolics. Tannins from degrading litter can react with protein sources in the soil to form complexes with proteins. It has been suggested (Northup et al. 1995, 1998; Inderjit and Mallik 1999; Kraus et al. 2003) that these nitrogen-rich complexes release inorganic nitrogen slowly and increase the production of dissolved organic nitrogen relative to that of NH_4^+ and NO_3^- . As ericoid mycorrhizal fungi are able to degrade these complexes (Bending and Read 1997), dwarf shrubs colonized by these fungi are able to absorb more organic nitrogen compared to non-colonized plants (Smith and Read 1997; Sokolovski et al. 2001). Grass species, on the other hand, predominantly colonized by arbuscular mycorrhizal fungi, are often assumed to be less able to utilize organic soil nitrogen. Although it has been shown that arbuscular mycorrhizal fungi can enhance amino acid absorption in plants, this seemed to occur only to a small extent (Hodge 2001; Hodge et al. 2001). Separate from this, there is evidence that grasses can absorb simple forms of organic nitrogen such as amino acids like glycine or methylamine (Näsholm et al. 1998; Falkengren-Grerup et al. 2000). It is, however, not yet shown whether grasses are also able to access more complex organic nitrogen sources. So, it is still unclear whether both *C. vulgaris* and *D. flexuosa* have equal access to the same sources of organic nitrogen when present in

more complex forms. An intriguing, but still unresolved issue is whether dwarf shrubs really gain a competitive advantage over dominant grasses by producing recalcitrant litter with high contents of phenolics that suppress grass growth.

To date, the effect of phenolics on the competition between heather and grasses has not been investigated. Previous experiments on competition between heather species and dominant grasses have focused primarily on the direct effect of additional inorganic nitrogen on the outcome of the competition (Berendse and Aerts 1984; Aerts and Berendse 1988; Aerts et al. 1990; Mickel et al. 1991; Alonso et al. 2001; Britton et al. 2003; Barker et al. 2004). Our hypothesis is that due to ericoid mycorrhizal fungi, ericaceous plants compete more efficiently with *D. flexuosa* on nutrient-poor soils amended with litter rich in phenolics than on nutrient-poor soils amended with litter with low phenolics concentrations. *D. flexuosa* roots are mainly colonized by arbuscular mycorrhizal fungi, so though the species is able to use dissolved organic nitrogen, we speculate it to do so less than *C. vulgaris*.

In the study reported here, we examined competition between *D. flexuosa* and *C. vulgaris* in a greenhouse experiment. We studied the effects of litter amendments with high and low concentrations of phenolics on this interaction. In many cases concentrations of phenolics and nitrogen are inversely correlated, but in an earlier field experiment with shading treatments we were able to produce *Calluna* litter with different concentrations of phenolics but similar nitrogen concentrations, so that we could avoid this important pitfall.

Materials & methods

Litter treatments

We performed a competition experiment between *D. flexuosa* and *C. vulgaris* in a greenhouse. As litter treatments we added grass and shrub litters with high and low concentrations of phenolics (Table 1). *C. vulgaris* litter was collected from *C. vulgaris* plants in shaded and non-shaded treatments (50% light reduction) in a field experiment in De Hoge Veluwe National Park that ran from September 2001 to March 2003. Leaf material of *D. flexuosa* was collected in

Table 1 Overview of litter treatments included in this study

Litter codes	Litter treatments
No litter	No litter
Cv-h	<i>C. vulgaris</i> (high phenolics)
Cv-l	<i>C. vulgaris</i> (low phenolics)
Df	<i>D. flexuosa</i>

Each treatment consisted of 2×6 monocultures of *C. vulgaris* or *D. flexuosa*, 6 mixtures of *C. vulgaris* and *D. flexuosa* and 6 pots without plants

the same area. The leaves were dried at 38°C for two days and sieved (1 mm mesh size) before grinding.

To measure concentrations of total phenolics and condensed tannins 20 ml of 50% (v/v) methanol was added to 0.19 g dry leaf material. The mixture was covered and placed in a water bath (75°C) for 1 h. The sample was then filtered through a glass filter and the extract adjusted to 50 ml with 50% (v/v) methanol. Total phenolics (measuring both concentrations of hydrolysable and condensed tannins) were analysed with the Folin Ciocalteu method as described in Waterman and Mole (1994). The concentration of condensed tannins were analysed following the butanol-HCl method described by Porter et al. (1986). Given the complexities of applying an appropriate standard for the proanthocyanidin method (Waterman and Mole 1994), the data are presented as final absorbance at 550 nm for the extracted sample of plant material (0.19 g). Absorbance values are not recalculated to standard units, since the relationship between absorbance and sample size is not linear.

Concentrations of phenolics and condensed tannins were much lower in the brown *Calluna* litter than in the green shoots collected from the field experiment. In the non-shaded treatment concentrations of total phenolics in the brown litter were 42 mg tae/g compared to 707 mg tae/g in the green shoots; concentrations of condensed tannins in the litter were 0.02 versus 0.04 A₅₅₀/0.19 g in the green material. In the shaded treatment similar differences were found. The measured concentrations of condensed tannins in *Calluna* material were extremely low compared to concentrations (0.15–0.18 A₅₅₀/0.19 g) measured in an earlier explorative field study (Zijlstra and Berendse 2009).

A subsample of the plant material was dried at 70°C and pulverized to measure the carbon and nitrogen

concentrations with an elemental analyser (Fisons Instruments, EA 1108). The ericaceous litter collected from the treatments was found to have similar nitrogen concentrations, but significantly different concentrations of total phenolics (Table 2).

Experiment

In the litter treatments, 5 g litter dry weight with particles smaller than 1 mm diameter was mixed with 1.7 kg soil and used to fill plastic pots (diameter 14 cm). The amount of litter added was equal to 325 g/m² which was comparable with the above-ground litter production in dry heathland that varies between 250 and 400 g m⁻² y⁻¹. The nutrient-poor soil substrate was prepared by mixing five parts sand without organic matter and one part black top soil. The organic matter content of the soil mixture (before litter addition) was 1.3%, and pH(H₂O) and pH(KCl) were 6.4 and 6.0, respectively. Total N and P contents were 345 and 163 mg kg⁻¹, respectively. Control pots were filled with soil without added litter. *C. vulgaris* seedlings up to 1-year old were collected from “De Hoge Veluwe National Park”, a dry sandy area in the central part of the Netherlands. *D. flexuosa* seeds supplied by a private company (De Cruydhoeck, the Netherlands) were germinated on soil and were 2 weeks old at the start of the experiment in May 2003. We assumed the young *Calluna* plants to be inoculated with mycorrhizal fungi, since they were transplanted from the field including some of the native soil adhering to their roots. The plants were grown either in monocultures or mixed with the other species at densities of six plants (six *C. vulgaris* plants, six *D. flexuosa* seedlings or three of each). At the start of the experiment the plants were sufficiently short (average height *C. vulgaris*, 3.8±0.5 cm, *D. flexuosa* 4.6±0.4 cm) and spaced far enough apart to ensure minimum competition for light. To avoid disturbing effects on plant growth (and for reasons explained in the next paragraph), we included control pots without plants in order to be able to measure the amounts of inorganic and dissolved organic soil nitrogen and pH-H₂O during the experimental period of 20 weeks. Each treatment was replicated six times. The treatments were set out randomly in a greenhouse with controlled climatic conditions (light/dark: 14/10 h, light intensity 50 W m⁻², temperature 20°C, 70%

Table 2 Overview of concentrations (means $\pm$ standard errors) of total phenolics, condensed tannins, carbon and nitrogen in the litter material of *C. vulgaris* and *D. flexuosa* used as treatments in the monocultures and mixed cultures

Litter	Total phenolics (mg tae/g dw)	Condensed tannins ($A_{550}/0.19$ g dw)	C (mg/g)	N (mg/g)
<i>D. flexuosa</i>	1.27 $\pm$ 0.04	0.00 $\pm$ 0.00	447 $\pm$ 1.6	24.3 $\pm$ 0.13
<i>C. vulgaris</i> (high phenolics)	42.2 ^b $\pm$ 0.35	0.02 ^{ns} $\pm$ 0.009	504 ^{ns} $\pm$ 3.1	16.4 ^{ns} $\pm$ 0.15
<i>C. vulgaris</i> (low phenolics)	27.1 ^a $\pm$ 0.23	0.00 ^{ns} $\pm$ 0.006	517 ^{ns} $\pm$ 3.6	18.4 ^{ns} $\pm$ 0.32

Concentrations of total phenolics are indicated as tannic acid equivalents (tae) per gram dry weight and concentrations of condensed tannins as the absorbance measured at 550 nm wavelength (A_{550}). Different letters indicate significant differences between *C. vulgaris* litters with high and low concentrations of total phenolics (Tukey test, $P < 0.05$)

ns non-significant

relative humidity). The pots were weighed and watered three times a week to keep the soil moisture at 60% of water saturation.

Measurements

During the experimental period we measured biweekly the amounts of inorganic and dissolved organic soil nitrogen and pH-H₂O in soil water suctioned from the pots. The pots were watered a day before the measurement and in the morning a syringe with suction cups was placed in a hole 3 cm above the bottom of each pot to collect sufficient water (30 ml) for the measurements. NH₄-N and NO₃-N in the soil solution were directly determined in the collected soil water. Total dissolved nitrogen (dissolved organic nitrogen (DON) + inorganic N) was determined conductimetrically after persulphate oxidation (Yu et al. 1993). DON was calculated by subtracting inorganic nitrogen from the total dissolved nitrogen. These measurements were taken in pots that did not contain any plants in order to estimate the amount of N available for plant uptake during the period of study.

At the end of the experiment heights of the individual plants (averaged over all treatments) of *Calluna* and *Deschampsia* had increased by 155 and 72% of their initial heights, respectively. Plants were harvested on 29 September 2003 and then dried at 70°C to measure dry weight. At harvest we distinguished between living and dead plant parts. Roots were carefully rinsed and cleaned from organic soil particles using tweezers. Carbon and nitrogen concentrations in the plant were measured with an elemental analyser (Fisons Instruments, EA 1108).

Statistical analyses

The effects of litter and competition on shoot and root biomass and soil nitrogen uptake were analysed by two-way ANOVA. The fixed factors were plant treatment (monoculture or mixture) and litter treatment (no litter, *C. vulgaris* high phenolics, *C. vulgaris* low phenolics and *D. flexuosa*). Block effects were initially included as random factor, but removed from further ANOVA analyses because they were found to be not significant. Multiple comparisons between treatments were made using Tukey's test ($P < 0.05$). We analysed the differences in shoot biomass production between the two species using the ratio between the biomass of the grass and the biomass of *C. vulgaris* in mixed culture. The same was done for the root biomass and shoot:root ratios. Effects of litter were analysed by one-way ANOVA (Tukey's test, $P < 0.05$). We used a repeated measures analysis for soil inorganic nitrogen, dissolved organic nitrogen, ratio organic nitrogen: inorganic nitrogen and pH-H₂O. To avoid confounding effects of plant growth, we measured control pots without plants only. Therefore there are no plant treatments in this analysis.

Results

Plant biomass

C. vulgaris plants in the monocultures produced significantly more shoot biomass on their own litter than on the grass litter and in the treatment without litter (Fig. 1, Table 3). Addition of *D. flexuosa* litter strongly reduced the amount of *C. vulgaris* shoot biomass. Moreover, the plants in this treatment

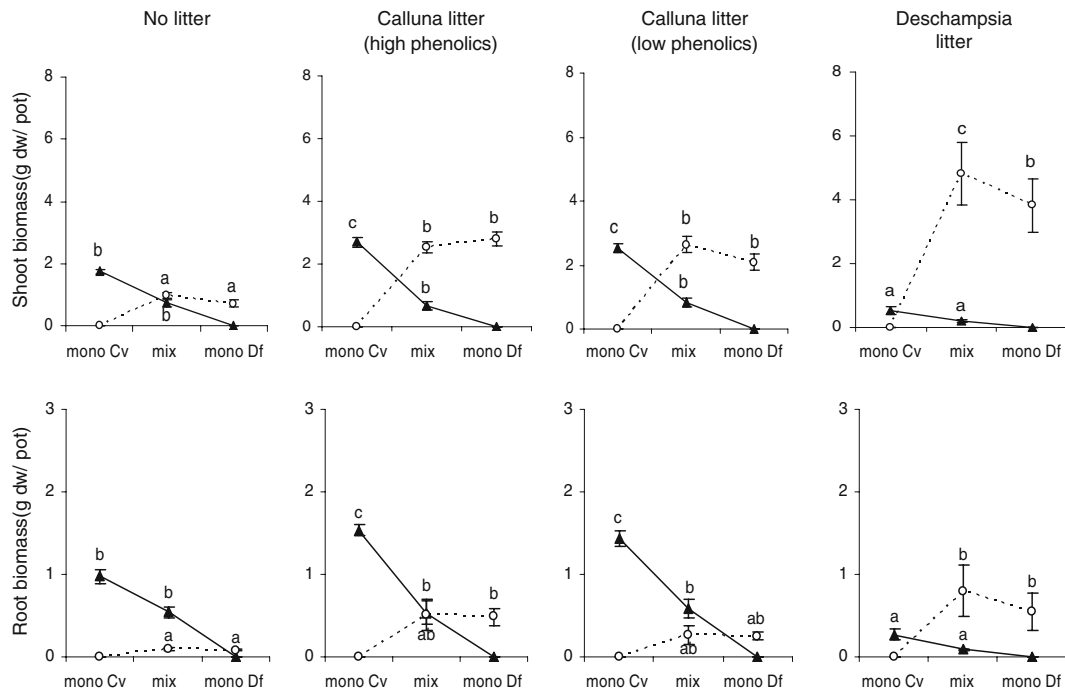


Fig. 1 Replacement diagrams showing the effects of litter treatments on the competition between plants of *C. vulgaris* and *D. flexuosa*. Mean shoot and root biomass per pot and their standard errors ($n=6$) are plotted versus the number of plants of the species involved (6 in monocultures and 3 per species in

mixtures). Different letters indicate significant differences in biomass among litter treatments (Tukey test, $P<0.05$). *C. vulgaris*—solid lines, *D. flexuosa*—broken lines. Calluna monocultures, Deschampsia monocultures, mixed cultures, litter treatments

contained the most dead shoot mass (Table 3, $P<0.05$). Also in the mixed cultures, *C. vulgaris* plants produced much less shoot biomass on *D. flexuosa* litter than in the other treatments. In the mixtures there was no positive effect of the *C. vulgaris* litter relative to the treatment without litter. We did not find any significant difference in shoot or root biomass between the two *C. vulgaris* litter types. The shoot: root ratio of *C. vulgaris* varied between 1.4 and 2.5 and tended to be highest in the *D. flexuosa* litter treatments.

Grass plants in mixture produced most shoot biomass on their own litter, but in the monocultures there were no significant differences between the grass and shrub litter treatments due to the large biomass variance in the grass treatment (Fig. 1). In both monocultures and mixtures, shoot biomass of grasses in the treatments with shrub litter was significantly greater than in the pots without litter. There were no differences in grass shoot or root mass between the two shrub litter treatments. In mixtures, the grass benefited greatly from the

addition of grass litter, but shrub growth was significantly suppressed (Fig. 3, Table 4). Overall, the shoot: root ratio of *D. flexuosa* was almost triple the shoot: root ratio of the *C. vulgaris* plants and varied between 7.2 and 15.5.

The effects of the treatments on the competitive balance between the two species was further analysed by calculating the ratios between the biomass of the two species in mixture (Table 4). The greatest biomass of *C. vulgaris* relative to *D. flexuosa* was found in the treatment without litter and the lowest nitrogen availability. The difference in shoot biomass between *C. vulgaris* and *D. flexuosa* plants in the mixed culture was much smaller on the two *C. vulgaris* litter types than in the *D. flexuosa* litter treatment. In the grass litter treatment *D. flexuosa* became strongly dominant.

Nitrogen uptake by plants

C. vulgaris plants grown in monocultures showed the highest nitrogen uptake on their own litter, but in the

Table 3 Effects of litter treatment and competition mode on shoot and root mass, shoot:root ratio, total nitrogen content of the plant and C:N ratio of total plant biomass in *C. vulgaris* and *D. flexuosa* plants

Plant	Litter F _{3, 15}	Competition F _{1, 15}	Litter × Competition F _{3, 15}
<i>C. vulgaris</i>			
green shoot mass	29.696***	21.559***	4.318**
dead shoot mass	17.071***	0.107 ^{ns}	0.955 ^{ns}
root mass	23.049***	2.932	1.343 ^{ns}
shoot:root ratio	2.247 ^{ns}	1.112 ^{ns}	0.076 ^{ns}
total nitrogen	20.954***	15.931***	1.955 ^{ns}
C:N ratio	35.463***	36.500***	3.636*
<i>D. flexuosa</i>			
green shoot mass	15.777***	31.124***	3.072*
dead shoot mass	4.530**	0.044 ^{ns}	1.233 ^{ns}
root mass	8.689***	8.825**	0.804 ^{ns}
shoot:root ratio	1.744 ^{ns}	3.663 ^{ns}	0.937 ^{ns}
total nitrogen	42.347***	48.449***	3.379*
C:N ratio	7.787***	17.781**	0.435 ^{ns}

Effects are analyzed using a two-way ANOVA with litter, competition and the interaction litter x competition as fixed factors. F-values with their degrees of freedom for these effects are given. Blocks did not have significant effects. Yields in monocultures and mixtures were calculated per plant. Results of two-way ANOVA: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

^{ns} non-significant

grass litter treatment the nitrogen uptake was strongly reduced (Fig. 2). In mixtures, the shrub plants were unable to absorb more nitrogen when grown with their own litter compared to the no litter treatment. In the grass litter treatment they barely absorbed any nitrogen. The combined nitrogen uptake of *C. vulgaris* and *D. flexuosa* was highest in the treatments with *D. flexuosa* litter ($P < 0.05$, Fig. 2). The total nitrogen uptake in the two *Calluna* treatments was higher than in the no litter pots. In both mono and mixed cultures, *D. flexuosa* plants were able to absorb

additional nitrogen released from *C. vulgaris* litter (Fig. 2).

Inorganic and dissolved organic nitrogen in the soil

In all the treatments the concentration of dissolved organic nitrogen is always more than 50% of the total dissolved soil nitrogen (Fig. 3). The production of inorganic nitrogen and dissolved organic nitrogen in the soil with *D. flexuosa* litter was significantly higher than in the treatments without litter or with *C.*

Table 4 Effects of litter treatment on the ratio between the shoot or root biomass of the two species (*D. flexuosa*:*C. vulgaris*) in mixtures (mean ± se)

Litter treatment	Biomass ratio in mixture shoot	Biomass ratio in mixture root
No litter	1.54±0.24 ^a	0.20±0.06 ^a
<i>C. vulgaris</i> (high phenolics)	5.14±1.61 ^b	2.43±1.30 ^a
<i>C. vulgaris</i> (low phenolics)	3.97±1.21 ^{ab}	0.86±0.58 ^a
<i>D. flexuosa</i>	17.93±2.98 ^c	10.96±4.01 ^b

Different letters indicate significant differences between litter treatments (Tukey's test, $P < 0.05$)

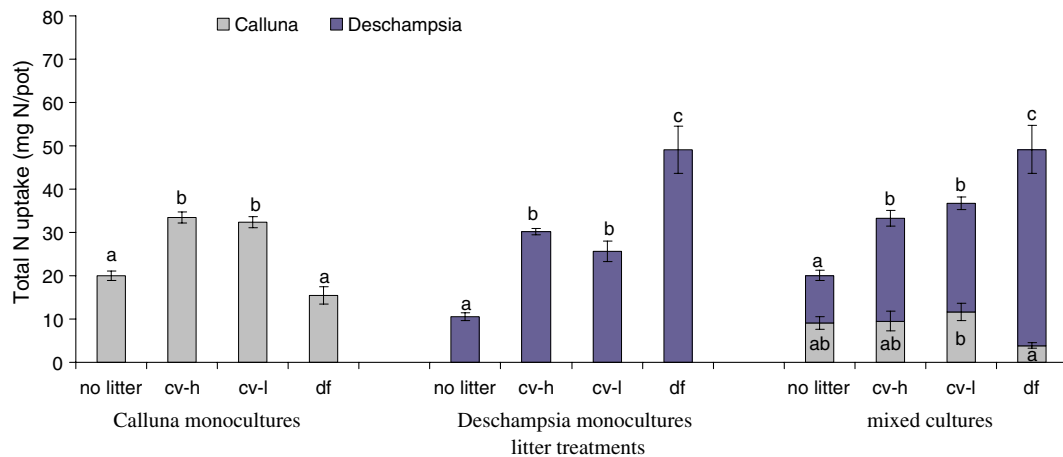


Fig. 2 Effects of litter treatments on the total amounts of absorbed nitrogen in the shoot and roots of *C. vulgaris* (light bars) and *D. flexuosa* (dark bars) per plant. Error bars show standard errors of the mean ($n=6$). Different letters indicate

significant differences between litter treatments (Tukey test, $P < 0.05$). Litter treatments: no litter = no litter applied, cv-h = *C. vulgaris* high phenolic concentration, cv-l = *C. vulgaris* low phenolic concentration, df = *D. flexuosa*

vulgaris litter ($P < 0.05$). After the first 2 months, *D. flexuosa* litter reduced the ratio DON: inorganic nitrogen, as can be seen from the significant interactions between time and litter (Table 5 and Fig. 3). In contrast, during the first two months *C. vulgaris* litter greatly increased this ratio. *C. vulgaris* litters with high and low concentrations of phenolics showed an immobilization of inorganic nitrogen during the first 8 weeks, as evidenced by the inorganic nitrogen being lower than in the control treatment (Fig. 3, $P < 0.05$).

Only after 20 weeks did the *C. vulgaris* litters produce significantly more inorganic nitrogen and organic nitrogen than the control treatments ($P < 0.05$).

Discussion

Our results show that *C. vulgaris* plants compete with *D. flexuosa* more efficiently on nutrient-poor soils (no litter amendment or amended with litter relatively rich

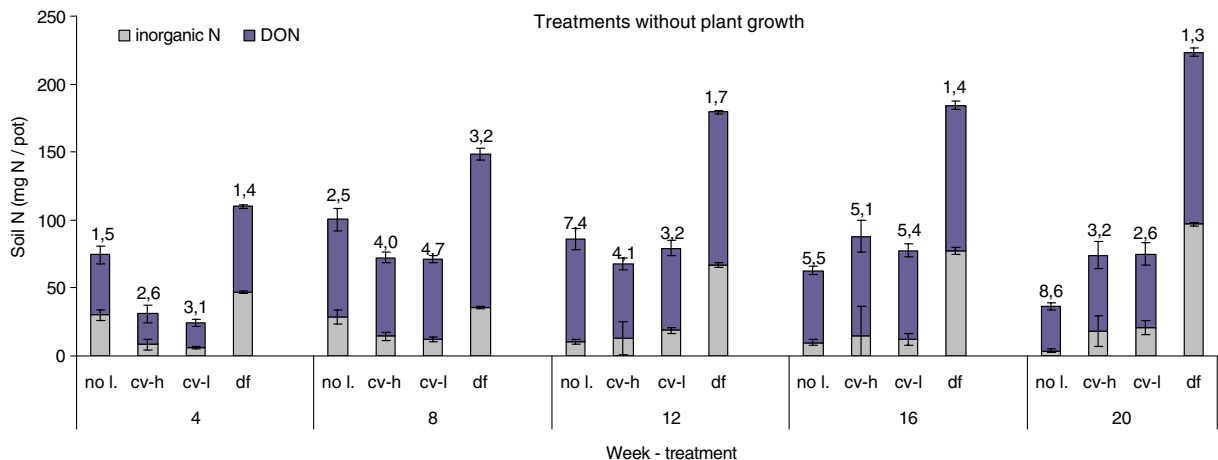


Fig. 3 Effect of litter treatments on the amounts of inorganic and dissolved organic nitrogen (DON) in treatments without plants during 20 weeks. Error bars show standard errors of the mean. At the top of each bar the ratio between DON and

inorganic nitrogen is given. Litter treatments: no l. = no litter applied, cv-h = *C. vulgaris* high phenolic concentration, cv-l = *C. vulgaris* low phenolic concentration, df = *D. flexuosa*

Table 5 Results of repeated measures two-way ANOVA of concentrations of inorganic N, DON, the ratio DON:inorganic nitrogen and pH (H₂O) in the control pots without plants

Source	Measure	df	F
Between-subject effects:			
Litter	Inorganic N	3	44.101***
	DON	3	72.704***
	DON: inorganic N	3	7.738***
	pH (H ₂ O)	3	0.542 ^{ns}
Within-subject contrasts:			
Time	Inorganic N	4	3.592*
	DON	4	43.818***
	DON: inorganic N	4	15.601***
	pH (H ₂ O)	3	181.560***
Time × Litter	Inorganic N	12	5.355***
	DON	12	2.811**
	DON: inorganic N	12	3.970***
	pH (H ₂ O)	9	2.219*

F-values and their degrees of freedom are given for effects of litter treatment, time and interaction time*litter

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

^{ns} non-significant

in phenolics) than on soils with grass litter. Only in treatments without competing grasses could *C. vulgaris* benefit from the nutrients in its own litter. It was striking that *Calluna* growth was strongly suppressed in treatments with grass litter, while *D. flexuosa* produced most biomass when growing on its own litter. Moreover, *D. flexuosa* was able to outcompete *C. vulgaris* both on shrub and grass litter. *C. vulgaris* was significantly less able to absorb nitrogen in the grass litter treatments than in the other treatments. In contrast, *C. vulgaris* growing in monocultures was able to use all the available nitrogen produced in the treatments with heather litter. The ratio between the biomass of the two species in mixtures shows that the difference in biomass production between the two plant species was largest on the grass litter and was much smaller in the treatment without litter.

Shrub plants were inhibited by the presence of grass plants and showed the highest production in the monocultures. The plants were not affected by the different concentrations of phenolics in the two shrub litter types they were growing on. Northup et al. (1995, 1998) argued that plant species with high concentrations of secondary plant metabolites could

absorb larger fractions of the organic nitrogen pool than species with lower concentrations, allowing such species to outcompete species less able to absorb organic nitrogen. If this were so, *C. vulgaris* plants should have been able to outgrow *D. flexuosa* plants on their own *Calluna* litter, which is rich in phenolics. Our replacement diagrams show, however, that *C. vulgaris* plants were not able to produce more biomass than *D. flexuosa* plants in any of the treatments. Only in monocultures *C. vulgaris* did benefit from its own litter. It seems that the hypothesized monopoly of *C. vulgaris* on the organic nitrogen pool does not increase the competitiveness of *C. vulgaris*.

One explanation of the above behaviour could be that *D. flexuosa* is also able to exploit complex sources of organic nitrogen. In this experiment we found that grass plants were able to use litter sources with high concentrations of phenolics equally well as the shrub plants. Previous competition studies between heather and grass and between *C. vulgaris* and *D. flexuosa* specifically, only considered competition for inorganic nitrogen sources (Berendse and Aerts 1984; Mickel et al. 1991; Berendse 1994; Alonso et al. 2001; Britton et al. 2003). The study by Näsholm et al. (1998) with dual-labelled glycine in a boreal forest provided the first evidence for significant organic nitrogen uptake by *D. flexuosa*, as 64% of the added glycine was taken up as intact glycine. It was shown that *D. flexuosa* absorbed the same amount (about 30%) of the dual-labelled glycine as the ericaceous shrub *Vaccinium myrtillus*.

As shown by the replacement diagrams of the shoot biomass (Fig. 1), *D. flexuosa* was not inhibited by the presence of *C. vulgaris* plants. We had expected that the high concentration of phenolics in *C. vulgaris* litter would lead to reduced nitrogen mineralization (Kraus et al. 2003; Bowman et al. 2004), subsequently inhibiting the growth of grass plants on shrub litters compared with the treatments without litter amendment. However, we found no such growth inhibition of the grass plants in the shrub litter treatments although they produced less biomass compared to grass plants in the grass litter treatments. The two *Calluna* litter types with different concentrations of phenolics did not affect the growth or the competitive ability of the grass plants.

Other competition studies have shown that grasses are able to benefit more than shrub plants from an

increased soil nitrogen supply (Mickel et al. 1991; Hartley and Amos 1999; Britton et al. 2003). Our results confirm this, as *D. flexuosa* absorbed most nitrogen and produced most biomass on its own litter, which had the highest nitrogen concentration.

We did not find any difference in plant growth on the two different *C. vulgaris* litter types, even though these varied significantly in the concentration of total phenolics. This lack of difference can be due to the low concentrations of condensed tannins in *C. vulgaris* litter, which did not differ between the two litter types. Condensed tannins in litter can inhibit mineralization rates significantly due to their higher protein-binding capacity compared to the hydrolysable tannins (Schimel et al. 1996, 1998; Bradley et al. 2000; Fierer et al. 2001; Kraus et al. 2003). Bradley et al. (2000) reported that adding condensed tannins from *Kalmia* and balsam fir foliage reduced mineral nitrogen availability in black spruce humus. In addition, Schimel et al. (1996, 1998) found that high molecular weight fractions of poplar tannins bound nitrogen-containing substrates and reduced mineral nitrogen pools, whereas the low molecular weight fractions acted as substrates or toxins leading to increased immobilization by the increased microbial growth. In our experiment we also found immobilization of inorganic nitrogen in the shrub litter treatments. The pots with *C. vulgaris* litter produced lower concentrations of soil nitrogen compared to the control pots and the pots with grass litter during the first 8 weeks (Fig. 3).

Arbuscular mycorrhizal fungi have not been found to degrade protein-phenolic complexes (Hodge et al. 2001). But molecular analysis based on the nuclear ribosomal internal transcribed spacer (ITS) region revealed that some roots of *D. flexuosa* plants are colonized by beneficial fungal endophytes belonging to the *Helotiales* (Zijlstra et al. 2005). The majority (68%) of the ericaceous root isolates analysed were found to belong to the *Helotiales*. Apparently, some grass root endophytes seem to be related to ericaceous root isolates forming ericoid mycorrhizae, e.g. *Hymenoscyphus ericae* complexes of fungal strains (Zijlstra et al. 2005). Such strains are able to degrade soluble phenolics (Bending and Read 1997; Zijlstra et al. 2005) and enhanced significantly nitrogen uptake in inoculated *D. flexuosa* and *C. vulgaris* seedlings compared to the inoculated control plants (Zijlstra et al. 2005).

Growth and nitrogen uptake in *Calluna vulgaris* was significantly inhibited in the grass litter treatments compared to the treatment without litter. At the start of the experiment, *C. vulgaris* plants established more slowly in this litter treatment—perhaps due to toxic effects of the grass litter on shrub roots. Sánchez-Moreiras et al. (2003) reported on allelopathic effects of grasses due to the presence of substances such as phenolic acids, hydroxamic acid, alkaloids and quinones. More than 40 years ago, Jarvis (1964) showed that *D. flexuosa* humus produces allelopathic effects on plant growth. In Jarvis's experiment with *D. flexuosa* and *C. vulgaris* litter, *D. flexuosa* litter inhibited the growth of oak roots drastically (to almost no root growth), whereas in contrast the oak roots developed well on *C. vulgaris* litter, achieving 90% of the growth of the control treatments. Furthermore, not only did the grass litter produce toxic effects on seedlings, the root exudates of *D. flexuosa* were also found to inhibit seedling growth (Jarvis 1964). Before the start of our experiment we measured the concentration of total phenolics in *D. flexuosa* litter and found it was very low compared to the concentrations found in *C. vulgaris* litter. It therefore seems unlikely that the phenolics in grass litter would be responsible for toxic effects. We cannot exclude the possibility that components other than phenolics could have been playing an inhibitory role. Clearly, the results of our experiment require further investigation to elucidate the possible allelopathic effects in litter of *D. flexuosa* as not only plant growth may be affected, but also mycorrhizal fungi present in the ericaceous roots could have been inhibited by the release of toxic substances or the activity of other litter-feeding organisms (Jarvis 1964; Rose et al. 1983).

Our results emphasize again that *C. vulgaris* plants are much less able to outcompete *D. flexuosa* under nutrient-rich conditions than under less fertile circumstances. Consequently, they confirm that increased atmospheric nitrogen deposition can weaken the competitive ability of *C. vulgaris* plants in heathland ecosystems. Concentrations of phenolics in ericaceous litter that are reduced due to increased atmospheric deposition of nitrogen do not have any effect on the competition between grass and heather. We conclude that the notion that *C. vulgaris* plants can dominate the organic nitrogen pool because they produce leaves rich in phenolics and have an exclusive symbiosis

with ericoid mycorrhizal fungi has to be revised. *D. flexuosa* seems to have adjusted to the nutrient-poor system too and is well able to use litter rich in phenolics. In addition, the fungal endophytes thought to be specific to ericaceous plants have turned out to be not that specific (Zijlstra et al. 2005). The expansion of grass species after an increased input of nitrogen can still be most properly explained by their ability to respond more rapidly than ericaceous dwarf shrubs to increased nutrient supply (Berendse and Elberse 1990).

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