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Soil Conditions in Natural, Declining and Restored Heathlands Influence Plant–Pollinator Interactions of *Calluna vulgaris*

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Abstract

We hypothesized that the contrasting soil conditions resulting from different historical land use in heathlands mediate the interactions of *Calluna vulgaris* with pollinators. We compared using a common garden experiment, the flowering phenology, the interaction with pollinators, and the colonization by ericoid mycorrhiza of mature *C. vulgaris* on three types of soils namely: (1) natural rhizospheric soil collected in a natural heath, (2) soil from an arable land recently restored into a heathland, and (3) soil of *C. vulgaris* from an area in which a high degree of heterospecific competition with perennial grasses occurred. The results of the experiment showed a strong effect of soil on flower phenology and synchrony. There was also an interaction with pollinators because not only did visitation rates depend on soil provenance but also the choice of plant by the pollinator, at least for honeybees, was affected by soil provenance. An a posteriori correlation analysis suggests that ericoid mycorrhizal fungi and not abiotic conditions across the different soil provenances may be involved in the interaction between plants and pollinators. The results obtained from this study highlight the importance of soil processes to understand plant–pollinator interactions and point at plant–soil feedbacks as an important mechanism for understanding heathland ecology.

Key words: ***Deschampsia flexuosa*, plant–soil feedback, ericoid mycorrhiza, competition, pollinators, *Apis mellifera*, soil biota, synchrony, above–below ground interactions.**

Introduction

From both an applied and fundamental point of view it is crucial to understand the factors that determine species interactions and drive community structure and diversity. Terrestrial ecosystems can be considered as two subsystems, above- and belowground, that are intimately connected by the plant community (Wardle et al. 2004). Although during the last decade there has been considerable evidence on how belowground biotic interactions affect interactions aboveground, their importance in restoration issues has been investigated less extensively (Sutherland et al. 2006).

In Western Europe, areas of heathland, dominated by ericaceous shrubs like *Calluna vulgaris,* have suffered a progressive reduction. For example, in central Europe only 5% coverage of *C. vulgaris* remains when compared with the distribution and dominance in the 19th and the first half of the 20th century (Odé et al. 2001). Moreover, 80% of the remaining heathlands are dominated by perennial grasses (e.g. *Molinia caerulea*, *Deschampsia flexuosa, Nardus* spp.) because of the high rates of atmospheric nitrogen deposition (Bakker & Berendse 1999). An important consequence of this decline is the increasing fragmentation of heathlands, resulting in a dramatic loss of species and ecological functions (Usher 1992; Webb 1998). *Calluna vulgaris* is the fundamental species for the maintenance of the unique associated biodiversity and there is currently considerable effort in central Europe to maintain and expand heathlands, namely by managing *C. vulgaris* formations (Webb 1998). In restored or declining heaths, such as recovered arable lands or areas where there is an increased atmospheric nitrogen deposition, soil conditions can be quite different to those of natural heath vegetation (Clarke 1997; Pywell et al. 1997; De Graaf et al. 1998; Allison & Ausden 2004, 2006). Nonetheless, reestablishment and maintenance of *C. vulgaris* is possible once suitable abiotic soil conditions are re-established (i.e. lowered pH values and reduced levels of nitrogen and phosphorus available to plants) and strict management regimes (e.g. by mowing, extensive burning, grazing) that keep under control perennial grasses are implemented (Owen & Marrs 2000; Alonso et al. 2001; Lawson et al. 2004; Hardtle et al. 2006). However, to what extent such different soil conditions affect plant performance and the interaction with other trophic groups has not yet been considered.

Calluna vulgaris is a mycorrhizal-dependent species (Gimingham 1960; Read & Stribley 1973). Several studies have indicated that ericoid mycorrhizal fungi is a fundamental factor for the successful restoration of heathlands (Genney et al.

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2000; Diaz et al. 2006; van der Wal et al. 2009). However, although these studies have highlighted the importance of ericoid mycorrhiza for restoration practices, the effect beyond plant growth has not been addressed. Once populations are established, as in successful restored sites, mycorrhiza might influence important traits that determine the long-term viability of populations.

Plant–pollinator interactions are crucial for the long-term sustainability of most plant populations since they assure interchange of genetic material between individuals of the population. That is the case of *C. vulgaris*, which is a cross-pollinating and partially self-incompatible species (Mahy & Jacquemart 1998; Mahy et al. 1998). There are several environmental factors that determine plant-pollinator interactions (e.g. degree of competition, landscape features); however, soil biotic and abiotic conditions on such interactions have not received much attention (Burkle & Irwin 2009). There are only a few studies on the impact of soil biota on plant–pollinator interactions or on flower characteristics. From these studies, it appears that changes in soil abiotic factors and also in the soil community may have cascading effects on plant–pollinator interactions (Poveda et al. 2003, 2005; Wolfe et al. 2005, Gange & Smith 2005). Such effects are mediated by either the modification of the plant floral display (e.g. the number or size of inflorescences) or by alteration of the quality of attractive traits, such as the number of flowers or the composition of pollen and nectar (Gange & Smith 2005; Wolfe et al. 2005). In this context, flowering phenology is particularly important because it determines reproductive synchrony with potential mates (Augspurger 1983; Marquis 1988). Whether changes in soil conditions may affect the phenology and interactions with pollinators has not yet been studied.

The main aim of this study was to address experimentally how the contrasting soil conditions found in heathlands with a different historical and ecological background would affect plant performance and the interaction with pollinators. We hypothesized that differences in soil attributes would affect the interaction of heath plants with pollinators by modifying basic floral traits and/or the flowering phenology. In a correlation analysis, we also investigated which soil attributes in the studied system may affect floral traits.

Methods

We conducted a "common garden" experiment by transplanting and growing mature *Calluna vulgaris* plant stands in soils from three different heathland provenances. That is, by growing plants in soils from different heaths but in a common environment (common garden) we can differentiate the effect of soil conditions on plant performance and the interaction with pollinators.

Collection of Material for Experiment

Calluna vulgaris plants were collected in the Nature reserve of the Hageven (Neerpelt, Province of Limburg, Belgium). This area was selected because there are three types of heaths, which differ in former land use, but are similar regarding soil type (i.e. sandy gleyic podzols), microclimate and abundance of pollinators (Dekoninck 2001). For the establishment of the experiment we used soil collected under monospecific stands of *C. vulgaris* growing on: (1) natural heath patches (pre-successional stadia toward a temperate cleared deciduous forest dominated by *Quercus robur* and *Betula pendula*), termed *reference* soil (coordinates of the site 51°16′35 76″N, 5°25′1954″E); (2) heath patches in serious decline due to intense competition with *D. flexuosa,* termed *competition* soil (coordinates 51°15′41 81″N, 5°25′09 76″E); and (3) heath patches in former agricultural fields product of a restoration effort by sod cutting (in the mid 1990s) termed *restoration* soil (coordinates 51°15′36 56″N, 5°24′ 58 90″E).

The rhizosphere soil used as substrate for the experiment was collected in the third week of June 2008. Eighteen blocks of 25×15 cm^2 were taken per site (reference, competition, and restored soils) and transported to the laboratory where they were homogenized. Plants for the experiment were collected at the same time, were circa 14 years old, which corresponds with the building phase of *C. vulgaris* (Gimingham 1972); they had similar size (i.e. diameter 17.31 ± 2.23 cm and 16 ± 1.32 cm height) and came from the population growing on natural heath patches. In the laboratory, plants were uprooted and gently washed, then plants were transferred to 1.7 L pots with rhizosphere soil (1.300 cm^3) from the reference soil, restored soil, or competition soil. To avoid bias in the establishment of the experiment all plants were transplanted; thus, for all treatments the same mechanical procedure was followed in the transplantation. Once plants were transferred to pots, these were taken to an experimental garden of Ghent University at Merelbeke (Belgium) and were left outdoors for the rest of the experiment. Plants were watered by natural rainfall and only during July received addition exogenous tap water (twice a week, 200 mL/pot). No fertilization was implemented during the course of the experiment.

Monitoring Interactions with Flower Visitors

The pollinator visitation experiment was conducted in the Heidebos Nature Reserve, East Flanders, Belgium. The plants were brought to this site on the third week of July. This natural reserve consists of 260 ha, and was included in the European habitat directory in 2001. The locality was chosen because, from preliminary studies, we knew that it contained a wide diversity of heath-related pollinators (Dekoninck 2001). The site choice avoided bias by performing the experiment with the pollinator community of our reference. When the plants started to flower, we proceeded with the observations to evaluate the interactions with pollinators. Details on the protocol used can be found in the Appendix S1, Supporting Information.

After the flowering season, the plants were grown for five additional weeks and then were harvested in the second week of November 2008. The total length of the experiment was 18 weeks.

Flower Synchrony

During the course of the experiment, we monitored the number of open flowers every other day. Flower synchrony was calculated for plants from each soil provenance. We used the S_A index proposed by Augspurger (1983) and the S_M index proposed by Marquis (1988), respectively. For details on the calculation and characteristics of synchrony indexes, see Appendix S1, Supporting Information.

Mycorrhiza Colonization

The percentage of mycorrhiza colonization was measured using only secondary plant roots (Johansson 2000). Root fragments were stained following Vierheilig et al. (1998). Percentage of root colonization was assessed by the intersect-grid method of Giovanetti and Mosse (1980), and mycorrhizal structures were classified according to Massicotte et al. (2005). Fifty 1-cm fragments were assessed per plant.

Soil Analysis

From each experimental pot we took a soil subsample of 200 g for analysis of the abiotic properties of the soil, i.e. nitrogen available to plants (NH_4), phosphorus available to plants, percentage of organic matter, pH, electroconductivity, and soil moisture. Details on methods used for soil nutrient analysis are found in the Appendix S1, Supporting Information.

Statistical Analysis

Flowering synchrony (i.e. S_M and S_A indexes), mycorrhizal colonization, and soil abiotic factors were analyzed by analysis of variance (ANOVA; SAS Institute 2003), with soil provenance as a factor. Post hoc comparisons between treatments were performed using Tukey's HSD test. Data on pollinator visits (i.e. number visits and duration of the visits) were analyzed with generalized linear mixed models (Proc Glimmix, SAS Institute 2003; Bolker et al. 2009). First, for each pollinator group a binomial mixed model with logit assuming Bernouilli distribution was used to assess the effect of soil provenance (used as main factor) on the chance of pollination visit by a group of pollinators (response variable). We corrected for eventual changes in the conditions under which the experiment was conducted (i.e. weather, shifts in the pollinator community per date) or individual plant traits (individual plants were repeatedly tested) and, in consequence, we used plant replication, date, and its interaction as random factors. The total duration of pollinator visits and the number of pollinator visits per plant followed a Poisson distribution and were consequently analyzed by GLMM, assuming Poisson-distributed error structure and log-link. Because pollinator visits may depend on the number of flowers and biomass, we modeled the number and duration of the pollination visits using soil provenance, number of flowers, and interaction as fixed effects and, again, date and experimental replicate as random factors. As the error structure of the models was already quite complicated and biomass was shown to be nonsignificant, it was removed from the model, as advised by Littell et al. (2006). In all cases, effective degrees of freedom were always estimated by the Satterthwaite procedure. A Mann–Whitney test was used to compare between treatments the number of days until the flowering peak.

Results

Flowering Phenology and Synchrony

All plants growing on competition soil flowered, whereas plants on reference and restored soil had a lower percentage of flowering (88 and 73%, respectively); therefore, the probability of flowering differed among the three soil provenances ($\chi^2_{2,50} = 6.98$ $p = 0.0305$).

Taking into account the whole flowering period, there was no significant difference between the different soil provenances in the total number of flowers produced ($F_{2,50} = 0.65$; $p = 0.5249$), although the average number of flowers per date measured was different ($F_{2,50} = 33.31$; $p = 0.0001$), which points to differences in flowering dynamics (see further). Also, the flowering period (i.e. number of days from the first flower observed till the last one) was different depending on soil provenance (Fig. 1a). The longest flowering period corresponded to plants growing in reference soil (94 days), while the flowering period of plants growing in restored and competition soil corresponded with 72 and 75 days, respectively. The flowering peak differed between soil provenances. While for plants growing in restored and competition soil the peak was reached on the 9th of August, for plants grown in the reference soil the peak was 5 days later (Fig. 1a, see arrows). When looking at the individual level, there were differences in the duration of the flowering period according to soil provenance ($F_{2,50} = 1.85$; $p = 0.016$; Fig. 1b). The average number of days of flowering for plants growing in the reference soil was 47.58 ± 5.4, while plants growing in soils coming from restored or competition soils had lower values on average, with a mean of circa 35 days (i.e. 38 days for plants grown in restored soil and 35 days in competition soil). The number of days that it took to get to the flowering peak (i.e. the day with the maximum number of flowers per plant) did not differ between soil provenances (Fig. 1c; $F_{2,50} = 0.85$, $p = 0.489$).

For both calculated S_M and S_A indexes, there were significant differences in the values according to soil provenance (Table 1). Both indexes were calculated taking into account different parameters (see Appendix S1, Supporting Information). While the S_A index does not correct for differences in the duration of flowering, the S_M does and, moreover, it only takes into account the presence or absence of flowers. For both indexes, plants growing in competition soil showed the highest levels of synchronization, with plants flowering at approximately at the same time and with the same duration. When correcting for flowering duration (by using the S_M index), the lowest level of synchrony was achieved in the reference soil (Table 1).

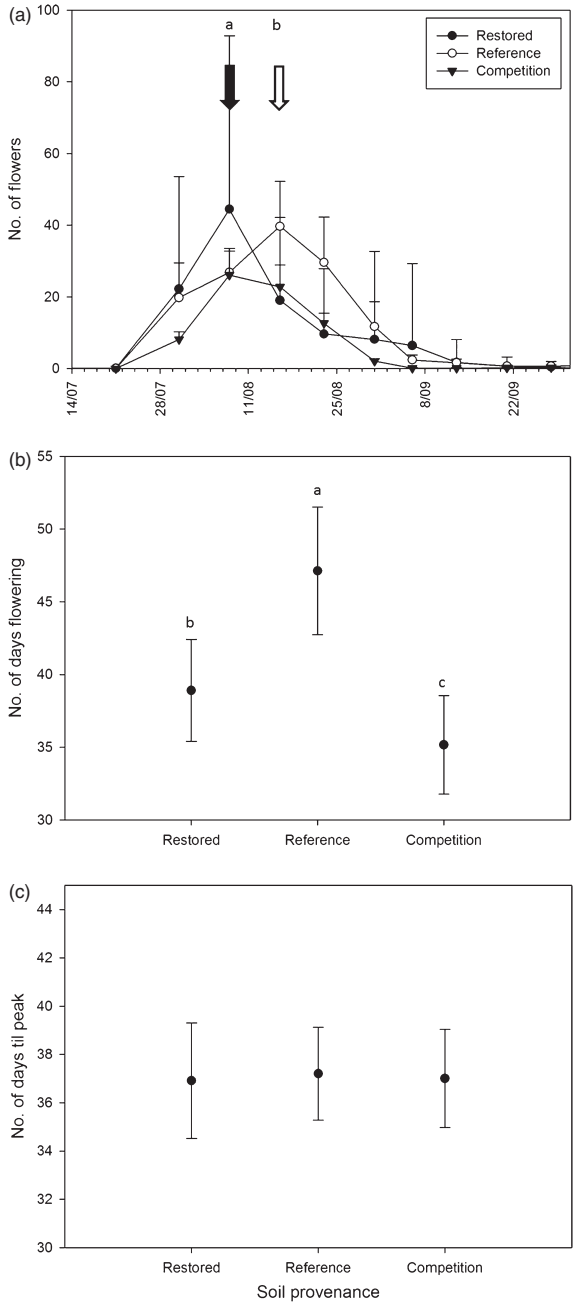

Figure 1. (a) Mean (±SE) number of flowers of *Calluna vulgaris* according to soil provenance, that is, natural, restored, and competition heaths (declining because of competition with *Deschampsia flexuosa*). Mean number of flowers is represented on a weekly basis. A black arrow indicates the flowering peak for plants in restored and competition soil and a white arrow indicates plants in reference soil. Different letters indicate significant differences between dates for the flowering peak after a Mann–Whitney nonparametric test ($p \leq 0.05$); (b) Mean (±SE) number of days that an individual plant was flowering according to soil provenance. Different letters indicate significant differences between soil provenances after a post hoc Tukey test ($p \leq 0.05$); (c) Mean (±SE) number of days until flowering peak (i.e. day with maximum number of flowers) according to soil provenance.

Effect of Soil Provenance on Flower Visitors

In total, 330 plant visits were recorded during the observation period. Insects belonging to eight different groups were found visiting the experimental plants (Fig. 2). The five most common flower visitors were syrphid flies (*Syrphidae*), flies (Empididae and Brachycera), honey bees (*Apis mellifera*), and bumblebees (*Bombus* spp.) (Fig. 2; Table S1, Supporting Information).

Flower visitors were affected by the soil provenance and number of flowers (Table 2). When testing visits regardless of the number of flowers, the effect of soil provenance also remained significant ($F_{2,93.14} = 4.09$, $p = 0.0198$). Plants growing in reference soil received the highest number of visits (mean ± SE, 3.44 ± 0.8), followed by restored soil (2.57 ± 0.67) and competition soil (1.36 ± 0.43). Overall, the more flowers that a plant had, the more visitors it received; however, the effect of the number of flowers differed across soil provenances (Table 2). Such differences are illustrated when the coefficients and slopes obtained from the generalized linear mixed model are plotted (Fig. 3a). For all soil provenances, there is a positive relation among the number of flowers; however, for the same number of flowers, plants growing in competition soil received fewer visits than those in the other two treatments (Table 2, Fig. 3a). The same results were obtained when the durations of the visits were compared (Table 2, Fig. 3b).

The results of the GLMM analysis show that there were no differences in plant preference according to soil provenance for most of the flower visitors (Table 3). However, for honey bees (*Apis mellifera*), there was a clear effect of soil treatment on plant choice, with a higher probability of plants being visited by bees if grown in reference soil than in the other two types of soils (Fig. 4; $F_{2,225} = 3.72$, $p = 0.0257$).

Colonization of Roots by Ericoid Mycorrhiza

All plants showed conspicuous infection by mycorrhizal structures, putatively attributed to the *Hymenoscyphus ericae* complex. A nearly significant effect of soil provenance on plant size was observed ($F_{4,50} = 2.73$, $p = 0.078$), with slightly larger plants for restored soils. There were no differences between the percentages of ericoid mycorrhizal colonization in experimental plants before and after the experiment for plants growing in reference soil ($p = 0.081$, $F_{2,50} = 3.253$, Fig. 5a), which indicates that there was no detrimental effect of transplantation on mycorrhizal colonization, and differences observed for the other soil provenances can likely be attributed to changes in soil conditions. Soil provenance had a significant effect on the colonization by ericoid mycorrhiza ($F_{2,50} = 5.72$, $p = 0.006$, Fig. 5b). The percentage of ericoid mycorrhiza colonization ranged between 58.55 and 26.09%. *Calluna vulgaris* plants growing on reference soil had a significantly higher percentage of colonization than plants growing on the other two types of soils.

Table 1. Comparison of flowering synchrony using the S_M (Marquis 1988) and S_A (Augspurger 1983) indexes of plants growing in soil from different provenances, that is, reference, restored, and competition.

				Soil Provenance		
Variable	*F*	*df*	*p*	*Restored*	*Reference*	*Competition*
S_M	25.73	2,41	<0.0001	0.608 ± 0.023^{b}	0.729 ± 0.020^{a}	0.815 ± 0.018^{c}
S_A	6.60	2,41	0.0035	0.664 ± 0.036^{ab}	0.594 ± 0.042^{b}	0.776 ± 0.032^{a}

The higher the index value (closer to 1), the more synchronous is the flowering within the experimental plants growing in a given soil provenance. Significant differences between provenances according to a post hoc Tukey's HSD test are shown with different letters.

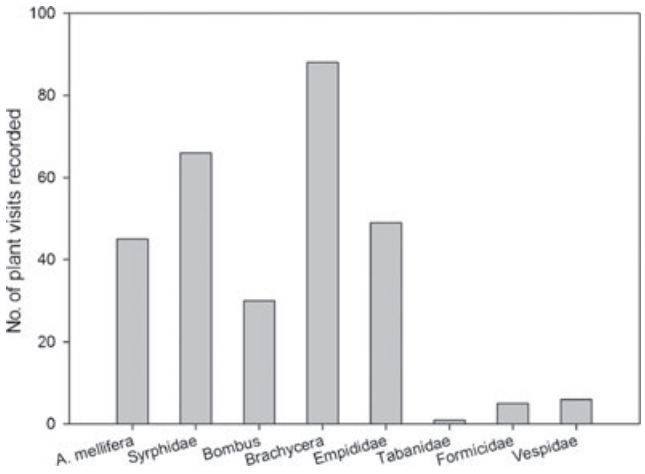

Figure 2. Total number of visits to plants by different flower visitors during the course of the experiment.

Soil Analysis

Soil attributes differed significantly between soil provenances. For nitrogen available to plants, soils from restored heathlands contained 3-fold more nitrogen than soils coming from natural or declining heathlands (Fig. S1A, Supporting Information, $F_{2,50} = 5{,}661.6$; $p = 0.0001$). The same pattern was found for phosphorus available to plants (Fig. S1B; $F_{2,50} = 491.1$, $p = 0.0001$), and pH (Fig. S1D; $F_{2,50} = 1{,}371.50$, $p = 0.001$). For all of these parameters, no differences were found between reference and competition soils. For the percentage of organic matter, there were significant differences between all compared soil provenances (Fig. S1C; $F_{2,50} = 34.6$, $p = 0.001$), and that was also the case for electroconductivity (Fig. S1E; $F_{2,50} = 122.495$, $p = 0.0001$). For both parameters, once again the highest values were found in soils coming from restored heaths. However, no significant differences were found between soil provenances in terms of soil moisture (Fig. S1F; $F_{2,50} = 0.702$, $p = 0.501$).

Correlation Analysis

While all abiotic variables tested were highly correlated with mycorrhizal colonization differences, the number of visits was only correlated with the percentage of mycorrhizal colonization and the number of flowers (Table 4). Floral traits (i.e. average number of flowers and maximal number of flowers) were also correlated (or marginally correlated) with the percentage of mycorrhizal colonization (Table 4), but not with any of the abiotic soil factors measured.

Discussion

Flowering Synchrony

Flowering phenology is considered a key characteristic in plant populations, as it influences many mutualistic and antagonistic interactions and thereby impacts on reproductive success. Although no differences were observed in the number of flowers produced, the number of days flowering and flowering synchrony differed between the three soil provenances compared. Plants grown in competition soil flowered most synchronically, experiencing the lowest visitation rates. The long-term consequences of a reduction in the number of visits need to be experimentally addressed, but because *C. vulgaris* experiences fitness reduction when self-pollinated, we could expect a negative effect (Mahy et al. 1998).

Interaction with Pollinators

There is abundant evidence in the literature indicating that changes in the floral traits of plants directly affect pollinators

Table 2. Statistics for the generalized mixed linear model (GLMM) of the effect of soil provenance (i.e. reference, restored, competition) (*S*) and number of flowers (*F*) per plant on the number of visits by flower visitors and on the duration of their visits.

	No. of Visits per Plant				*Duration of the Visit*			
	F	*Numerator df*	*Denominator df*	*p*	*F*	*Numerator df*	*Denominator df*	*p*
F	33.31	1	58.82	**0.0001**	28.32	1	51.5	**<0.0001**
S	3.98	2	35.28	**0.0276**	4.99	2	37.9	**0.0119**
$F \times S$	5.46	2	49.09	**0.0072**	7.68	2	41.5	**0.0015**

Significant differences are highlighted in bold.

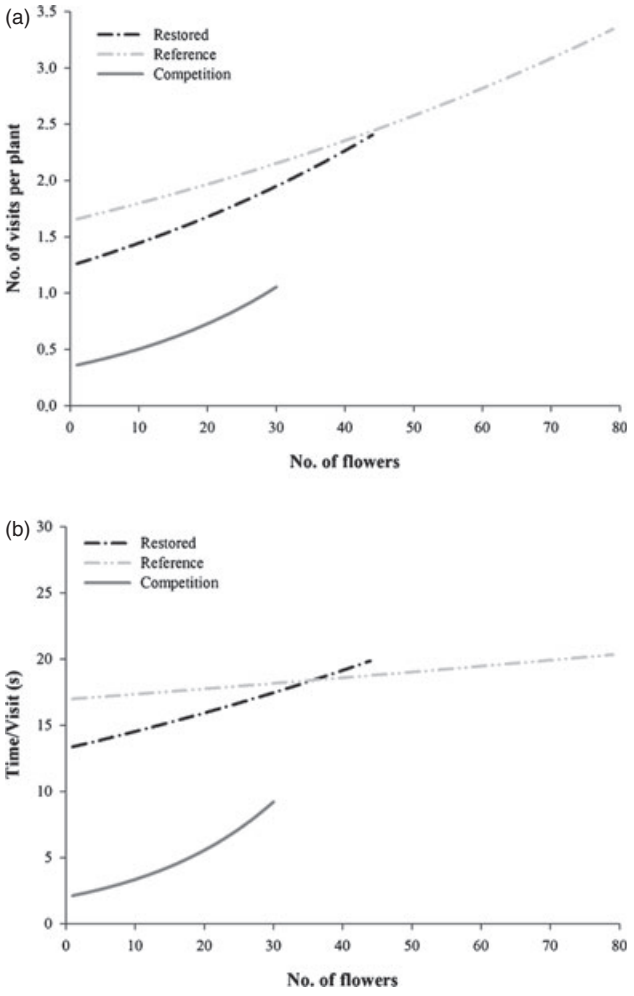

Figure 3. Modeled number of visits (a) and duration of visits (b) as a function of soil provenance and number of flowers. Parameters are estimated from the generalized mixed models with log normal distribution where the variables (i.e. duration of pollinators' visits and number of visits) depend on soil treatment and number of flowers. Differences in the length of dashed lines between soil provenances reflect differences in the average number of flowers in the original data.

Table 3. Statistics for the generalized mixed linear model (GLMM) analyzing the effect of soil provenance (reference, restored, competition) on the probability of a visit by different flower visitors.

Pollinator	*F*	*Numerator df*	*Denominator df*	*p*
Apis mellifera	3.72	2	225	**0.0257**
Syrphidae	0.83	2	225	0.4380
Brachycera	1.58	2	225	0.2074
Bombus spp.	1.33	2	225	0.2674
Empididae	0.70	2	225	0.4974

Significant differences are highlighted in bold.

(Thompson 2001). In our experiment, soil provenance influenced the mean number of flowers per date of observation (with the greatest number of flowers for plants growing in reference soil and the lowest in competition soil) and, moreover,

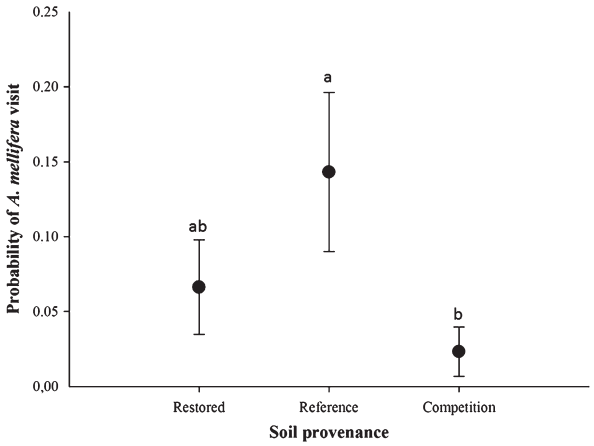

Figure 4. Mean (±SE) probability of receiving a visit by *Apis mellifera* per hour of observation in experimental *Calluna vulgaris* plants according to soil provenance. Significant differences according to Tukey's HSD post hoc comparisons between soil provenances are indicated with different capital letters.

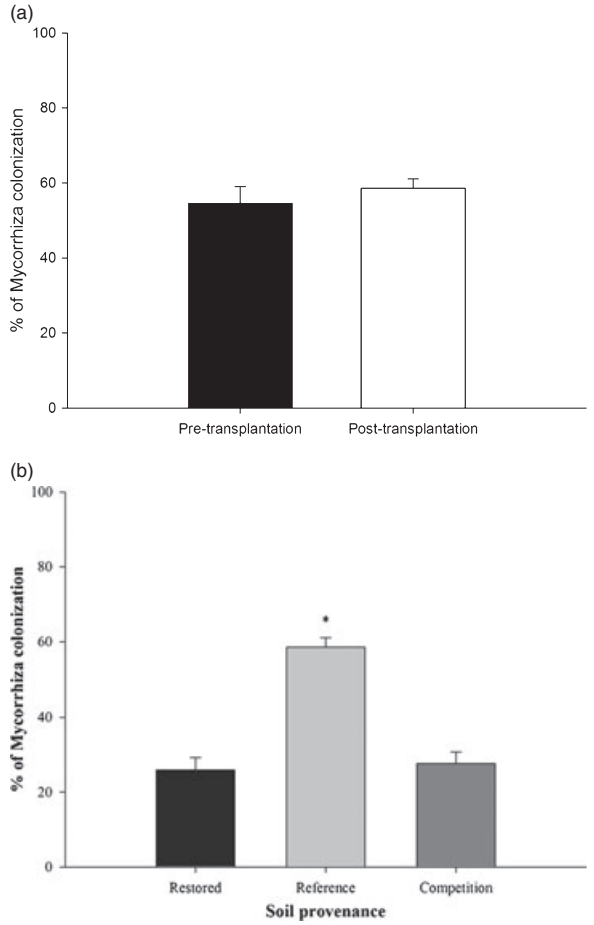

Figure 5. (a) Mean (±SE) percentage of ericoid mycorrhizal colonization in plants growing in the reference soil before and after transplantation; (b) mean (±SE) percentage of ericoid mycorrhizal colonization of roots of the experimental plants at harvest in relation to soil provenance. Significant differences according to one-way ANOVA are indicated with an asterisk ($p \leq 0.001$).

Table 4. Statistics (i.e. Pearson's coefficient correlation, *C*, and *p* values) after pair-wise correlations for different variables measured: percentage of mycorrhizal colonization, visits by pollinators, mean number of flowers (Mean_flowers), maximal number of flowers (Max_flowers), and abiotic factors (i.e. NH_4, pH, electroconductivity [EC], phosphorus [P], percentage of organic matter [OM%], and soil humidity).

		Myc.	*Visits*	*Mean_flowers*	*Max_flowers*	*NH_4*	*pH*	*EC*	*P*	*OM%*	*Humidity*
Myc.	*C*	1	0.297	0.335	0.248	−0.465	−0.534	−0.394	−0.514	−0.125	−0.117
	P	—	**0.031**	**0.014**	**0.074**	**0.001**	**0.001**	**0.04**	**0.001**	0.374	0.406
Visits	*C*	0.297	1	0.919	0.217	0.005	0.002	0.087	−0.018	0.038	−0.029
	P	**0.031**	—	**0.000**	0.118	0.969	0.986	0.534	0.898	0.789	0.838
Mean-flowers	*C*	0.335	0.919	1	0.209	−0.060	−0.077	−0.010	−0.083	0.003	−0.065
	p	**0.014**	**0.000**	—	0.133	0.668	0.584	0.942	0.554	0.985	0.642
Max_flowers	*C*	0.248	0.217	0.209	1	−0.023	−0.024	0.040	−0.063	−0.113	−0.233
	p	*0.074*	0.118	0.133	—	0.869	0.865	0.777	0.653	0.421	0.094
NH_4	*C*	−0.465	0.005	−0.060	−0.023	1	0.978	0.900	0.973	0.685	0.173
	p	**0.001**	0.969	0.668	0.869	—	**0.001**	**0.001**	**0.001**	**0.001**	0.214
pH	*C*	−0.534	0.002	−0.077	−0.024	0.978	1	0.916	0.951	0.619	0.165
	p	**0.001**	0.986	0.584	0.865	**0.000**	—	**0.001**	**0.001**	**0.001**	0.238
EC	*C*	−0.394	0.087	−0.010	0.040	0.900	0.916	1	0.825	0.501	0.251
	p	**0.004**	0.534	0.942	0.777	**0.001**	**0.001**	—	**0.001**	**0.001**	0.070
P	*C*	−0.514	−0.018	−0.083	−0.063	0.973	0.951	0.825	1	0.682	0.142
	p	**0.001**	0.898	0.554	0.653	**0.001**	**0.001**	**0.001**	—	**0.001**	0.309
OM%	*C*	−0.125	**0.038**	0.003	−0.113	0.685	0.619	0.501	0.682	1	0.163
	p	0.374	0.789	0.985	0.421	**0.001**	**0.001**	**0.001**	**0.001**	—	0.243
Humidity	*C*	−0.117	−0.29	−0.065	−0.233	**0.173**	**0.165**	**0.251**	**0.142**	0.163	1
	p	0.406	0.838	0.642	0.094	0.214	0.238	0.070	0.309	0.243	—

Significant differences are highlighted in bold.

for the same number of flowers among treatments visitation rates varied, which suggests that differences in reward quality (either quantity or quality) is the underlying mechanism.

Several types of pollinators, mainly Hymenoptera (i.e. bees and bumblebees) and Diptera, visited the experimental plants, which is in agreement with previously observed heaths for the same geographical region (Gimingham 1960; Mahy et al. 1998). Strikingly, soil provenance did affect plant choice by the common honeybee *Apis mellifera*, which is a key pollinator of *C. vulgaris* (Mahy et al. 1998). Because different pollinators used different morphological traits to distinguish flowers, small changes in floral traits will differently influence the behavior of pollinators (Martin 2004).

While the strength of our approach is that we are able to demonstrate the effect of soil properties on plant–pollinator interactions, we are careful in extrapolating these mechanisms toward population-level effects. Changed soil properties cannot be decoupled from other environmental effects. For instance, under grass encroachments, not only are the rhizosphere characteristics altered, but also the structure of the vegetation. Under such conditions, flowering *C. vulgaris* may actually attract more pollinators compared to relatives in heath patches due to concentration effects (Bossuyt 2007), eventually compensating for the decreased attractiveness with increased visitation rates.

Role of Ericoid Mycorrhizal Fungi

There are several studies on *C. vulgaris* in which interactions at different plant levels have been described; however, most of these studies have investigated how aboveground changes, mainly produced by grazers on the microbial community, affect below-ground interactions (Tracey & Frank 1998; Hartley & Amos 1999; Hartley et al. 2003). In this study, we looked at the inverse path. We assessed colonization by mycorrhiza before and after the transplantation experiment and, in consequence, we attribute (based on a correlation analysis) changes in phenology and the interaction with pollinators to changes in mycorrhizal colonization. Different studies have shown that changes in mycorrhizal colonization and soil abiotic factors can affect floral traits by increasing the number of flowers, pollen grains, and nectar production (Lau et al. 1995; Poulton et al. 2002; Wolfe et al. 2005). Although with our experimental design we are not able to infer whether changes in ericoid mycorrhizal colonization are the result of different initial biotic or abiotic factors, nonetheless we can hypothesize about some of the observed differences. Firstly, the low percentage of colonization of plants growing in soil from former arable lands can be attributed to the high concentration of nitrogen and phosphorus in those soils (Yesmin et al. 1996; Hofland-Zijlstra & Berendse 2009). Secondly, competition soils are very similar in abiotic factors to reference soils (only the percentage of organic matter and the pH are slightly different), and we can attribute the low levels of mycorrhizal colonization to other causes. Ericoid mycorrhiza produce a low number of spores, which results in very low expansion rates (Bergero et al. 2003; Diaz et al. 2006) and in consequence an active spore bank is only possible with the long-time persistence of the host plant (van der Wal et al. 2009). Therefore, the higher percentages of ericoid mycorrhizal colonization of *C. vulgaris* on natural soils, compared with restored and competition soils, might be the product of fewer spores in the latter (Allison & Ausden 2004; Diaz et al. 2006) or different types

of ericoid mycorrhiza present according to soil provenance (Helgason et al. 1998).

There is increasing recognition of the feedback between below- and aboveground biotic interactions for ecosystem processes. The contrasting soil conditions found in the different soils used in our transplantation experiment affected not only plant phenology (i.e. flowering synchrony) but also the interaction between *C. vulgaris* and pollinators.

Restoration practices in heathlands focus on the reintroduction and/or maintenance of certain key species (e.g. *Calluna vulgaris*), but they often neglect to assess whether key interactions are completely restored. Our study goes in this direction and reveals intricate processes that highlight complex interactions between the soil, the plant community and the pollinators that associate with *C. vulgaris.*

Implications for Practice

- The results of our common garden experiment indicate that flowering synchrony is affected by soil conditions and that this has cascading effects on the interaction between *Calluna vulgaris* and pollinators.
- Plant–soil feedback in heathlands is likely to be driven by ericoid mycorrhiza.
- In order to understand the functioning and ensure the long-term restoration and management of heathlands, plant–soil feedback needs to be taken into account, not only to understand plant responses, but also the interaction of heather with other tropic groups.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary material and methods and complementary results of pollinator visits and soil analysis

Table S1. Mean (±SE) duration of a visit to a flower and number of flowers visited per plant according to type of flower visitor.

Figure S1. Abiotic factors according to soil provenance, i.e. natural, restored and declining heathland to competition with *Deschampsia flexuosa.*: A. total nitrogen available to plants; B. phosphorus available; C. organic matter; D. pH; E. electroconductivity; and F. soil humidity. All graphs show mean (±SE) and different letters indicate significant differences after post-hoc Tukey test ($P \leq 0.0001$).

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