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**The effects of forest floor organic matter, charcoal and light availability on the establishment and growth of *Pulsatilla patens* (L.) Mill. and *Pulsatilla pratensis* (L.) Mill.**

Master of Science Thesis

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# Contents

1. Introduction	3
1.1. The aims of the study	6
2. Materials and methods	8
2.1. Study species	8
2.2. Seed material	9
2.3. Experimental design	9
2.4. Plant measurements	11
2.5. Data analysis	11
3. Results	13
4. Discussion	21
Summary	27
Kokkuvõte	29
Acknowledgements	31
References	32

# 1. Introduction

The causes of rarity are not always easy to explain as there are usually several factors and processes influencing plant performance. In some cases, the reason behind rarity can be easily guessed, for example while the considered species is at the margin of its distribution or when it exclusively inhabits very rare habitats (Rabinowitz 1981; Kunin *et al.* 1997). However, in other cases, there is frequently no consistent and unambiguous explanation to why some species are less abundant than others (Rabinowitz *et al.* 1984; Baskauf *et al.* 1994; Bevill *et al.* 1999; Lloyd *et al.* 2002; Murray *et al.* 2002). Still, understanding the causes of rarity is essential for determining the conservation strategy in species' protection (Pärtel *et al.* 2005).

While considering the rarity of a species which inhabits rare habitats, two kinds of rarity have been notified – “natural” rarity and “anthropogenic” rarity (Fiedler 2001; Pärtel *et al.* 2005). In the first case, species is believed to be rare because it lives in a very limited habitat and has always been rare during its evolutionary history. In the second case, species may have been widespread in the historic past, but, through relatively recent negative human-imposed interactions, its habitat has been destroyed or converted into a different one. For instance, the current forestry suppresses forest fires and that results in uniform dense stands with rich above-ground litter layer, without natural gaps in the tree layer. Such conditions may hinder the regeneration of species whose regeneration is favoured by the suppression of competition due to local disturbances (Uotila 1996; Pilt *et al.* 2002; Pärtel *et al.* 2005; Reier *et al.* 2005). Although, as for most species, fire itself is not needed, the opening of mineral soil patches for regeneration is still crucial.

There has been a growing interest in ecological importance of wildfires in the last decades. Forest fires are considered as primary type of disturbance in natural boreal forests (Zackrisson *et al.* 1996; DeLuca *et al.* 2002; Berglund 2004; Czimczik *et al.* 2005), with return-interval from 80 to 100 years (Zackrisson 1977; Berglund *et al.* 2004). The coniferous canopy, as well as the rich above-ground litter layer, makes boreal coniferous forests relatively fire-prone. Historically, the primary source of ignition has been lightning (Latham 2003; Pitkänen *et al.* 2003; Czimczik *et al.* 2005),

and the spread of fire has been hindered only by the availability of fuel (litter, needles, twigs *etc.*), weather, and natural barriers (Brown *et al.* 2000).

Fire is likely to enhance light conditions for field-layer herbs and decrease competition intensity among herbaceous species by destroying the moss and litter layer (as well as field layer dwarf shrubs), thus providing favourable conditions for seed germination and seedling establishment. Changes in the availability of photosynthetically active radiation (PAR) have frequently been reported to cause multiple and strong adaptive morphological responses in plants (Dorn *et al.* 2000; Steinger *et al.* 2003). As the availability of light is one of the most limiting factors while considering plant growth (Valladares *et al.* 1998; Maddonni *et al.* 2002), improved light conditions must be of crucial value, since these can modify species associations.

Fires often apply significant rejuvenating effects on soil properties. Given that a substantial proportion of nutrients, especially nitrogen, may be locked in forest floor organic matter, and not readily available for plants and micro-organisms, forest fires are of high importance for nitrogen cycling by increasing decomposition of plant litter and humus (Schimmel *et al.* 1996; Pietikäinen *et al.* 2000; Prescott *et al.* 2000; Czimeczik *et al.* 2005; Gonzalez-Perez *et al.* 2004). In addition, forest fires may improve soil properties by also increasing the pH value or increasing microbial activity (Schimmel *et al.* 1996; Pietikäinen *et al.* 2000; Gonzalez-Perez *et al.* 2004).

The reason behind the fact that most of the nitrogen in forest floor organic matter is bound in complex molecules, and thus relatively immobile, probably lies in the chemical nature of the forest floor organic matter in boreal forests. The latter is shown to be rich in various phenolic compounds, especially this, produced by ericoid dwarf shrubs (*Calluna vulgaris*, *Empetrum nigrum*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *etc.*; Rice 1984; Gallet *et al.* 1997; Jäderlund *et al.* 1998; Hättenschwiler *et al.* 2000; Mallik *et al.* 2000). These compounds are believed to seriously affect nitrogen cycling (Aerts 1995; Northup *et al.* 1995; Inderjit *et al.* 1999; Hättenschwiler *et al.* 2000; Kruse *et al.* 2000; Kraus *et al.* 2004). Phenolic compounds are capable for binding proteins and other organic substrates into

insoluble complexes (Northup *et al.* 1995; Hättenschwiler *et al.* Vitousek 2000; Prescott *et al.* 2000; Vitousek *et al.* 2002) which in turn are usually hardly degradable and thus responsible for changes in soil properties (Inderjit *et al.* Mallik 1997).

In addition to the effect of phenolic compounds on nutrient cycling, these can have some direct negative effects to plants as well. It has been demonstrated that phenolic compounds from forest floor organic matter are responsible for poor germination and seedling establishment (Pellissier 1994; Jäderlund *et al.* 1998; Wardle *et al.* 1998; Wu *et al.* 1998; Gallet *et al.* 1999; Reigosa *et al.* 1999; Kruse *et al.* 2000; Mallik *et al.* Pellissier 2000; Séné *et al.* 2000; Souto *et al.* 2000; Muscolo *et al.* 2001; Wallstedt *et al.* 2001; Hille *et al.* den Ouden 2005). Moreover, they may inhibit the growth of mycorrhiza as well (Robinson 1972; Kruse *et al.* 2000; Souto *et al.* 2000).

Forest fires leave ash, charcoal, and other fire-altered material on the ground. These are usually charred or partially burned plant remains, which chemical profile is determined by the intensity of fire (Perez-Fernandez *et al.* Rodriguez-Echeverria 2003). One of the important properties of charcoal is its porous structure (Zackrisson *et al.* 1996) that provides a good adsorbing ability. Accordingly, charcoal is described to efficiently adsorb secondary metabolites, such as humus phenols produced by the ericaceous vegetation (Zackrisson *et al.* 1996; Wardle *et al.* 1998; Pietikäinen *et al.* 2000; DeLuca *et al.* 2002; Berglund *et al.* 2004). In addition, the porous structure of charcoal may offer favourable microsites for microbes that can utilize the adsorbed compounds as substrate (Zackrisson *et al.* 1996; Pietikäinen *et al.* 2000). The levels of charcoal after forest fires in boreal forests are sufficient to have possible ecological effect (Zackrisson *et al.* 1996).

Charcoal is very persistent in boreal forests. However, it has been shown that charcoal from sites with a recent fire history has considerably greater capacity to adsorb phenolic metabolites compared to sites with less recent fires (Zackrisson *et al.* 1996). Zackrisson *et al.* (1996) demonstrated that charcoal has its sorptive abilities for the first 100 years following its formation. This could be so as charcoal from sites with recent fires appears to have a clearer surface while that from sites with less recent fires is largely occluded by humic debris and senescent hyphae. Although it sorptive

ability weakens with time passed by last forest fire, this effect can be restored by subsequent fires.

The stimulus behind this study was a concern about clarifying the possible causes why *Pulsatilla patens* (L.) Mill. (synonym *Anemone patens* L.; Ranunculaceae), a well-known and much discussed herb, which has experienced an increased decline during the last decades and is now considered a threatened plant species in all European countries in which it occurs, is much less abundant than its close relative *Pulsatilla pratensis* (L.) Mill. (Ranunculaceae). The populations of these species, but especially of *P. patens*, have diminished appreciably (Uotila 1996; Pilt *et* Kukk 2002). One possible reason behind this could be an increase in moss layer density (Uotila 1996; Pilt *et* Kukk 2002).

### ***1.1. The aims of the study***

The purpose of this study was to elucidate the effect of phenolic compounds and charcoal on the growth of *P. patens*. As the previous study of Kalamees *et al.* (2005) showed, the charcoal, produced by forest fires, could enhance the conditions for germination and seedling establishment of *P. patens*. In the current study, the aim was to test experimentally the hypothesis that the germination and growth of *P. patens* is retarded by forest floor organic matter (from what phenolic compounds are believed to leach out), whereas charcoal is able to improve its negative impact. Since forest wildfires, besides altering forest floor environment and nutrient cycling, also strongly alter the light environment, the availability of PAR was also manipulated in the experiment.

Another purpose of the experiment was to elucidate whether the two *Pulsatilla* species, present in Estonia – *P. patens* and *P. pratensis* – differ in their response to environmental changes which are characteristic to those caused by forest wildfires. Positive answer to this question would possibly help to explain the differential ecological success of the two species in the last decades. Since both *Pulsatilla* species

can be considered as potentially disturbance-related boreal herbs, I also included third species from the same family – *R. polyanthemos* L. The latter is a widespread herb, inhabiting grasslands, forest-edges and road-edges, that has not been included among so-called gap species (disturbance-dependent boreal herbs). Thus, when *P. pratensis* was included as a close relative with similar ecological preferences, *R. polyanthemos* was grown as a phylogenetically relatively close species with different ecological performance, which is not expected to have complex adaptations to the integrated environmental effects associated with forest wildfire.

## 2. Materials and methods

### 2.1. Study species

*P. patens* is a perennial that shows widespread circumpolar distribution, growing in Europe as well as in North America (Hultén *et* Fries 1986). In Europe, *P. patens* subsp. *patens* ranges from Eastern Europe to Central Siberia (Hultén *et* Fries 1986; Jalas *et* Suominen 1989). In Estonia the species is at its north-western border of continuous distribution (Pilt *et* Kukk 2002). *P. pratensis* is an European endemic, growing mainly in Eastern and Central Europe (Jalas *et* Suominen 1989).

In several isolated localities in central and north-western Europe populations of *P. patens* are considered to be relicts (Uotila 1996). *P. patens* is protected as a rapidly declining species in all those European countries in which it occurs. The decline of the species is presumably to great extent related to changes in land use, especially in forestry practices where efficient wildfire prevention and termination of cattle grazing in forests has led to the formation of a continuous moss layer or a strongly grass-dominated vegetation, that severely hinders the regeneration of the species (Uotila 1969, 1996).

In Estonia the habitat of *P. patens* is usually dry boreal forest with limited distribution while *P. pratensis* being found both in forest and grassland locations (Pilt *et* Kukk 2002). They usually grow in well-illuminated locations such as forest edges, clearings, and slopes of southern exposition, where the vegetation layer is sparse or disturbed (Uotila 1969; Pilt *et* Kukk 2002). Both species tend to grow also in open anthropogenic habitats where human influence is frequently evident such as road and railroad edges, logged and grazed areas, excavations for sand, *etc.* (Uotila 1969; Uotila 1996). In Estonia several mixed stands have been found, with both species sharing the same habitat.

*R. polyanthemos* is a perennial grassland species which is phylogenetically as well as morphologically close to *Pulsatilla* species. In addition, it might, to a certain extent,

be also considered as disturbance-dependent species since it inhabits semi-natural communities, altered by grazing, mowing or logging. However, *R. polyanthemos* has never been considered a fire-related herb.

## **2.2. Seed material**

Seeds of these three study species were each collected from two different sites in Estonia in the summer of 2004. Seeds of *P. patens* were collected from south-eastern Estonia, Palojärve; and northern Estonia, Aegviidu. Seeds of the second study species, *P. pratensis* were collected from north-western Estonia, Alliklepa; and northern Estonia, Kurblu. And finally, seeds of *R. polyanthemos* were collected from south-eastern Estonia, Trassi grassland; and south-western Estonia, Varbla.

Seeds were stored in paper envelopes at room temperature. Only presumably fertile seeds (those not visibly immature or damaged) were selected for sowing. Before sowing the elongated feathery beaks were removed from the achenes of *P. patens* and *P. pratensis* (as in the study of Kalamees *et al.* 2005).

## **2.3. Experimental design**

To evaluate the effect of forest floor organic matter, charcoal, and light availability on plant performance, a laboratory experiment was conducted. Plants were maintained at 16-h light/8-h darkness regime in a growth-chamber, daytime full light level being  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  (OSRAM Fluora<sup>®</sup> fluorescent lamps).

Three treatments – addition of forest floor litter and decay (+/-), addition of charcoal (+/-), and shading (50% and 100% of original illumination in the growth chamber) – were combined in a factorial design. In the variants where organic matter and charcoal

were not added, sand was used as a substitute. In addition, sand was also added as a component of soil mixture in every treatment. Soil treatments were as follows: (1) charcoal and organic matter added (ratios in soil mixture 1:1:1, charcoal, organic matter, and sand); (2) only charcoal added (ratios in soil mixture 1:2, charcoal and sand, respectively); (3) only organic matter added (ratios in soil mixture 1:2, organic matter and sand, respectively); and (4) control (only sand).

Organic matter was collected from boreal pine forest in south-western Estonia, Soomaa, where both *Pulsatilla* species occur naturally. The main dominating species in the ground layer were *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Calluna vulgaris*, *Pleurozium schreberi* and *Hylocomium splendens*. Organic matter, approximately with thickness 1-2 cm, was gathered from beneath the vegetation layer. To imitate charcoal produced by forest fires, commercial charcoal was shattered into small particles. All components of soil were homogenized.

Shade was provided by tents made of aluminium-coated shade cloth (spectrum neutral; Ludvig Svensson, Kinna, Sweden), which allowed us to achieve 50% of the original illumination of the growth chamber.

Each treatment variant was represented by five replicate plants. The plants were grown in 0.38-litre plastic pots (diameter 7 cm, height 7.6 cm). Plants were watered regularly and uniformly, to keep the soil moist.

Seeds were sown on moist soil in a growth chamber at the beginning of December, 2004. Initially, five seeds were sown into each pot; after germination all but one randomly chosen healthy seedling were removed.

Plants of *R. polyanthemos* were harvested at the end of March, 2005, and plants of *P. patens* and *P. pratensis* at the end of May, 2005. Different harvesting times were practiced so that plants of different species could be compared at a similar phenological stage.

## ***2.4. Plant measurements***

The number of germinated seeds was recorded per each pot. After harvesting, the number of leaves and the length of petiole were recorded. Length was measured to the nearest millimetre.

Plants were dried at 80°C for 24 hours. Above-ground and below-ground parts were weighed separately to an accuracy of 10<sup>-4</sup> g. Total vegetative biomass (above-ground vegetative biomass + root biomass) and the ratio between below-ground biomass and above-ground biomass (root:shoot ratio) was also calculated.

Leaf area estimates were obtained using a scanner (CanoScan LIDE 30), Adobe Photoshop 7.0 and Pindala 1.0 software. As a result, total photosynthetic area and specific leaf area (SLA, leaf area per unit leaf mass cm<sup>2</sup> g<sup>-1</sup>) was found.

## ***2.5. Data analysis***

For data analysis, a factorial three-way Type 1 ANOVA model was used for each species with organic matter addition (two levels: present/absent), charcoal addition (two levels: present/absent), and shading (two levels: 50% and 100% of full-light) serving as fixed independent variables. Dependent variables were: number of germinated seeds; number of leaves; root:shoot ratio; average length of the petiole; total photosynthetic area; specific leaf area; and total vegetative biomass.

Tukey HSD test was used to identify differences between trait means. Plant counts were transformed (log; square root) to meet assumptions of normality.

In addition, having some pre-information from aforementioned ANOVA, to elucidate the adaptations of *Pulsatilla* species to environment, characteristic to this caused by

forest fires, General Linear Model (GLM) that included *Species* as a categorical factor was used. Also, t-test was performed to find if trait means differ among two *Pulsatilla* species.

All analysis were performed by using software system STATISTICA 7.1.

### 3. Results

The results of all main effects are shown in Table 1.

The applied experimental treatments had no effect on seed germination rate in any of the three studied species.

Table 1. Analysis of variance for phenotypic traits of *P. patens*, *P. pratensis* and *R. polyanthemos* in different treatments. *p*-values are shown, with significant effects in boldface.

Trait	Treatments	p-values of species		
		<i>P. patens</i>	<i>P. pratensis</i>	<i>R. polyanthemos</i>
Number of germinated seeds	Organic matter	0.806	0.751	0.933
	Charcoal	0.766	0.812	0.628
	Light availability	0.181	0.239	0.628
Total vegetative biomass	Organic matter	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Charcoal	0.198	0.140	0.876
	Light availability	<b>0.004</b>	0.099	<b>&lt;0.001</b>
Root:shoot ratio	Organic matter	0.489	0.200	0.725
	Charcoal	0.140	0.063	0.862
	Light availability	<b>0.012</b>	0.059	<b>&lt;0.001</b>
Number of leaves	Organic matter	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Charcoal	0.755	0.187	0.407
	Light availability	0.113	0.802	<b>0.049</b>
Average length of petioles	Organic matter	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.003</b>
	Charcoal	0.383	<b>0.016</b>	0.557
	Light availability	<b>0.014</b>	<b>0.002</b>	<b>&lt;0.001</b>
Total photosynthetic area	Organic matter	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Charcoal	0.368	0.757	0.996
	Light availability	0.261	0.810	0.656
Specific leaf area	Organic matter	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.095
	Charcoal	<b>&lt;0.001</b>	<b>0.030</b>	0.520
	Light availability	<b>&lt;0.001</b>	<b>0.022</b>	<b>&lt;0.001</b>

The presence of forest floor organic matter significantly affected almost all measured traits, mostly in all three species. Plants had more total vegetative biomass, higher number of leaves, and larger total photosynthetic area when organic matter had been added. The average length of petioles was also greater in the presence of forest floor organic matter. The effect of forest floor organic matter on the thickness of leaves (as indicated by SLA) was registered only in *Pulsatilla* species. Leaf area per unit leaf mass was larger when organic matter was absent from soil. The only measured trait that was not affected by the addition of forest floor organic matter into the soil was root:shoot ratio (ratio of below-ground and above-ground biomasses).

The addition of charcoal into the soil had a main (direct) effect on SLA in both *Pulsatilla* species – plants grew thinner leaves in the presence of charcoal – but had no effect on *R. polyanthemos*. Also, charcoal shortened the average length of petioles in *P. pratensis* but remained non-significant in other two species.

Light availability had also certain predictable main effects on plant performance. In conditions where the amount of photosynthetically active radiation was lower (50% of full-light treatment), plants elongated their petioles and increased their leaf area per unit leaf mass (SLA). Such plastic responses were observed in all species. In *P. patens* and *R. polyanthemos* better light availability resulted also in higher biomass, and in an increased allocation of biomass into below-ground parts (increased root:shoot ratio). The latter species had also more leaves in high light.

There was a significant *Forest floor organic matter\*Charcoal* interactions on plant performance (Table 2). In both *Pulsatilla* species total vegetative biomass, average length of petioles, and SLA the effect of the addition of forest floor organic matter depended on whether charcoal was added or not (or *vice versa*). Plants had the lowest total vegetative biomass (Figure 1. a, b) and shorter petioles (Figure 2. a, b) when soil contained charcoal but forest floor organic matter was absent. Leaf area per unit leaf mass (SLA) was highest only when charcoal was added to the soil without the presence of forest floor matter (Figure 3. a, b). On the other hand, if the soil contained both charcoal and forest floor organic matter, *Pulsatilla* plants had vigorous growth – their total vegetative biomass was the highest and petioles were the longest.

Table 2. Analysis of variance for phenotypic traits of *P. patens*, *P. pratensis* and *R. polyanthemus* in different treatment interactions. *p*-values are shown, with significant effects in boldface.

Trait	Treatments	p-values of species		
		<i>P. patens</i>	<i>P. pratensis</i>	<i>R. polyanthemus</i>
Number of germinated seeds	Organic matter*Charcoal	0.415	0.751	0.112
	Organic matter*Light availability	0.175	0.210	0.902
	Charcoal*Light availability	0.295	0.579	0.097
Total vegetative biomass	Organic matter*Charcoal	<b>&lt;0.001</b>	<b>0.005</b>	0.607
	Organic matter*Light availability	0.215	0.210	0.413
	Charcoal*Light availability	0.372	0.531	0.213
Root:shoot ratio	Organic matter*Charcoal	0.246	0.625	0.238
	Organic matter*Light availability	0.654	<b>0.003</b>	0.397
	Charcoal*Light availability	0.148	<b>0.006</b>	0.652
Number of leaves	Organic matter*Charcoal	0.157	0.159	0.857
	Organic matter*Light availability	0.828	<b>0.046</b>	0.938
	Charcoal*Light availability	0.717	0.283	0.192
Average length of petioles	Organic matter*Charcoal	<b>0.002</b>	<b>0.003</b>	0.792
	Organic matter*Light availability	0.099	0.335	0.483
	Charcoal*Light availability	0.120	0.787	0.324
Total photosynthetic area	Organic matter*Charcoal	<b>0.007</b>	0.053	0.968
	Organic matter*Light availability	0.234	0.054	0.696
	Charcoal*Light availability	0.367	0.624	0.926
Specific leaf area	Organic matter*Charcoal	<b>&lt;0.001</b>	<b>0.003</b>	0.811
	Organic matter*Light availability	0.455	0.857	0.087
	Charcoal*Light availability	0.407	0.710	0.059

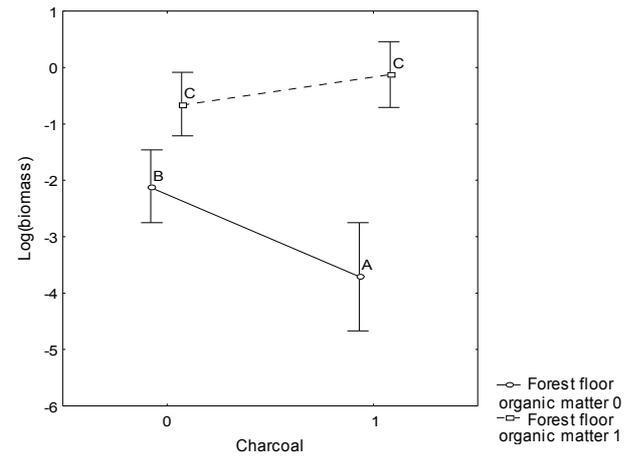
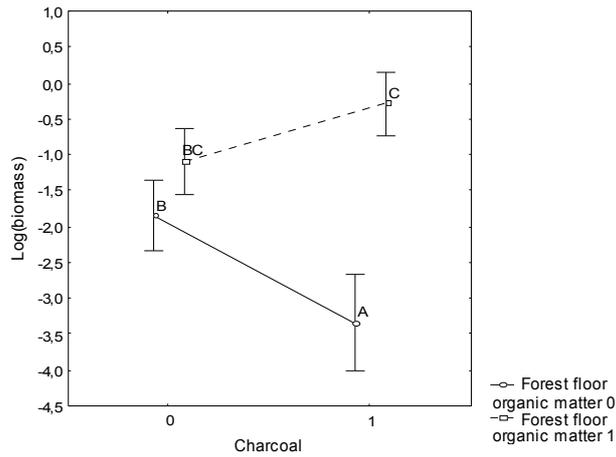
Also, *Pulsatilla* plants produced the thickest leaves when soil contained both forest floor matter and charcoal. Forest floor organic matter and charcoal interactively affected total photosynthetic area in *P. patens* but not in *P. pratensis*. While forest floor organic matter and charcoal were both present in the soil, *P. patens* plants had the highest total photosynthetic area (Figure 4.).

There was a significant *Forest floor organic matter\*Light availability* interaction only in *P. pratensis* (Table 2, Figure 5. and Figure 6.). Plants that experienced low illumination (50% of full light), allocated more resources to above-ground structures, and produced more leaves in case the forest floor organic matter was added to the soil.

A significant *Charcoal\*Light availability* interactive effect on root:shoot ratio in *P. pratensis* was detected (Table 2, Figure 7.) – at low illumination (50% of full light) plants allocated more resources to below-ground structures in case the charcoal was added to the soil.

As for *R. polyanthemus*, there were no significant interactive effects of the applied experimental treatments.

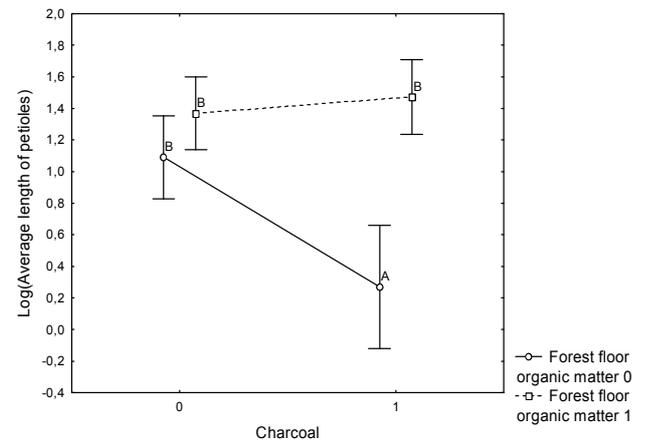
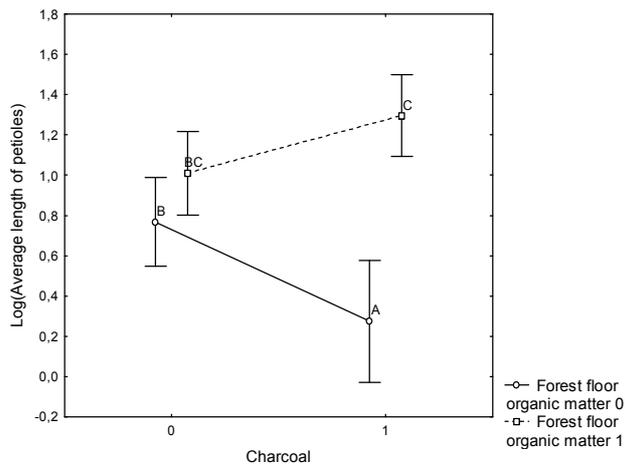
As the two *Pulsatilla* species demonstrated remarkably similar responses to the main and interactive effects of *Forest floor organic matter* and *Charcoal* treatments, I was interested this would be confirmed by exploring the data with the help of a General Linear Model that included *Species* as a categorical factor. The results of the analysis showed no significant effect of *Species*. Regarding this, and also the fact that I could not detect any significant differences among trait means of the two species (t-test, data not shown), I thought it possible to observe certain traits of the two *Pulsatilla* species so that the data was pooled together. This study revealed a strong dual effect of charcoal addition, in case forest floor matter was not present – plants had thinner leaves and grew more roots in such treatment. Figure 8. demonstrates that only-charcoal-treatment clearly contrasts with other treatment variants.



**a**

**b**

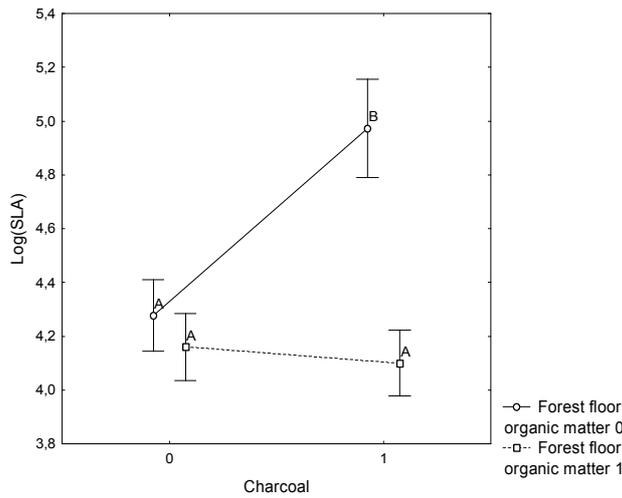
Figure 1. *Forest floor organic matter\*Charcoal* effect to the total vegetative biomass of *P. patens* plants (a) and of *P. pratensis* plants (b). The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.



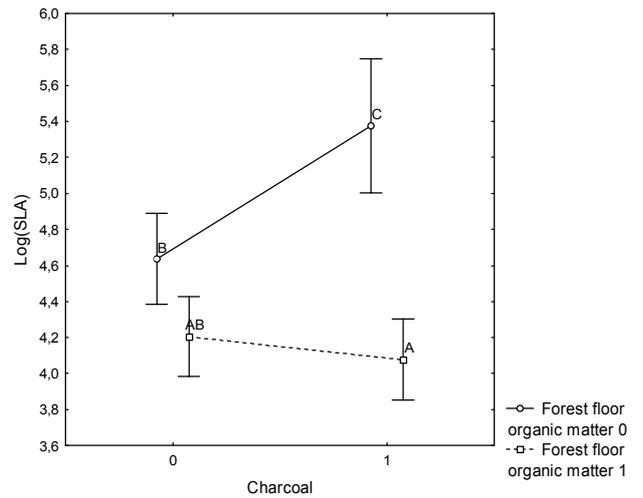
**a**

**b**

Figure 2. *Forest floor organic matter\*Charcoal* effect to the average length of petioles of *P. patens* plants (a) and of *P. pratensis* plants (b). The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.



**a**



**b**

Figure 3. *Forest floor organic matter\*Charcoal* effect to specific leaf area (SLA) of *P. patens* plants (a) and of *P. pratensis* plants (b). The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.

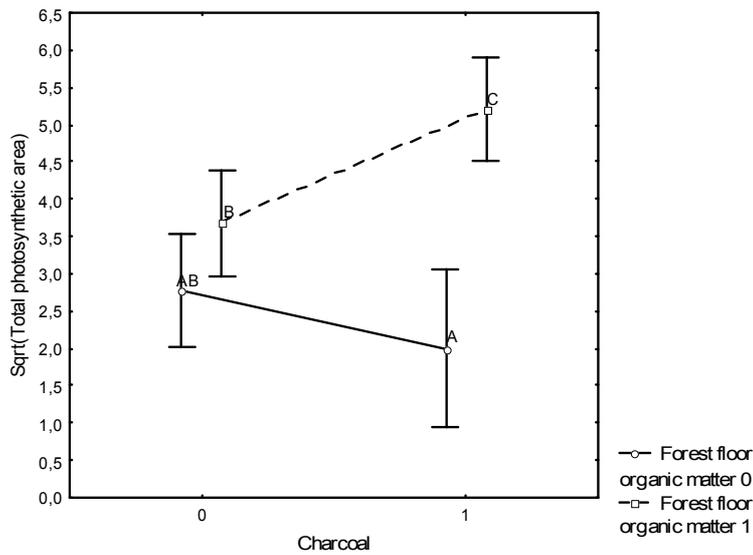


Figure 4. *Forest floor organic matter\*Charcoal* effect to total photosynthetic area of *P. patens* plants. The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.

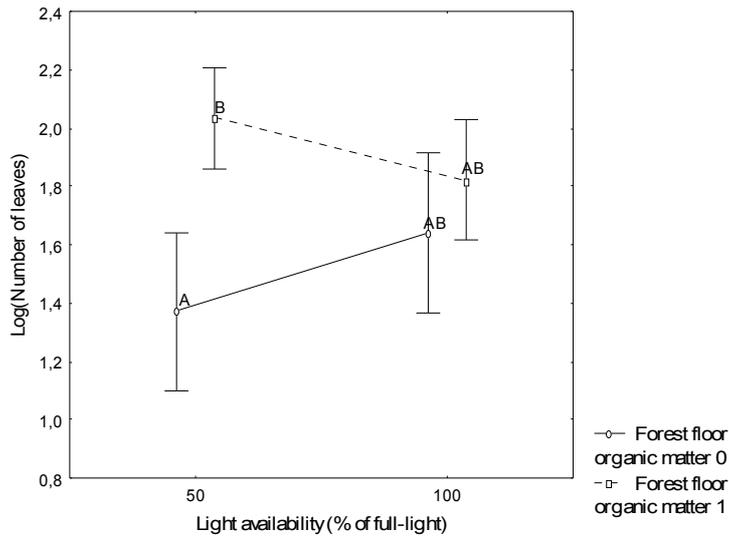


Figure 5. *Forest floor organic matter\*Light availability* effect to the number of leaves of *P. pratensis* plants. The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.

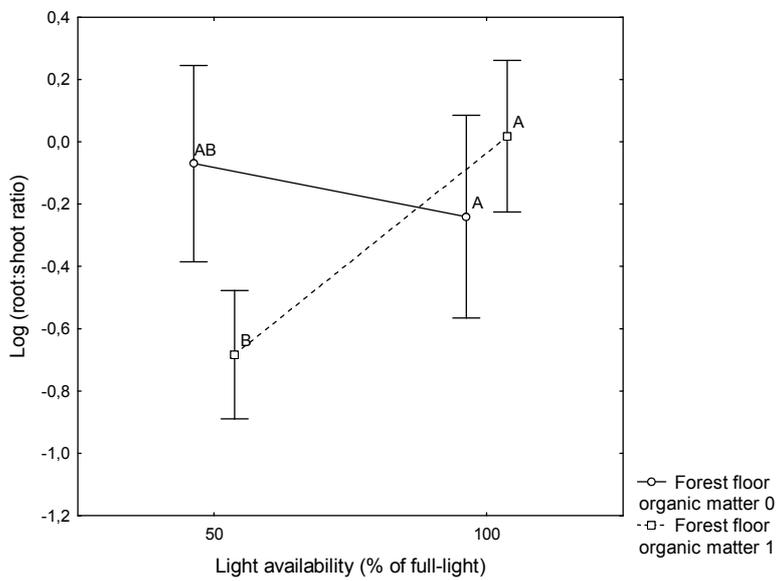


Figure 6. *Forest floor organic matter\*Light availability* effect to the root:shoot ratio of *P. pratensis* plants. The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.

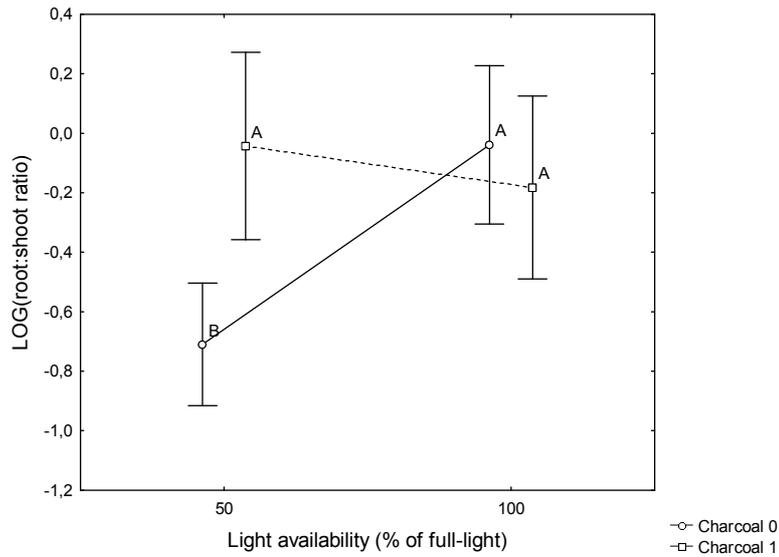


Figure 7. *Charcoal\*Light availability* effect to the root:shoot ratio of *P. pratensis* plants. The error bars denote 95% confidence intervals for the mean. Different letters indicate homogeneous groups within a treatment variant (Tukey HSD). Symbol "0" marks absence and "1" presence of parameter.

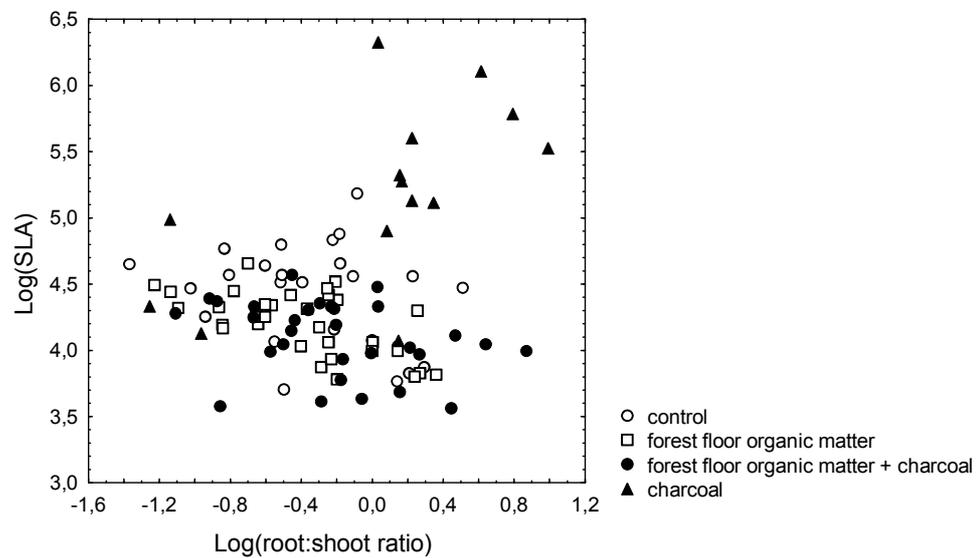


Figure 8. Allometry between specific leaf area and root:shoot ratio in different soil treatments in *Pulsatilla* species. Four symbols denote different treatments.

## 4. Discussion

In a way, the results of the experiment are rather unexpected, especially in what concerns the effects of forest floor organic matter. So far there has not been evidence of a positive effect of ericoid-rich boreal forest floor litter on plant performance. Quite the other way round, ericoid-rich litter has been found to inhibit seed germination and plant growth. The notion of negative effect of ericoid litter stems from several studies that show that ericaceous forest floor organic matter contains several phytotoxic phenolic compounds that inhibit the growth of plants (Pellissier 1994; Jäderlund *et al.* 1998; Wardle *et al.* 1998; Gallet *et al.* 1999; Prescott *et al.* 2000). In this study, the presence of forest floor organic matter in soil mixture affected almost all measured traits positively (*i.e.* increased growth), independent of the species. Also, leaves grew relatively thicker when forest floor litter was added, except in *R. polyanthemos*. It should be noted here that most of the studies that have examined the effect of phenolic compounds on plant performance, have not used forest floor organic matter as a source of these compounds. Instead, water-diluted extracts from green leaves of ericaceous plants have been used (see Zackrisson *et al.* 1996; Nilsson *et al.* 2000; Hille *et den Ouden* 2005; Keech *et al.* 2005) with greatly varying concentrations of the phenolic compounds (Pellissier 1994; Reigosa *et al.* 1999; Hille *et den Ouden* 2005). Thus, these studies have not adequately mimicked the actual effect of ericoid litter since the concentrations in most of the experiments have been much higher than the expected concentrations in the nature. This can radically change the ecological interpretation of the mentioned experiments since Reigosa *et al.* (1999) have demonstrated that in low concentrations phenolic compounds may even promote the establishment of plants.

Thus, these findings suggest that there might be some other aspects in the integrated effect of ericoid forest floor organic matter than the so far discussed phytotoxic effects. Probably there exist some ameliorating effects on plant growth that are not thoroughly studied yet. It has been proposed that forest floor organic matter may reduce the fluctuation of temperature, and, in addition, provide moister conditions by increasing moisture-holding capacity of the soil (Ibañez *et Schupp* 2002).

Despite the fact that in this experiment forest floor litter had clear positive effects on plant performance, in the nature it may still represent a barrier to regeneration by physically keeping the seeds apart from mineral soil surface and also by hindering seedling emergence (Facelli *et Pickett* 1991; Ibañez *et Schupp* 2002).

Some direct (main) effects of charcoal addition on plant performance were detected. In an intriguing way charcoal significantly increased SLA in both *Pulsatilla* species, but had no effect on *R. polyanthemos*. Furthermore, charcoal shortened the average length of petioles in *P. pratensis*. Already Tryon (1948) noticed that the presence of charcoal shortened plant height. He argued that such response could be the result of an increased salt concentration in the soil. This is quite probable as salinity can lead to reduced growth (Crawley 1997). Tryon (1948) claimed these salts might originate from the pieces of fine-grained or crushed charcoal. I propose that there might be other mechanisms as well. The addition of charcoal into soil mixture turns soil surface towards darker, increases radiation absorption capacity and causes a rise of soil temperature. This, in turn, could be responsible for increasing specific leaf area (Woodward 1979; DeLucia *et al.* 1992).

As expected, plants generally grew better in high light. This effect was detectable in most of the measured traits in *R. polyanthemos*. Probably, in *R. polyanthemos* the availability of light could be one of the key factors for growth and survival since it is considered to be a disturbance-dependent grassland species. In both *Pulsatilla* species there was a response to light availability in SLA and in petiole length. These are expected plastic responses to light, aimed at better light capture in low light (higher SLA) and at vertical elongation with the aim to reach better radiation conditions in competitive situations (longer petioles). In *P. patens*, better illumination resulted also in higher biomass and increased allocation to roots. It shows that well illuminated conditions are favoured by *Pulsatilla* species, and are crucial for vigorous growth of *P. patens*.

While exploring forest floor organic matter and charcoal interactive effects, I was able to reveal certain trends of plant performance that were characteristic to both *Pulsatilla* species but absent in *R. polyanthemos*. In all but one interactive effects the nature and extent of responses were almost identical in both *Pulsatilla* species, demonstrating a

remarkably similar ecological performance of the two. When soil contained charcoal but the forest floor litter was absent, plant growth was severely inhibited. When forest floor organic matter and charcoal both were added to the soil, plant growth was the most intense. This can be seen in plant biomass as well as in petiole length data (see Figure 1. and Figure 2.). The only difference between the two *Pulsatilla* species was the above-mentioned interactive effect on total photosynthetic area, this was present in *P. patens* but absent in *P. pratensis* (Figure 4).

Despite demonstrating clear interactive effects of forest floor organic matter and charcoal, there is little, if any, support in favour of the initial work hypothesis. The aim was to test for the negative effect of forest floor organic matter and for the compensating effect of simultaneously added charcoal. Such interactions have been demonstrated in several previous studies in other species (Wardle *et al.* 1998; Pietikäinen *et al.* 2000; DeLuca *et al.* 2002; Berglund *et al.* 2004). In this study the results were quite different – charcoal had a strong negative and forest floor litter strong positive direct impact on plant performance.

It remains unclear why charcoal, if added to the soil alone, affected plant growth negatively, when, at the same time, if applied together with forest floor organic matter, had no negative effect whatsoever (or even non-significant but consistently positive effect). Still, some speculations could be made here. If one considers two types of forest fire – one that does not destroy all of the organic matter layer and in which most of the vegetation survives, and the other that is more severe by killing most of the above-ground vegetation and leaving only charcoal on the ground – one might expect that *Pulsatilla* species have a sensing mechanism which makes the plants respond differently, depending on the severity of damage caused by the fire. If the plant detects that the fire has not completely harmed the forest floor organic matter, it will be beneficial to intensify above-ground vegetative growth since most of the surrounding environment is still viable and vigorous growth of the herbaceous layer will be expected after moderate wildfire. In this case the best competitive strategy is fast vegetative growth with the aim of capturing as much of the radiation resource as possible. On the other hand, if fire has also removed the organic litter layer, increased environmental stress can be expected in the nearest future (due to removed organic layer soil temperature would rise and soil moisture content would

sharply decrease). In such stressful environment large plant size would be harmful because of high transpiration and relatively higher water content of plant tissues in the case of rapid growth.

There may also somewhat different explanations to the differential response to charcoal and forest floor organic matter addition than the above-presented hypothesis. Environment containing only charcoal and no forest floor organic matter generally signals a situation after recent forest fire. At the same time, environment with the presence of both forest floor organic matter and charcoal would indicate an absence of forest wildfires for a long time. Soil without charcoal could therefore imply to an environment with no forest wildfires in the past. Thus, if *Pulsatilla* plants experience an environment in which only charcoal is present, indicating a recent forest fire, they form relatively cheap leaves (see Figure 2. and Figure 3.) and allocate more resources to below-ground parts (see Figure 8.). Such a response might help to survive possible further disturbances as it has been demonstrated that *Pulsatilla* plants have dormant buds in a considerable depth which can be activated to form new above-ground structures as a result of damage (Wildeman *et Steeves* 1982). As the life-span of *Pulsatilla* leaves is relatively brief (Wildeman *et Steeves* 1982) and there exists a strong need to invest to below-ground structures, it could be reasonable to invest as little as possible to light capturing organs. The strategy in which plants can survive forest fires by allocating more resources to below-ground structures to produce buds, which stay dormant until activated by disturbance, has been described by many researchers (Brown *et Smith* 2000; Vesik *et Westoby* 2004; Falster *et Westoby* 2005; Knox *et Clarke* 2005). Thus, the “negative” effect of charcoal may conceal a ‘cautious’ response to a signal of forest wildfire and an enhanced investment to stress-tolerant survival strategy. At the same time the vigorous growth of *Pulsatilla* species in the presence of forest floor organic matter (or the absence of both – forest floor organic matter and charcoal) could indicate that there is no obvious threat and the plant can continue its growth without any large-scale arrangements on its ontogeny.

In addition, I was able to demonstrate the interactive effect of forest floor organic matter and light availability in *P. pratensis*. Plants that experienced low illumination (50% of full light) produced the greatest number of leaves when forest floor organic matter was added to the soil and hence, allocated more resources to above-ground

biomass (see Figure 5. and Figure 6.). However, the number of leaves was the lowest and the allocation to below-ground parts highest in 50% shade and without forest floor organic matter in the soil. Biomass partitioning was influenced also by interactive effects of charcoal and light availability. Plants which experienced low illumination (50% of full light) allocated more resources to below-ground structures in case the charcoal was added to the soil (see Figure 7.). However, the allocation to roots decreased in 50% shade and when charcoal was absent. On this basis one may assume that these allocation responses are related to each other and may be explained by the above-described *Forest floor organic matter\*Charcoal* effects. In other words, the absence of forest floor organic matter, as a whole, might signal about a situation where charcoal is present and *vice versa*.

The initial aim of this study was to explore which factors could cause the rarity of *P. patens*. However, current study seems to indicate that at least the chemical properties of forest floor organic matter, which I believed to affect the growth of *P. patens* negatively, are not responsible for the poor regeneration of *P. patens*. I propose that forest floor organic matter is more likely a physical barrier that hinders germination and seedling establishment as shown also in Kalamees *et al.* (2005). In other words, I believe that putative phytotoxic effects of forest floor organic matter can not be responsible for the rarity of *P. patens*. The relationship between forest fires and seed germination in *P. patens* (see Uotila 1996) is not due to phytotoxic effects of forest floor organic matter but due to the fact that wildfires destroy the moss and litter layer in boreal stands allows for successful germination and establishment of seeds. The results of this study raise a question whether forest floor organic matter really contains any compounds that are capable of retarding the regeneration of *P. patens*.

While comparing rare *P. patens* and common *P. pratensis*, I was unable to detect major differences in their performance as far as the integrated effects of charcoal and forest floor litter was concerned. However, there were certain differences in biomass partitioning and leaf production in responses to the interactive effects of illumination and forest floor litter. As demonstrated in earlier studies (Uotila 1969; Uotila 1996; Pilt *et al.* 2002, Kalamees *et al.* 2005) and in this study, it is clear that benign light conditions are crucial for both *Pulsatilla* species. This study demonstrated that *P. pratensis* is somehow able to withstand low illumination in case forest litter is

included in the soil. This ability is absent in *P. patens*. Therefore it is possible that the differences between the regeneration of the more common *P. pratensis* and the rare *P. patens* are in some way induced by interactive effects of forest floor organic matter and light availability.

It is important to note that none of the above-discussed interactive responses could be observed in *R. polyanthemos*, a species that inhabits environments which are not prone to forest wildfires. This confirms that the complex responses of *Pulsatilla* species to forest litter and charcoal are adaptive and related to regular forest wildfires in boreal forests.

In conclusion, this study strongly indicates that other aspects than phytotoxic ones should be considered when relating plant performance to the presence of ericoid litter in future studies. Thus, more experiments must be conducted to clarify the exact impact of forest floor organic matter to the growth of plants. Similarly, further experiments are required to ascertain the effect of charcoal and especially the interactive effect of charcoal and forest floor organic matter. Finally, it can be said that research to elucidate possible causes for the rarity of *P. patens* should be continued, as so far there is no clear evidence that would explain the poor regeneration of *P. patens*.

## Summary

This study was carried out as a laboratory pot-experiment in 2005.

The aim of this study was to elucidate whether forest floor organic matter as a potential source of phytotoxic phenolic compounds is responsible for the poor regeneration of *P. patens* in nature, and whether charcoal, produced by forest fires, expected to adsorb phenolic compounds, is capable of reducing the negative effects provided by the organic matter. Light availability was also considered.

Three treatments – addition of forest floor litter and decay (+/-), addition of charcoal (+/-) and shading (50% and 100% of original illumination in the growth chamber) – were combined in a factorial design. The same treatments were applied to *P. pratensis*, which is phylogenetically closely related to and shares similar habitat with *P. patens* but is much more common; and to *R. polyanthemus*, which is disturbance dependent but not fire-dependent grassland species. The different responses of *Pulsatilla* species and *R. polyanthemus* to forest floor organic matter, charcoal and light availability were expected to indicate adaptations to habitats, and different responses of *P. patens* and *P. pratensis* were expected to give a sight to the successive ability of these two species in forest.

The results showed that the addition of forest floor organic matter alone influenced positively almost all measured traits notwithstanding the species, however the effect of charcoal was rather hindering. Light availability changed plants morphology in an expected way.

Two *Pulsatilla* species demonstrated similar responses while exploring forest floor organic matter and charcoal interactive effects. When soil contained charcoal but the forest floor litter was absent, plant growth was severely inhibited. When forest floor organic matter and charcoal both were added to the soil, plant growth was the most intense. Possible hypothesis for such responses were proposed.

In addition, current study seems to indicate that at least the chemical properties of forest floor organic matter, which were believed to affect the growth of *P. patens* negatively, are not responsible for the poor regeneration of *P. patens*. However, it is possible that the differences between the regeneration of the more common *P. pratensis* and the rare *P. patens* are in some way induced by interactive effects of forest floor organic matter and light availability.

Thus, more experiments are needed to clarify the exact effect of forest floor organic matter and charcoal on the regeneration and growth of *Pulsatilla* species.

## Kokkuvõte

### Kõdu, söe ja valguse kättesaadavuse mõju palu-karukella (*Pulsatilla patens*) ja aas-karukella (*Pulsatilla pratensis*) kasvule

Käesolev töö viidi läbi 2005. aastal potikatsena Tartu Ülikooli botaanika ja ökoloogia instituudi laboris.

Töö eesmärgiks oli uurida metsakõdu kui potentsiaalse allelopaatiliste fenoolide allika ja söe kui metsapõlengute tagajärjel tekkinud potentsiaalse absorbendi mõju palu-karukella (*Pulsatilla patens*) kui ohustatud ja looduses halvasti uueneva liigi seemnete idanemisele ja taimede kasvule erineva valguse kättesaadavuse tingimustes.

Katses kasutati kombineeritult kolme töötlust – kõdu lisamine (+/-), söe lisamine (+/-) ja varjutamine (50% ja 100% valgustatusest). Lisaks palu-karukellale kasutati neid töötluseid ka aas-karukella (*Pulsatilla pratensis*) ja mitmeõiese tulika (*Ranunculus polyanthemos*) puhul. Aas-karukell on palu-karukellale fülogeneetiliselt lähedalseisev liik ning eelistab sisuliselt kasvada palu-karukellale sarnastes kasvukohtades. Mitmeõiene tulikas seevastu kasvab pigem niidukoosluses, olles seega küll ilmselt kohastunud häiringutele, kuid mitte põlengutele. Seega peaksid kahe karukella ja tulika vahelised erinevad reaktsioonid metsakõdule, söele ja valguse kättesaadavusele viitama kasvukohaga seotud kohastumuslikele eripäradele, ning kahe karukellaliigi omavahelised erinevad reaktsioonid nende kahe liigi levikusageduse võimalikele põhjustele.

Käesoleva töö tulemused näitasid, et kõdu lisamine peamõjuna mõjutas positiivselt peaaegu kõiki mõõdetud tunnuseid hoolimata liigist, samas mõjus süsi taimede kasvule pigem pärssivalt. Valguse kättesaadavus mõjutas taimi oodatud viisil.

Kahe karukellaliigi reaktsioon kõdu ja söe koosmõjule oli sarnane. Kui muld sisaldas sütt, aga kõdu ei lisatud, oli taimede kasv pärsitud. Kui mullasegusse lisati nii kõdu kui ka sütt, oli taimede kasv tunduvalt jõulisem. Sellise käitumise seletamiseks pakuti välja mitu hüpoteesi.

Käesoleva töö põhjal võib väita, et kõdus ilmselt ei ole karukellade jaoks kasvu pärssivaid ühendeid, seega ei ole kõdu keemilised omadused ka tõenäoliselt palu- karukella harulduse põhjustajaks. Samas ei saa välistada võimalust, et kahe karukella liigi erineva levikusageduse taga on kõdu ja valguse koosmõjud.

Kõdu ja söe täpsem mõju karukelladele, aga ka nende kahe liigi erineva ohtruse põhjuste selgitamine, vajab täiendavat uurimist.

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