

Population structure and distribution of
the declining endangered forest plant
Chimaphila umbellata

by
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The Department of Ecology,
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Cover: Flowering *Chimaphila umbellata*. Photo by Margareta Edqvist.

Summary

The occurrence of the rare forest plant *Chimaphila umbellata* has decreased with approximately 80 % in Uppland and its decline is also reported for other regions in Sweden. Suggested causes of decline include the increasingly shaded conditions in understory habitats and increased competition from *Vaccinium myrtillus* and graminoid species. The aim of the study was to investigate the effects of various biotic and abiotic conditions on *C. umbellata* populations with regard to population size, flowering frequency, fruit set and seed production. Conditions analyzed include light inflow, coverage of competitive species, soil nitrogen, continuity of forest cover and soil texture, which were investigated in 38 *C. umbellata* sites in Uppland and Södermanland, Sweden. Results showed that population size was negatively affected by the coverage of competitive species. Population size was not affected by light availability, but increased shading resulted in a decreased flowering frequency. Fruit set decreased with increasing coverage of competitive species and seed production per capsule decreased with increasing soil nitrogen content. This study shows that the overall changes in present-day forest conditions have negative effects on *C. umbellata*. Longevity and clonal recruitment may act as buffers of these unfavourable environmental conditions, which might contribute to an extinction debt in the species. There is a need for urgent and targeted management in forest habitats, including moderate disturbance regimes to reduce competition and increase light inflow, to maintain viable populations of *Chimaphila umbellata* in our forests.

Sammanfattning

Ryl (*Chimaphila umbellata*) är en vintergrön växt tillhörande tribus Pyroleae inom familjen Ericaceae. Artens utbredning i Sverige är begränsad till de södra och centrala delarna, med nordlig gräns längs kusten i Medelpad. Ryl förekommer främst i barrskogar dominerade av tall, och den anses generellt föredra sandiga och näringsfattiga jordar. Arten är klonbildande och reproducerar sig följaktligen både asexuellt och sexuellt. Ryl har i likhet med övriga Pyrolaarter mycket små frön med en mycket liten näringsreserv, s.k. "dust-seeds", vilka produceras i stort antal. Arten är därför beroende av mykorrhizasvampar som den parasiterar på för att kunna gro och etablera sig, medan den som vuxen planta är autotrof.

Rylen har minskat kraftigt under de senaste decennierna. En studie från Uppland rapporterar en minskning på ca 80 %, och arten är numera rödlistad ("starkt hotad") i Sverige. En hypotes är att minskningen orsakas av förändringen i markanvändning och skogsbruk under det senaste seklet, vilket har medfört att skogarna har blivit tätare och skuggigare. Ytterligare en hypotes är att rylen har minskat på grund av ökad konkurrens från blåbär och gräs. Dessa arter har gynnats av den ökade tillförseln av näringsämnen till skogsekosystemen från luftföroreningar och jordbruk, samt av skogsbetets upphörande.

Studiens syfte var att undersöka effekten av olika miljöfaktorer på ryl med avseende på populationsstorlek, blomningsfrekvens, fruktsättning och fröproduktion. De undersökta miljöfaktorerna inkluderade ljusinsläpp, täckningsgrad av blåbär och gräs, jordens näringshalt och textur samt skogens ålder. Studien genomfördes under sommaren 2013 och omfattade 38 ryl-lokaler i Uppland och Södermanland.

Resultaten visade att populationsstorleken minskade då täckningsgraden av blåbär och gräs ökade. Populationsstorleken påverkades inte av ljusinflödet, det gjorde däremot blomningsfrekvensen som minskade i skuggigare miljöer. Vidare visade resultaten att fruktsättningen minskade med ökande täckningsgrad av blåbär och gräs och att fröproduktionen minskade då kvävehalten i jorden ökade.

Resultatet tyder således på att förändringarna av skogarna som skett under det senaste seklet, genom intensivare skogsbruk och ökade kväveutsläpp, har haft en negativ påverkan på ryl. Arten minskade i antal när konkurrensen från blåbär och gräs ökade, och blomningen avtog i skuggiga miljöer. Populationsstorleken påverkades däremot inte av ljusstillgången. En möjlig orsak till att någon sådan effekt inte kunde påvisas är att klonbildande arter kan fortleva under

långa perioder av ogynnsamma ljusförhållanden genom asexuell reproduktion. Det är möjligt att det hos rylen finns en utdöendeskuld d.v.s. att de för arten ogynnsamma miljöförhållandena ännu inte resulterat i ett utdöende på grund av lång livslängd och klonal tillväxt.

Sammantaget visar studien på behov av förändringar i skogsmiljön för att rylen ska kunna fortleva. Skötselåtgärder som ökar ljusinsläppet och minskar konkurrensen i fältskiktet från bland annat blåbär och gräs skulle främja arten. Minskade storskaliga utsläpp av näringsämnen kan också antas viktiga för dess långsiktiga bevarande. Ytterligare studier är nödvändiga, bland annat med fokus på rylens demografi, för att kunna utröna vilka livsstadier som är mest sårbara för de miljömässiga förändringarna. Dessutom behövs mer kunskap om rylens beroende av mykorrhizasvampar. Sammantaget skulle detta öka kunskapen om utdöenderisken och vilka skötselåtgärder som är mest effektiva för att bevara ryl i våra skogar.

Introduction

The loss of biodiversity and transformation of the composition of biological communities due to human influence on earth's ecosystems is of rising concern both locally and globally (Vitousek et al. 1997). Ecosystem properties depend highly on biodiversity and knowledge of the processes and factors that affect species extinctions is hence essential (Hooper et al. 2005). This study concerns the population structure and distribution of one rare and declining plant, *Chimaphila umbellata* (Pipsissewa, Sw. Ryl). The species is believed to decline because of overall changes in present-day forest conditions, and it is currently red-listed (endangered) in Sweden. Below I will first give a general presentation of the background of the changing forest conditions, before describing *C. umbellata*, and the specific objectives of this study.

Changes of forest habitats

Before commencement of substantial anthropogenic influence, a large proportion of Sweden was covered with extensive forests subjected to natural disturbances like fires, storms and grazing by wild animals (Bengtsson et al. 2000). Humans began to modify the landscape through agriculture and other human activities in the beginning of the millennia BCE (before common era). This influence was small-scale and relatively stable, with minor fluctuations of resource use, up to the 18th and 19th centuries when modernization of the agricultural system yielded dramatic land use changes, i.e. grasslands and wetlands were converted to arable land (Eriksson and Cousins 2014). The pre-industrial agricultural landscape (c 1850) was dynamic and characterized by fenced infields, where crops and fodder were cultivated, and outfields that provided additional resources. The forests, as part of the outfields, were utilized for grazing of the livestock and collection of timber for household needs (Dahlström et al. 2006).

To improve the grazing habitat, forests were subjected to small-scale management actions like occasional clearing and burning (Ericsson 2001). In addition, forest ecosystems were subjected to natural fire regimes (Ericsson et al. 2000). Industrialization led to an increased exploitation of forests in the late 19th century (Östlund et al. 1997). However, the extent and intensity of forest use has varied across boreal regions and time – in the central parts of Sweden the human impact was substantial already from medieval times due to mining activities (Ericsson 2001; Kardell 2003). The removal of biomass by fires, grazing and logging resulted in sparse and sun exposed forests and stands that were uneven-aged and

multilayered (Ericsson et al. 2000). Also, it led to the loss of nitrogen from the forest ecosystem (Kellner and Redbo-Torstensson 1995).

The gradual introduction of modern forest management in the beginning of the 20th century combined with an intensified forestry after the 1950's has entailed major transformations of forest ecosystems. Management includes practices like clear-cutting, thinning, fertilisation, draining and plantations of pine and spruce. This has resulted in stands that are relatively dense, young, even-aged and single-layered, and dominated by one species i.e. pine or spruce (Östlund et al. 1997). The grazing of livestock in forests was banned in 1928, however, it still occurred for some decades until gradually decreasing in the middle of the 20th century and the practise was eventually abandoned (Eriksson and Cousins 2014). Also, forest fires were suppressed (Ericsson et al. 2000). Overall, this has resulted in the fast regrowth of forests and hence darker understory conditions (Maad et al. 2009). The light availability in forests has been further reduced by the large-scale planting of Norway spruce (*Picea abies*), a late-successional species with a great shading capacity (Hedwall et al. 2013).

Thus, the present management regimes disfavors species that are dependent on features of old-growth forests and those adapted to sparse, light forests (Berg et al. 1994; Bengtsson et al. 2000; Ericsson et al. 2000). Changes in forest use have consequently led to a reduction in many forest species populations and a loss of biodiversity (Berg et al. 1994; Östlund et al. 1997; Bengtsson et al. 2000). Furthermore, clearcut logging has been found to influence species composition and diversity of ectomycorrhizal fungal communities (Jones et al. 2003). Sweden is dominated by coniferous forests where impacts from forestry have been and continue to be major (Larsson and Thor 2010) and a high number of species are hence affected. There are currently 2,131 species associated to forest habitats on the Swedish national red-data list, corresponding to 52 % of the listed species (Gärdenfors 2010).

The changes in land use have been accompanied with a large increase in the use of fossil fuels and consequently atmospheric emissions of nitrogen and sulphur compounds. The atmospheric deposition of nitrogen and the resulting nutrient input to terrestrial and marine ecosystems has risen sharply since the 1950's (Lövblad 2000). The annual deposition of nitrogen has been highest in the most southern parts and the western region of Sweden, in 2011 it was 500-1000 mg/m². In Uppland and Södermanland, in the east central of Sweden, the deposition was lower, 200-500 mg/m² (SMHI 2013). In addition, nitrogen compounds are released from agriculture and livestock production (Bertills and Grennfelt 2000).

Swedish boreal forests are nitrogen limited and species native to these habitats are hence adapted to low levels of nutrients (Tamm 1991). Input of nutrients will alter the species composition due to differences in competitive ability, i.e. species adapted to environments with nutrient limitations are replaced with species that can utilize high nutrient levels (Kellner and Redbo-Torstensson 1995). Shifts from lichen-dominated flora to dominance by dwarf shrub flora, like *Vaccinium* species, and then to graminoid species can hence be expected and have also been demonstrated (Tamm 1991; Kellner and Redbo-Torstensson 1995; Strengbom et al. 2001; Manninen et al. 2009). Nitrogen deposition has also been found to influence the species composition of mycorrhizal communities (Wallenda and Kottke 1998; Lilleskov et al. 2002). Furthermore, the deposition of nitrogen has a positive effect on forest productivity with denser canopies and subsequently less light inflow to the understory vegetation (Kellner and Redbo-Torstensson 1995).

There are long-term studies showing shifts in species composition, which can be derived to changes in forest management and nitrogen deposition, although determining the extent of each effect separately can be difficult (Falkengren-Grerup et al. 2000). A study over southern England demonstrated that the observed reorganisation of woodland plant communities since the 1930's was an effect of increased levels of nutrients and a higher degree of shading (Keith et al. 2009). Similar findings were reported by Maad et al. (2009) in their mapping of changes in the plant species distribution in the province of Uppland, in east central Sweden, during the last century. They found that several factors significantly explained the observed frequency changes, highest explanatory power was reported for species biotope affiliation, regional distribution, soil fertility, light and dependence of management. Species with a northern distribution, and species favoured by management and light had decreased their distribution while species favoured by soil nitrogen had increased it. The study included 451 species from various biotopes, of which six species were native to coniferous forests. Results show a sharp and statistically significant decline, on average 40 %, in five of these species. One of the largest reductions was observed in the focal species of this study, *C. umbellata*, which had decreased with 75-81 % in Uppland since the beginning of the 20th century. *Chimaphila umbellata* is currently red-listed (EN) in Sweden. Decline of *C. umbellata* and additional forest species are reported also for the province of Södermanland, Sweden (Rydberg and Wanntorp 2001).

The study species: Chimaphila umbellata

The genus *Chimaphila* is placed in the tribe Pyroleae along with the genera *Pyrola*, *Orthilia* and *Moneses*, within the family Ericaceae (Kron et al. 2002). It consists of approximately seven species (Freudenstein 1999) that are native to the temperate regions of the Northern Hemisphere, of which one, *C. umbellata* ((L.) W.P.C. Barton), occurs in Sweden. The distribution in Sweden is limited to the southern and central parts, with the northern range found along the coastline of Medelpad (Delin 2013).

Chimaphila umbellata is a perennial, evergreen dwarf shrub, 10-20 centimetres in height. The leaves are coriaceous, bright green and shiny, shallowly serrated and typically arranged in whorls, in general two on each stem (Fig. 1). The coming annual shoot is covered by bud shells (Fig. 1). The long, creeping root is yellowish with growths of thin roots (Fig. 2). The flowers are pink and produced 3 to 6 in an umbel (Fig. 1) (Barton 1817; Warming 1918).



Photos: Anna Lundell

Photo: Margareta Edqvist

Figure 1. Morphology in *Chimaphila umbellata*, from left to right: stem and leaves; top whorl and the coming annual bud; flowers and fruit capsules.

Chimaphila umbellata reproduces both by seeds and clonally by rhizomes (Fig. 2). Thus, a population consists of one or more genets (genetic individuals) that are producing ramets (vegetative units) (Kays and Harper 1974).

The habitat preference of the species is generally described as sparse pine forests or old growth boreal forests and in sandy, calcareous and nutrient-poor soils (Rydberg and Wanntorp 2001; Jonsell 2010).



Photos: Anna Lundell

Figure 2. Ramets (vegetative units) of *Chimaphila umbellata*

An analysis of the geographic distribution of *C. umbellata* in the provinces of Uppland and Södermanland in relation to soil type supports the claims of the species' affiliation to sandy soils. The analysis was based on reported findings to the Species Observation System (Artportalen 2013) of *C. umbellata* in Uppland and Södermanland between the years 1913-2013. Included were sites where the coordinate precision was at minimum 100 meters. An area (approximately 35 ha) in northern Uppland, where *C. umbellata* is abundant, had been inventoried thoroughly and thus had many reported findings. Four sites were included from this area: those situated in the far east, west, south and north. The final compilation included 214 sites (Fig. 3).

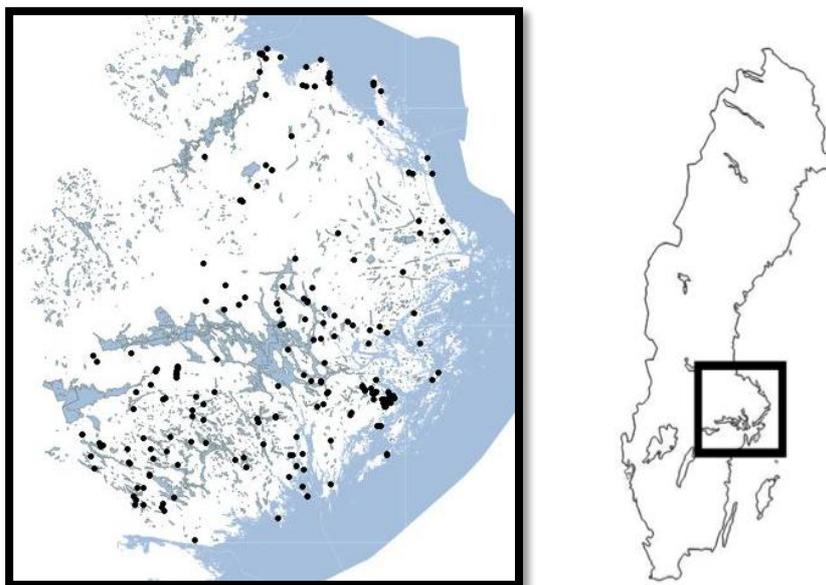


Figure 3. 214 reported sites of *Chimaphila umbellata* in Uppland and Södermanland, Sweden.

Data on soil type (scale 1:50 000) was provided by the Geological Survey of Sweden (SGU, Uppsala, Sweden, <http://www.sgu.se>). The soil type of each site was then extracted using QGIS (Quantum GIS Development Team 2013). Results showed that 51.4 % of the reported *C. umbellata* sites were in sandy soils, 6.1 % were in clay soils and 11.2 % were in mixed soils (Table 1).

Table 1. Distribution of 214 reported *Chimaphila umbellata* sites in Uppland and Södermanland, Sweden, on various soil types.

Soil type ^a	%	n
Sandy soils ^b	51.4	110
Clay soils ^c	6.1	13
Mixed soils ^d	11.2	24
Bedrock	23.4	50
Peat	3.7	8
Other	4.2	9

^a At 50 cm depth

^b Sandy moraine; glaciofluvial sediment of fine sand, sand, medium sand, gravel; postglacial sediment of fine sand, medium sand to coarse sand, gravel, cobble to boulder; dunes; postglacial sand.

^c Glacial clay; postglacial fine clay; clay; sandy silty moraine

^d Glaciofluvial sediment of coarse silt to boulder.

The feature of having both asexual reproduction and sexual reproduction is commonly seen in clonal plants (Eriksson 2011). The research regarding the recruitment of *C. umbellata* is scarce, but typically, clonal growth is more local and yields a higher offspring survival than recruitment from seeds (Eriksson 2011). Silvertown et al. (1993) presented a review on demographic plant studies and concluded that the local population growth in clonal species is to a larger extent influenced by asexual, rather than sexual, reproduction. Eriksson (1989) found that recruitment by seedlings occurred regularly in approximately 30 % of the clonal species native to forest habitats. Still, genetic diversity does not seem to be lower in populations of clonal plants than in populations of non-clonal plants (Ellstrand and Roose 1987). In simulations, Watkinson and Powell (1993) showed that genetic diversity of a population could still be maintained with only a low frequency of seedling recruitment. *Chimaphila umbellata* is self-compatible (Knudsen et al. 1993) and in a review of mating system and genotypic diversity in clonal plants it was found that self-incompatible species

had a higher genetic diversity than self-compatible species (Honnay and Jacquemyn 2008). While self-incompatible species may suffer from mate-limitation, a possible fitness cost in self-compatible species is inbreeding depression. However, Honnay and Jacquemyn (2008) found no support for this in their review. *Chimaphila umbellata* is mainly pollinated by bumblebees and nectar is produced in order to attract pollinators. The species is hence not buzz-pollinated as other studied Pyroleae (Knudsen et al. 1993).

Species of Pyroleae have extremely small seeds, i.e. dust seeds, which are produced in large numbers. Johansson et al. (2014) estimated the seed production in six Pyroleae species and found the highest number in *C. umbellata*, with an average of 7,882 seeds/capsule. Dust seeds, being the smallest seeds that can be found in angiosperms, have evolved in at least 12 families (Eriksson and Kainulainen 2011). There is not yet a clear definition in the literature regarding the upper limit in size of dust seeds, but see Eriksson and Kainulainen (2011) for a comprehensive summary of previous descriptions and categorization of small seeds. Seeds of *C. umbellata*, with a mean size of 0.55 x 0.10 mm, are among the smallest of the Pyroleae (Johansson et al. 2014).

Due to their small size, dust seeds contain only a minimal nutrient reserve and are hence dependent on fungal hosts to germinate and develop into seedlings. Pyroleae are, however, photosynthetic as adults. This strategy is termed mixotrophy and enables the species to establish in deeply shaded understory habitats (Tedersoo et al. 2007; Zimmer et al. 2007). Whether *C. umbellata* continues to parasitize on fungi as an adult is unclear. Zimmer et al. (2007) reported that 40 % of the nitrogen in leaves of *C. umbellata* was gained from fungi, and Tedersoo et al. (2007) found, in one of their two study sites, a 29 % carbon gain. Both studies showed a specialization to ectomycorrhizal fungi (Tedersoo et al. 2007; Zimmer et al. 2007). However, Hynson et al. (2012) found no support for carbon gain in the species and a recent study by V. Johansson (unpubl.) also supports that *C. umbellata* is fully autotrophic as an adult.

Results from a study of dispersal in *Pyrola chlorantha* indicate limited ability of long-distance dispersal in Pyroleae (Johansson et al. 2014). It was found that 82.5 % of the seeds were dispersed within 1 meter from the source, and 95.7 % were dispersed within 5 meters.

Recruitment of plants can be limited by both seed and microsite availability (Eriksson and Ehrlén 1992). A study by Johansson and Eriksson (2013) showed that microsite availability was the main limitation in *C. umbellata*, as the fraction of germinated seeds was higher at

sites where adults were present (18 %) than in sites where they were absent (8.7 %). Furthermore, increased levels of soil nutrients (phosphorous) had a negative effect on seedling size, and the authors suggest that also the continued development might be affected. Johansson and Eriksson (2013) hypothesized that the observed decline in *C. umbellata* might be caused by a reduction in microsites that meets the requirements of the species, e.g. competition from other species may prevent the contact with suitable fungi.

The increased competition from *V. myrtillus* and graminoids, species that are favoured by the cessation of forest grazing and eutrophication, are commonly suggested as a cause of decline in *C. umbellata*, together with increasingly shaded conditions in forest habitats due to modern forestry practises (Rydberg and Wanntorp 2001; Maad et al. 2009)

The main objectives of this study were:

(1) to investigate the effects of various biotic and abiotic conditions on *C. umbellata* populations with regard to population size, flowering frequency, fruit set, seed production and leaf length. Examined conditions included: (i) light inflow, (ii) coverage of *V. myrtillus* and graminoid species, (iii) soil nutrients, (iv) continuity of forest cover and (v) soil texture.

(2) to examine the impact of population size on fruit set and seed production.

Material and methods

Data collection

Findings of *C. umbellata* in the provinces of Uppland and Södermanland were retrieved from the Species Observation System (Artportalen 2013) or by personal communication from the discoverers of sites. Potential sites of extant and extinct populations were visited in May, when the evergreen *C. umbellata* plants were relatively easy to track. In total, 38 sites were selected for the study (Fig. 4), including 34 sites with extant *C. umbellata* populations and four sites with extinct populations. Only sites where the estimated locations of extinct populations were certain were included.

The study area is part of the boreonemoral vegetation zone. The sites consisted of mixed coniferous forests of Scots pine (*Pinus sylvestris*) and Norway spruce (*P. abies*) to a varying extent intermixed with deciduous tree species, or pure pine stands. The most common

deciduous trees included *Betula pendula*, *Populus tremula*, *Quercus robur* and *Sorbus aucuparia*. Ground vegetation was typically dominated by dwarf shrubs (*V. myrtillus*, *Vaccinium vitis-idea*, *Calluna vulgaris*), reindeer lichens (*Cladina* spp.), feather mosses (*Pleurozium schreberi* and *Hylocomium splendens*) and graminoids.

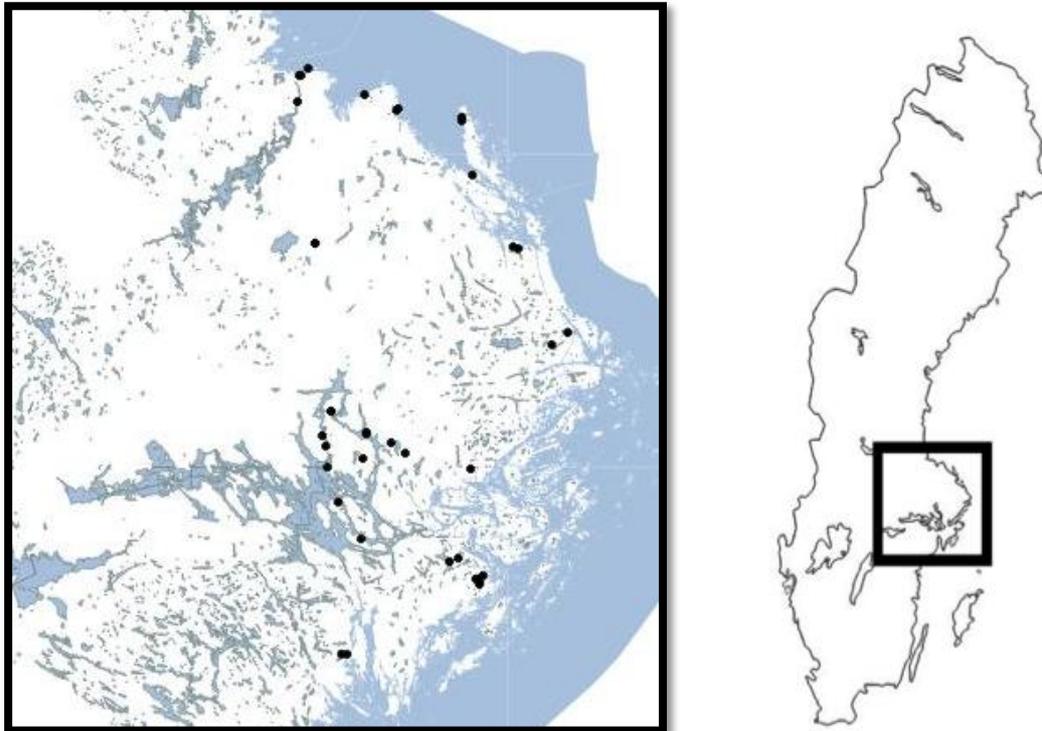


Figure 4. The 38 study sites of *Chimaphila umbellata* in Uppland and Södermanland, Sweden.

The field sites were visited on two occasions in 2013, at flowering time in July and at fruit set in September.

July

The patch of the *C. umbellata* population was delineated and the patch area was measured. When sites consisted of multiple patches, the one that was estimated to have the largest population size was chosen. All shoots, as well as all the flowering shoots, were counted. In one site, the population was very large and the flowering frequency was high. In this case, all shoots were counted, but to estimate flowering frequency, 100 shoots were sampled by placing a yardstick through the patch and noting at every 10th cm if the closest shoot was flowering or not. To estimate forthcoming fruit set, 25 of the flowering shoots were randomly selected, marked and the number of flowers was noted. The length of the longest leaf was

measured for 25 shoots. In large populations, 25 shoots were sampled by placing a yardstick through the patch and at every 10th cm the leaf length of the closest shoot was measured .

To estimate light inflow, the canopy cover was photographed in five directions: upwards and at 45 degrees tilt at north, west, south and east. The percentage canopy cover in the photos was analyzed using the software ImageJ (Rasband 2013) and the mean canopy cover of the photos was calculated. In larger patches, photos were taken at up to five random locations and the mean canopy cover of the points was then calculated. The parameter “light inflow” was computed by subtracting the mean value of percentage canopy cover from 100.

A transect (40 × 40 cm) was placed randomly on the ground at five locations in the patch. The coverage of the species in the field vegetation was estimated and a mean value of coverage for each species was calculated for the site. In subsequent statistical analysis, the coverage of *V. myrtillus* and graminoid species was grouped together. The following graminoids were identified: *Agrostis capillaris*, *Agrostis vinealis*, *Calamagrostis arundinacea*, *Carex digitata*, *Carex* spp, *Carex vesicaria*, *Dactylis glomerata*, *Deschampsia cespitosa*, *Deschampsia flexuosa*, *Festuca ovina*, *Luzula pilosa*, *Luzula* spp, *Melica nutans* and *Poa nemoralis*.

Increment cores were taken at breast height from the three thickest trees in 5-meter radius of the *C. umbellata* population, using an increment borer. The age of the oldest tree was included in the statistical analysis to account for minimum continuity of forest cover at the site.

September

The number of fruits on the marked flowering shoots was counted and five seed capsules were collected randomly from each site. The seeds were dissolved in a solution of water and detergent to remove surface tension. From this seed solution, ten subsamples of 50 µl were taken, and mature and aborted seeds were counted. To calculate the total number of seeds collected from the site, the mean number of seeds in the subsamples was multiplied with the total volume of the seed solution. The seed production per capsule was then calculated by dividing the total number of seeds with the number of capsules collected from the site.

Soil samples were collected at three locations from just under the forest litter (at approximately 10 cm depth) and pooled to a total of approximately 0.4 L. The samples were kept cool in freezers to halt microbial activity and avoid evaporation of gaseous nitrogen. The amounts of ammonium nitrogen (mg/100 g dry mass) and nitrate nitrogen (mg/100 g dry

mass) were analyzed, and were then summarized to total soil nitrogen (mg/100 g dry mass). The analysis was performed by an accredited lab (Eurofins, Kristianstad, Sweden, <http://eurofins.se>).

To determine soil texture, additional soil samples were collected and sieved into four grain size classes (according to the international scale of identification and classification of grain size, ISO 14688-1): coarse sand (0.63 mm - 2.00 mm), medium sand (0.2 - 0.63 mm), fine sand (0.063 - 0.2 mm), silt and clay (< 0.063 mm). Organic material was removed from the samples and the different classes were weighed. Adding together the weights of medium and coarse sand, and dividing the value with the total weight of the soil sample calculated the proportion of coarse grain size. Soil samples that consisted of mainly organic material (n = 13) were not sieved and excluded from further analysis.

Data analyses

Generalized linear models were used to investigate the effects of the environmental conditions on population size, flowering frequency, fruit set and seed production per capsule. Analyses of correlations between the environmental conditions showed that all pair-wise correlations (Pearson's r) were < 0.5. Light inflow, coverage of competitive species, soil nitrogen and tree age were used as explanatory variables in negative binomial generalized linear models with population size and seed production per capsule as response variables, and in generalized linear models (family = quasibinomial) with flowering frequency and fruit set as response variables. The models were reduced step-wise by removal of the explanatory variable with the highest p-value, and the simplified model was then compared to the fuller model using a likelihood ratio test. Thus, in the model selection procedure, an explanatory variable was only included if it significantly improved the fit of the model. The effect of proportion coarse grain size was analyzed separately in negative binomial generalized linear models and generalized linear models (family = quasibinomial), due to a smaller sample size of soil texture.

To analyze the effect of population size on fruit set and seed production, a generalized linear model (family=quasibinomial) was performed with fruit set as response variable, and a negative binomial generalized linear model was performed with seed production per capsule as response variable.

A linear model was fitted with leaf length as response variable and light inflow, coverage of competitive species, soil nitrogen and tree age as explanatory variables. Leaf length was log transformed to meet the assumption of normality. Model selection was performed as described above. Due to the smaller sample size of soil texture, effect of proportion coarse grain size was tested separately in a linear model.

All analyses were performed in R 3.0.2 (R Development Core Team 2013).

Results

Population size

Of the 38 surveyed sites, 34 had extant populations with population sizes ranging between one and 1,776 shoots. Densities in the larger populations (> 150 shoots) ranged between 4.9 shoots/m² and 98 shoots/m². When aggregations of shoots occurred, densities in the densest part ranged between 32.5 shoots/m² and 217.9 shoots/m².

Population size decreased with increasing coverage of competitive species (Table 2, Fig. 5). There were no significant effects on population size of light inflow, soil nitrogen, continuity of forest cover and soil texture ($p > 0.05$).

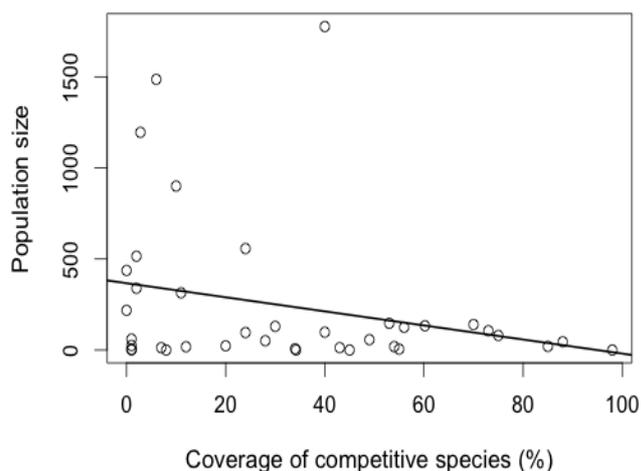


Figure 5. Relationship between coverage of competitive species (*Vaccinium myrtillus* and graminoids) and population size in *Chimaphila umbellata* at study sites in Uppland and Södermanland, Sweden.

Flowering frequency

Of the 34 sites with extant populations, 23 were flowering while 11 were sterile. In the flowering populations, the flowering frequency ranged between 0.18 % and 38 %. Flowering frequency increased with increasing light inflow (Table 3, Fig. 6 a). Flowering frequency was also affected by continuity of forest cover (Table 3, Fig. 6 b). There were no significant effects of coverage of competitive species, soil nitrogen and soil texture ($p > 0.05$).

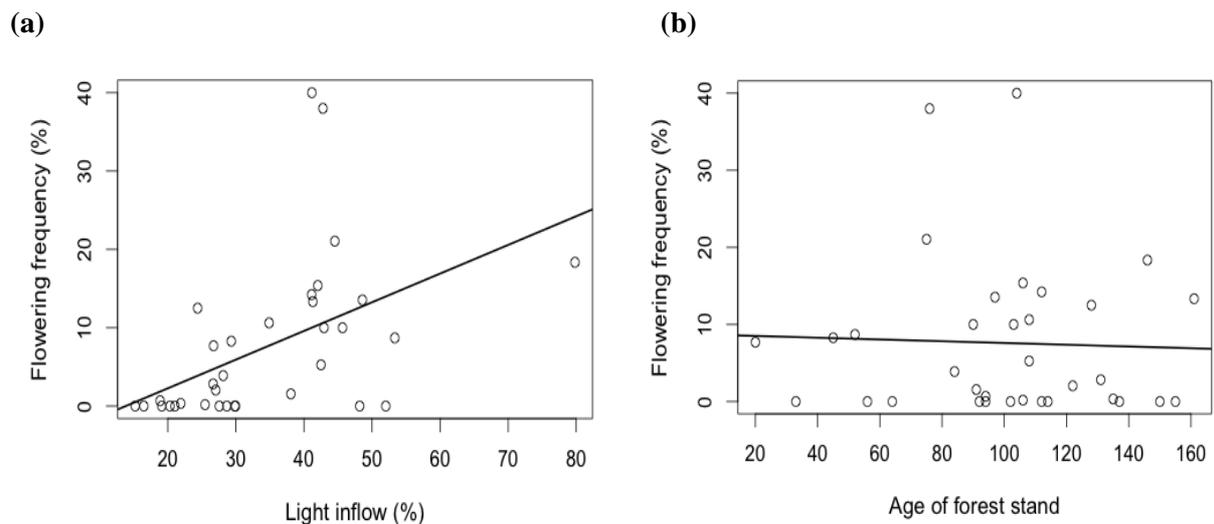


Figure 6. Relationship between (a) light inflow; (b) age of forest stand and flowering frequency in *Chimaphila umbellata* at study sites in Uppland and Södermanland, Sweden.

Fruit set

Out of 23 flowering populations, 18 populations produced fruits with the fruit set ranging between 3.7 % and 100 %. Fruit set was negatively affected by the coverage of competitive species (Table 3, Fig. 7 a). There were no significant effects on fruit set of light inflow, soil nitrogen, continuity of forest cover and soil texture ($p > 0.05$).

Seed production

In the 18 populations that set fruit, numbers of seeds per capsule ranged between 1,600 and 18,960. Seed production per capsule decreased with increasing soil nitrogen content (Table 2, Fig. 7 b). There were no significant effects on seed production per capsule of light inflow, soil nitrogen, continuity of forest cover and soil texture ($p > 0.05$).

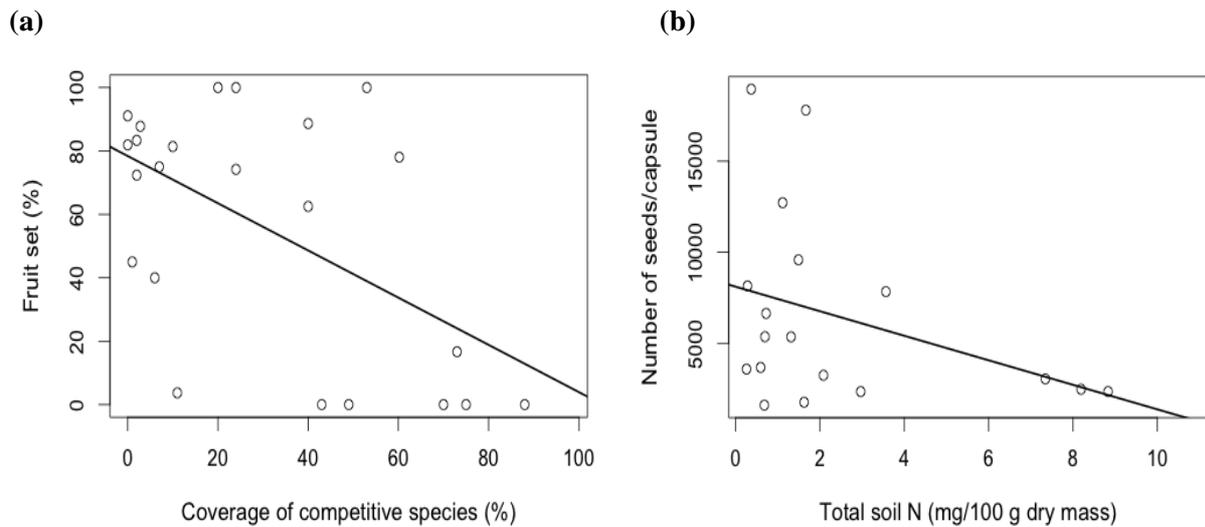


Figure 7. Relationship between (a) coverage of competitive species and fruit set; (b) soil nitrogen and seed production per capsule in *Chimaphila umbellata* at study sites in Uppland and Södermanland, Sweden.

Relationship between population size, fruit set and seed production

Fruit set and seed production per capsule increased with increasing population size (Table 2, Table 3, Fig. 8). The significance of these relationships remained unchanged or increased when only populations with a flowering frequency > 1 % were included in the analysis (Table 2, Table 3).

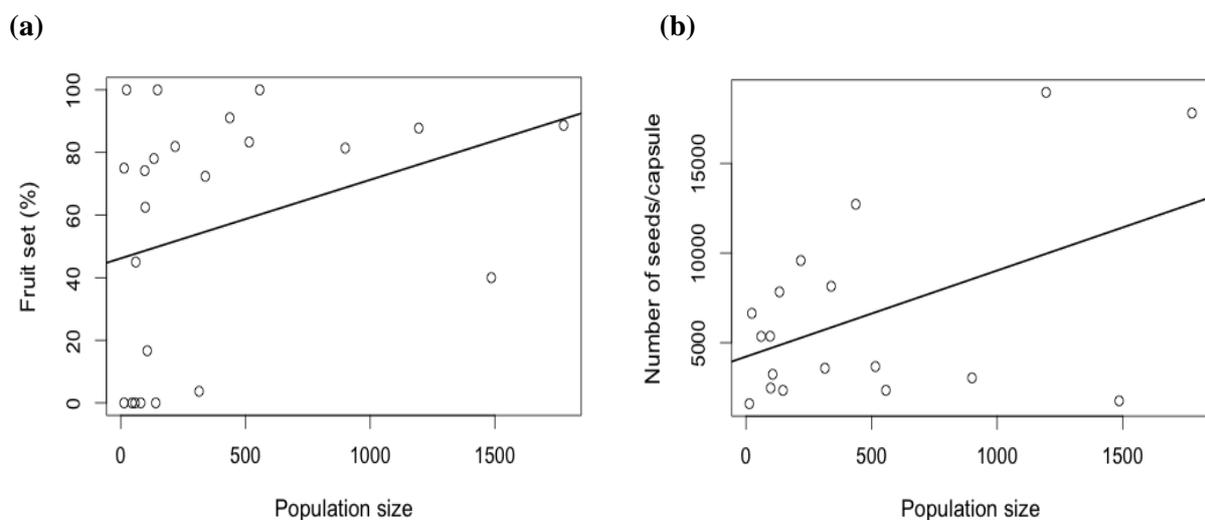


Figure 8. Relationship between population size and (a) fruit set; (b) seed production per capsule in *Chimaphila umbellata* at study sites in Uppland and Södermanland, Sweden.

Table 2. Negative binomial generalized linear models describing the effects of environmental conditions on population size and seed production per capsule, and the effect of population size on seed production per capsule, in *Chimaphila umbellata*.

		Estimate	SE	z value	Pr(> z)
Population size	~ Coverage of competitive species	-2.43	0.92	-2.65	0.008
Seed production/capsule	~ Soil nitrogen	-0.14	0.06	-2.56	0.010
Seed production/capsule	~ Population size ^a	0.0006	0.0003	2.04	0.041
Seed production/capsule	~ Population size ^b	0.0008	0.0003	2.87	0.004

^a including all flowering populations

^b including populations with a flowering frequency > 1 %

Table 3. Binomial generalized linear models describing the effects of environmental conditions on flowering frequency and fruit set, and the effect of population size on fruit set, in *Chimaphila umbellata*.

		Estimate	SE	t value	Pr(> t)
Flowering frequency	~ Light inflow	5.73	1.25	4.57	<0.001
	Tree age	-0.02	0.007	-2.31	0.028
Fruit set	~ Coverage of competitive species	-2.88	1.95	-2.75	0.012
Fruit set	~ Population size ^a	0.002	0.0007	2.32	0.031
Fruit set	~ Population size ^b	0.002	0.0008	2.34	0.031

^a including all flowering populations

^b including populations with a flowering frequency > 1 %

Leaf length

Results showed no significant effects on leaf length of light inflow, coverage of competitive species, soil nitrogen, continuity of forest cover and soil texture ($p > 0.05$).

Discussion

This study shows several significant effects on *C. umbellata* populations from several environmental factors. The effects can be related to some key factors influencing the current decline of this endangered species.

Coverage of *V. myrtillus* and graminoid species had a negative effect on population size, indicating that *C. umbellata* may be outcompeted by these species. Since population dynamics have not been investigated here, it is not possible to conclude which processes that underlie the observed effect on population size. Johansson and Eriksson (2013) hypothesized that the contact with suitable fungi may be prevented which in turn hampers recruitment from seeds. Also clonal recruitment might be hindered if the field vegetation is too extensive, especially if perennial grasses are abundant, as they are effective competitors due to their growth form, i.e. comprehensive tillering and high root:shoot ratios (Wilson 1998). Furthermore, coverage of *V. myrtillus* and graminoid species had a negative effect on fruit set, indicating that the competition prevents detection by pollinators. No influence from competition was however observed regarding frequency of flowering or seed production per capsule.

Population size was not affected by variation in light availability whereas flowering frequency was negatively affected by increased shading. The results suggest that the species is able to reproduce clonally in habitats with darker conditions while sexual reproduction occurs to a lesser extent in such habitats. The lack of flowering under low-light conditions is a common characteristic of shade-tolerant woodland species (Grime 2001) and has been reported for other clonal forest species. For example, Lezberg et al. (2001) demonstrated very limited flowering and seed production in the herb *Maianthemum dilatatum* growing in the low-light conditions of young, dense stands when compared to populations growing in mature stands. Regarding the herb *Uvularia perfoliata* it was found that the clonal structure and reproductive strategy of the species differed in sites with open and closed canopies (Kudoh et al. 1999). Populations in gap habitats consisted of several or many genets, indicating an on-going recruitment from seeds while populations in closed habitats more often consisted of a single genet. Kudoh et al. (1999) suggested a “waiting strategy” in populations under closed canopies i.e. mainly clonal reproduction until light conditions become favourable enough to initiate sexual reproduction.

Ehrlén and Lehtilä (2002) found in their meta-analysis that ramets of clonal plants had shorter life spans than non-clonal plants. However, longevity of genets can be substantial. De Witte and Stöcklin (2010) show in a review that the age of genets of clonal shrubs ranged from approximately 50 years to many thousands years (there is currently no data on longevity in *C. umbellata*). Ozinga et al. (2007) showed that the local aboveground persistence was extended when the clonal connections are long lasting (>1 year), which they are in *C. umbellata*. Furthermore, woody plants have been found to have longer life spans than non-woody plants (Ehrlén and Lehtilä 2002).

Life-cycle characteristics like clonality and (the likely) longevity of genets in *C. umbellata* may buffer habitat deterioration and a limited recruitment from seeds, and reduce the risk of local extinction. Some of the populations of the species may therefore exist as remnant populations, i.e. they persist despite a population growth rate (λ) below 1 because of population inertia (Eriksson 1996). This type of population dynamics resembles source-sink populations but on a temporal rather than a spatial scale, and due to the extended time frames population decline is not readily observed. Eriksson (1996) concluded that most evidence of remnant populations is reported in research on long-lived, clonal plants. Johansson et al. (2011) investigated the relationship between plant traits and development of remnant populations in grasslands after abandonment. They found that several traits were characteristic of remnant populations, including clonality, a perennial life cycle and the absence of long-distance dispersal.

The possible development of remnant populations in *C. umbellata* may contribute to an extinction debt in the species, i.e. a time lag in the response to unfavourable conditions (Tilman et al. 1994). There are few empirical studies demonstrating extinction debts in species, mainly due to the lack of data required i.e. long-term data of species losses or comparisons of similar habitats with varying land use histories (Vellend et al. 2006). One of these demonstrated that extinction debt persisted more than a century after forest fragmentation in slow forest species, i.e. species with low rates of population extinction and colonization (Vellend et al. 2006), .

The use of simulation models, in spite of their constraints, can overcome the obstacles of long time-scales in the analysis of population dynamics of clonal species. Eriksson (1994) estimated time to extinction in the clonal woodland herb *Rubus saxatilis* to nearly 100 years, when the initial population consisted of 1,000-1,500 ramets and under unfavourable

conditions, i.e. closed canopies. Time to extinction was 55 years when the starting population consisted of 125 ramets. These simulations demonstrate that time-spans can be considerable even in moderately sized and small populations. The same study examined how variation in recruitment influenced time to extinction of genet populations. The basis used for the transition matrices was the life cycle of a hypothetical genet population, but founded to a large extent on field data from several clonal species. Simulations with a starting population of 1,000 genets showed that a reduction of recruitment with 50 % had small effects on population growth rate (λ) and population structure. However, larger reductions in recruitment had negative effects on population growth rate ($\lambda = 0.96$) with the consequence that the population consisted of only adults after approximately 100 years. Time to extinction was estimated to 169 years and occasional recruitment pulses could not compensate for an overall reduction in recruitment. Eriksson (1994) hence concluded that populations without seed recruitment are eventually doomed to extinction.

Forest continuity is probably an important factor to explain and understand the long-term development of *C. umbellata* populations. A study by Halpern et al. (1995) found both higher persistence and greater coverage of *C. umbellata* in old-growth forests (> 210 years) compared to mature and younger stands. In addition, they showed that the species was sensitive to disturbance by fire. These findings indicate that reestablishment and growth of *C. umbellata* is slow (Halpern et al. 1995). An insufficient range in the age material could explain the limited effect of continuity of forest cover in this study – the maximum tree age recorded was 160 years. The extensive forest exploitation in Uppland and Södermanland in the previous centuries might be the reason why no old-growth forests (> 200 years) were included in the study. Another possible reason for the lack of effect of forest age might be that forest conditions before establishment of the oldest tree has not been considered here. Moreover, impacts of previous land use and management (e.g. cattle grazing and fires) have not been accounted for in detail.

Both fruit set and seed production per capsule were increased with increasing population size, indicating that pollinators are attracted to larger populations. Additional studies with the focus on pollination are needed in order to clarify the underlying cause of the effect of population size. Also studies including the genetic diversity of the population would be valuable since this is not necessarily related to population size in clonal species.

Plants growing in the shaded understory conditions of closed-canopy stands can allocate resources to leaf growth to enhance light capture (Grime 2001). However, this study found no support for increased leaf length in *C. umbellata* under low light conditions. This result is in line with Lezberg et al. (2001) who studied the leaf area of *M. dilatatum* in forests with various structure and light conditions. They suggested that the lack of response might be attributed to the physiological adaptations of the species to tolerate low-light environments and that the shaded conditions were not extreme enough to initiate allocation of resources to leaves. This might apply also for this study on *C. umbellata*. Grime (2001) notes that morphological plasticity under shaded conditions are less pronounced in shade-tolerant species which tend to grow slowly, than in species characteristic of lighter environments. Also, evergreen plants in cool, temperate regions have a photosynthetic advantage during a large part of the year and photosynthesis might instead be limited by leaf temperature. This is indicated by the placement of the leaves in *C. umbellata* and other Pyroleae close to the litter layer which is the warmest place in the understory habitat (Givnish 1982). Thus, there are competitive constraints in the species and it cannot readily respond to high summer understory coverage by increased leaf height.

Conclusions and conservation applications

Populations of *C. umbellata* were found to be affected by the light availability, the competition from *V. myrtillus* and graminoid species, continuity of forest cover and soil nitrogen. Population size decreased with increased competition and the sexual reproduction was hampered under shaded conditions. The possible development of remnant populations, where longevity and clonal recruitment buffers the effects of unfavourable environmental conditions, might contribute to an extinction debt in the species. These results indicate the need of urgent and targeted management to restore forest habitat quality, including moderate disturbance regimes to increase light inflow and reduce competition in the field layer. Furthermore, given the general effects of eutrophication, actions for reduced large-scale emissions of nutrients are important for long-term preservation of the species.

More research is needed on *C. umbellata*'s association with mycorrhizal fungi as soil nutrient increase and clearcut logging has been shown to change the species composition of the mycorrhizal community. For instance, it is important to assess host specificity in *C. umbellata* to properly evaluate the potential risk of coextinction (Colwell et al. 2012).

Additional studies, with emphasis on demography of *C. umbellata* and the identification of limiting life history stages, are needed to clarify the impacts of environmental conditions on population vital rates. Also, the assessment of potential remnant populations and extinction debt in the species is of critical importance. This approach would give a fuller understanding of extinction risks and which management actions that would be most effective to maintain viable populations of *Chimaphila umbellata* in our forests.

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References

- Artportalen (2013). Rapportsystem för växter, djur och svampar. Artdatabanken, SLU. <http://www.artportalen.se>.
- Barton, W.P.C. (1817) *Vegetable materia medica of the United States, or, Medical Botany*. H.C. Carey & I. Lea – Chestnut Street, Philadelphia.
- Bengtsson, J., Nilsson, S.G., Franc, A. & Menozzi, P. (2000) Biodiversity, disturbances, ecosystem function and management of European forests. – *Forest Ecology and Management* 132: 39-50.
- Berg, A., Ehnström, B., Gustafsson, L., Hallingbäck, T., Jonsell & Weslien, J. (1994) Threatened plant, animal, and fungus species in Swedish forests - distribution and habitat associations. – *Conservation Biology* 8: 718-731.
- Bertills, U. & Grennfelt, P. (2000) International negotiations and national environmental objectives. In Bertills, U. & Näsholm, T. (eds.) *Effects of nitrogen deposition on forest ecosystems*. Naturvårdsverket, Stockholm, pp 13-20.
- Colwell, R.K., Dunn, R.R. & Harris, N.C. (2012) Coextinction and persistence of dependent species in a changing world. – *Annual Review of Ecology, Evolution, and Systematics* 43: 183-203.
- Dahlström, A. (2006) Pastures, livestock number and grazing pressure 1620-1850. Ecological aspects of grazing history in south-central Sweden. PhD Thesis, Swedish University of Agricultural Sciences.
- Delin, A. (2013) Ryl – gynnad eller missgynnad av brand? – *Svensk Botanisk Tidskrift* 107: 252-263.
- Ehrlén, J. & Lehtilä, K. (2002) How perennial are perennial plants? – *Oikos* 98: 308-322.
- Ellstrand, N.C. & Roose, M.L. (1987) Patterns of genotypic diversity in clonal plant-species. – *American Journal of Botany* 74: 123-131.
- Ericsson, S., Östlund, L. & Axelsson, A.L. (2000) A forest of grazing and logging: Deforestation and reforestation history of a boreal landscape in central Sweden. – *New Forests* 19: 227-240.
- Ericsson, T.S. (2001) Culture within nature. Key areas for interpreting forest history in boreal Sweden. PhD Thesis, Swedish University of Agricultural Sciences.
- Eriksson, O. (1989) Seedling dynamics and life histories in clonal plants. – *Oikos* 55: 231-238.
- Eriksson, O. (1994) Stochastic population dynamics of clonal plants: Numerical experiments with ramet and genet models. – *Ecological Research* 9: 257-268.
- Eriksson, O. (1996) Regional dynamics of plants: A review of evidence for remnant, source-sink and metapopulations. – *Oikos* 77: 248-258.

- Eriksson, O. (2011) Niche shifts and seed limitation as mechanisms determining seedling recruitment in clonal plants. – *Preslia* 83: 301-314.
- Eriksson, O. & Ehrlén, J. (1992) Seed and microsite limitation of recruitment in plant-populations. – *Oecologia* 91: 360-364.
- Eriksson, O. & Kainulainen, K. (2011) The evolutionary ecology of dust seeds. – *Perspectives in Plant Ecology Evolution and Systematics* 13: 73-87.
- Eriksson, O. & Cousins, S.A.O. (2014) Historical landscape perspectives on grasslands in Sweden and the Baltic region. – *Land* 3: 300-321.
- Falkengren-Grerup, U., Ericson, L., Gunnarsson, U., Nordin, A., Rydin, H. & Wallén, B. (2000) Does nitrogen deposition change the flora? In Bertills, U. & Näsholm, T. (eds.) *Effects of nitrogen deposition on forest ecosystems*. Naturvårdsverket, Stockholm, pp 77-104.
- Freudenstein, J.V. (1999) Relationships and character transformation in Pyroloideae (Ericaceae) based on ITS sequences, morphology and development. – *Systematic Botany* 3: 398-408.
- Givnish, T.J. (1982) On the adaptive significance of leaf height in forest herbs. – *The American Naturalist* 120: 353-381.
- Grime, J.P. (2001) *Plant strategies, vegetation processes, and ecosystem properties*. John Wiley & Sons Ltd, Chichester.
- Gärdenfors, U. (ed.) (2010) *Rödlistade arter i Sverige 2010*. [The 2010 Red List of Swedish Species]. ArtDatabanken, Uppsala.
- Halpern, C.B. & Spies, T.A. (1995) Plant species diversity in natural and managed forests of the Pacific Northwest. – *Ecological Applications* 4: 913-934.
- Hedwall, P.O., Brunet, J., Nordin, A. & Bergh, J. (2013) Changes in the abundance of keystone forest floor species in response to changes of forest structure. – *Journal of Vegetation Science* 24: 296-306.
- Honnay, O. & Jacquemyn, H. (2008) A meta-analysis of the relation between mating system, growth form and genotypic diversity in clonal plant species. – *Evolutionary Ecology* 22: 299-312.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. – *Ecological Monographs* 75: 3-35.
- Hynson, N.A., Mambelli, S., Amend, A.S. & Dawson, T.E. (2012) Measuring carbon gains from fungal networks in understory plants from the tribe Pyroleae (Ericaceae): a field manipulation and stable isotope approach. – *Oecologia* 169: 307-317.
- Johansson, V.A., Cousins, S.A.O. & Eriksson, O. (2011) Remnant populations and plant functional traits in abandoned semi-natural grasslands. – *Folia Geobotanica* 46: 165-179.

- Johansson, V.A. & Eriksson, O. (2013) Recruitment limitation, germination of dust seeds, and early development of underground seedlings in six Pyroleae species. – *Botany* 91: 17-24.
- Johansson, V.A., Müller, G. & Eriksson, O. (2014) Dust seed production and dispersal in Swedish Pyroleae species. – *Nordic Journal of Botany* 32: 209-214.
- Jones, M.D., Durall, D.M. & Cairney, W.G. (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. – *New Phytologist* 157: 399-422.
- Jonsell, L. (2010) *Upplands flora*. [The Flora of Uppland]. SBF-förlaget, Uppsala.
- Kardell, L. (2003) *Svenskarna och skogen - från ved till linjeskepp*. Skogsstyrelsen, Jönköping.
- Kays, S. & Harper, J.L. (1974) The regulation of plant and tiller density in a grass sward. – *Journal of Ecology* 62: 97-105.
- Keith, S.A., Newton, A.C. Morecroft, M.D., Bealey, C.E. & Bullock, J.M. (2009) Taxonomic homogenization of woodland plant communities over 70 years. – *Proceedings of the Royal Society* 276: 3539-3544.
- Kellner, O. & Redbo-Torstensson, P. (1995) Effects of elevated nitrogen deposition on the field-layer vegetation in coniferous forests. – *Ecological Bulletins* 44: 227-237.
- Knudsen, J.T. & Olesen, J.M. (1993) Buzz-pollination and patterns in sexual traits in north European Pyrolaceae. – *American Journal of Botany* 80: 900-913.
- Kron, K.A., Judd, W.S., Stevens, P.F., Crayn, D.M., Anderberg, A.A., Gadek, P.A., Quinn, C.J. & Luteyn, J.L. (2002) Phylogenetic classification of Ericaceae: Molecular and morphological evidence. – *Botanical Review* 68: 335-423.
- Kudoh, H., Shibaike, H., Takasu, H., Whigham, D.F. & Kawano, S. (1999) Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. – *Journal of Ecology* 87: 244-257.
- Larsson, A. & Thor, G. (2010) Pressures in the landscapes - the forests. In Gärdenfors, U. (ed.) *The 2010 Red List of Swedish Species*. Artdatabanken, Uppsala, pp 85-92.
- Lezberg, A.L., Halpern, C.B. & Antos, J.A. (2001) Clonal development of *Maianthemum dilatatum* in forests of differing age and structure. – *Canadian Journal of Botany* 79: 1028-1038.
- Lilleskov, E.A., Fahey, T.J., Horton, T.R. & Lovett, G.M. (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. – *Ecology* 83: 104-115.
- Lövblad, G. (2000) Nitrogen deposition now and in the future. In Bertills, U. & Näsholm, T. (eds.) *Effects of nitrogen deposition on forest ecosystems*. Naturvårdsverket, Stockholm, pp 21-28.

- Maad, J. Sundberg, S. Stolpe, P. & Jonsell, L. (2009) Floraförändringar i Uppland under 1900-talet – en analys från Projekt Upplands Flora. [Floristic changes during the 20th century in Uppland, east central Sweden.] – *Svensk Botanisk Tidskrift* 103: 67-104.
- Manninen, O.H., Stark, S., Kytoviita, M.M., Lampinen, L. & Tolvanen, A. (2009) Understorey plant and soil responses to disturbance and increased nitrogen in boreal forests. – *Journal of Vegetation Science* 20: 311-322.
- Ozinga, W.A., Hennekens, S.M., Schaminee, J.H.J., Smits, N.A.C., Bekker, R.M., Romermann, C., Klimes, L., Bakker, J.P. & van Groenendael, J.M. (2007) Local above-ground persistence of vascular plants: Life-history trade-offs and environmental constraints. – *Journal of Vegetation Science* 18: 489-497.
- Quantum GIS Development Team (2013). Quantum GIS Geographic Information System. Open source geospatial foundation project. Available from <http://qgis.osgeo.org>.
- Rasband, W.S. (2013). ImageJ, version 1.47v. National Institutes of Health, Bethesda, Maryland, USA. Available from <http://imagej.nih.gov/ij>.
- R Development Core Team (2013). R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. Available from <http://www.R-project.org>.
- Rydberg, H. & Wanntorp, H-E. (2001) *Sörmlands flora*. [The Flora of Sörmland.] Botaniska Sällskapet i Stockholm, Västervik.
- Silvertown, J., Franco, M., Pisanty, I. & Mendoza, A. (1993) Comparative plant demography - relative importance of life cycle components to the finite rate of increase in woody and herbaceous perennials. – *Journal of Ecology* 81: 465-476.
- Strengbom, J., Nordin, A., Näsholm, T. & Ericson, L. (2001) Slow recovery of boreal forest ecosystem following decreased nitrogen input. – *Functional Ecology* 15: 451-457.
- SMHI (2013). Swedish Meteorological and Hydrological Institute. Klimatdata. Atmosfärs kemi. Total deposition of nitrogen. <http://www.smhi.se/klimatdata/miljo/atmosfarskemi> 2013-12-09
- Tamm, C.O. (1991) *Nitrogen in terrestrial ecosystems*. Springer Verlag, Berlin.
- Tedersoo, L., Pellet, P., Kõljalg, U. & Selosse, M.A. (2007) Parallel evolutionary paths to mycoheterotrophy in understorey Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyroleae. – *Oecologia* 151: 206-217.
- Tilman, D., May, R.M., Lehman, C.L. & Nowak, M.A. (1994) Habitat destruction and the extinction debt. – *Nature* 371: 65-66.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997) Human domination of earth's ecosystems. – *Science* 277: 494-499.
- Vellend, M., Verheyen, K., Jacquemyn, H., Kolb, A., Van Calster, H., Peterken, G. & Hermy, M. (2006) Extinction debt of forest plants persists for more than a century following

habitat fragmentation. – *Ecology* 87: 542-548.

Wallenda, T. & Kottke, I. (1998) Nitrogen deposition and ectomycorrhizas. – *New Phytologist* 139: 169-187.

Warming, E. (1918) *Om jordudløbere*. A. F. Høst og Søn, Copenhagen.

Watkinson, A.R. & Powell, J.C. (1993) Seedling recruitment and the maintenance of clonal diversity in plant populations: a computer simulation of *Ranunculus repens*. – *Journal of Ecology* 81: 707-717.

Wilson S.D. (1998) Competition between grasses and woody plants. In Cheplick, G.P. (ed.) *Population biology of grasses*. Cambridge University Press, Cambridge, pp 231-254.

de Witte, L.C. & Stöcklin, J. (2010) Longevity of clonal plants: why it matters and how to measure it. – *Annals of Botany* 106: 859-870.

Zimmer, K., Hynson, N.A., Gebauer, G., Allen, E.B., Allen, M.F. & Read, D.J. (2007) Wide geographical and ecological distribution of nitrogen and carbon gains from fungi in pyrolids and monotropoids (Ericaceae) and in orchids. – *New Phytologist* 175: 166-175.

Östlund, L., Zackrisson, O. & Axelsson, A.L. (1997) The history and transformation of a Scandinavian boreal forest landscape since the 19th century. – *Canadian Journal of Forest Research* 8: 1198-1206.