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Effects of Forest Succession on the Occurrence of Orchid Species

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BB, WD and MM designed the study, collected the data, performed the statistical analysis, managed the analysis, wrote the protocol and wrote the manuscript. Authors MP and KL collected the data, managed the analysis and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The *orchid* species, *Neottia cordata* and *Goodyera repens* found in large parts of Europe and North America, but rarely found in the Netherlands. Hence, the Forestry Services of Netherlands is working to preserve these endangered orchid species by maintaining the current forest structure and applying different forest management strategies. The aim of the study were, (1) to identify an environmental factors which determine the occurrence of the orchid species, mainly *G. repens* and *N. cordata, (2) to* recommend management options to preserve identified suitable environmental factors in a mixed forest and forest structure. The study was conducted in Hoornsebos. In each sample plot, we recorded the vegetation composition, forest structure and composition, humus and litter layer, and canopy openness. In the study forest, we found two plant communities (i,e, Vaccinio-Piceetalia and Quercetalia-roboris). Both *N. cordata* and *G. repens were abundant* in

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Vaccinio-Piceetalia plant community, but were rare in the Quercetalia-roboris plant community. Our results showed that both orchids' species are dependent on the occurrence of needle litter and need to grow in low productive environment, where they are not outcompeted by other plants. There is also a general trend of development towards more broadleaved tree composition which has a negative effect on the occurrence of both orchid species. The development of a shrub layer leads to increased shading and canopy closure and likely to decrease the abundance of *N. cordata* in the study forest. Aging of coniferous forests will lead to further build-up of the litter layer which negatively affects *G. repens*. Without good forest management, the existing suitable environmental conditions for both *G. repens* and *N. cordata*, will likely to degrade, hence active management is required for sustainable conservation of these orchid species.

Keywords: Neottia cordata; Goodyera repens; mixed forest; broadleaved forest; Netherlands.

1. INTRODUCTION

The orchid species, *Neottia cordata* and *Goodyera repens* occur in large parts of Europe [1,2] and North America [3], but rarely found in the Netherlands [4]. *Neottia cordata* and *Goodyera repens* occur in *Pinus nigra* plantation forests of the Dutch Wadden island of Terschelling. . *G. repens* also occurs on the mainland of the Netherlands along the Dutch west coast and inland pine forests [4], and *N. cordata* is only found on two locations on the mainland [4]. More importantly, these *orchid* species are classified under Dutch red list of vascular plants species [5]. As the result, they are one of highly target species for nature management because of their rarity in the Netherlands [6]. Both *G. repens* as *N. cordata* are bound to coniferous forests, becousethe occurrence of micro-habitat with a relatively moist needle litter layer is crucial for the orchids species [7].

The State Forestry Service (NL: Staatsbosbeheer; SBB) have been working to conserve *G. repens* and *N. cordata* in Terschelling plantation forest. To keep the forests suitable for *G. repens* and *N. cordata,* SBB designated core areas where they do not apply any management to preserve the pure *Pinus nigra* forests [8]. This forest is reach in *G. repens* and *N. cordata*, but apart from these species the forest is relatively species poor [9]. Apart from the SBB goals the island of Terschelling is ranked as a Natura 2000 area. The *G. repens* and *N. cordata* are although no specific target species within the Natura 2000 management plan, but there is a maintenance obligation for the habitat (H2180_A, Dry forests of the dunes) in where the species occur [10].

For high species diversity as desired by SBB and Natura 2000, forests should consist of mixed forests with broadleaved trees [11] and parts of the forest structure should be more open and include gaps [12,13]. Also for the recreational value of forests, species diversity and complex forest forest structure is important [14]. However, the requirements on structure and species composition for this habitat type are not suitable for *G. repens* and *N. cordata* because of the requirements on high occurrence of broadleaved trees and a more open forest structure [10]. This is in conflict with the requirements of *G. repens* and *N. cordata.* Therefore, the SBB management on *G. repens* and *N. cordata* to maintain the status in the unmixed, even-aged *Pinus nigra* forests are conflicting with the other goals of SBB and the goals of Natura 2000 in the forests on Terschelling.

Therefore, it is important to know the environmental factors that determine the occurrence and establishment of these red listed orchid species (i.e. *G. repens* and *N. cordata*). Such information might help to handle the conflicting interests of SBB and the goals of Natura 2000, within Terschelling forest areas. The information might also help to understand changing environmental factors which is suitable for orchid species under changing forest.

Succession and dynamics when there is an understanding of these factors, the possibilities to maintain the orchid species under a mixed forest can be achieved. The main objectives of this study is, to come with effective forest management recommendations that influence the forest structure and forest dynamics, in a way that the environmental circumstances are suitable for the orchids species and other goals of the forest management can also be met. To come to appropriate recommendations considering the management of the forests, the following research questions were addressed.

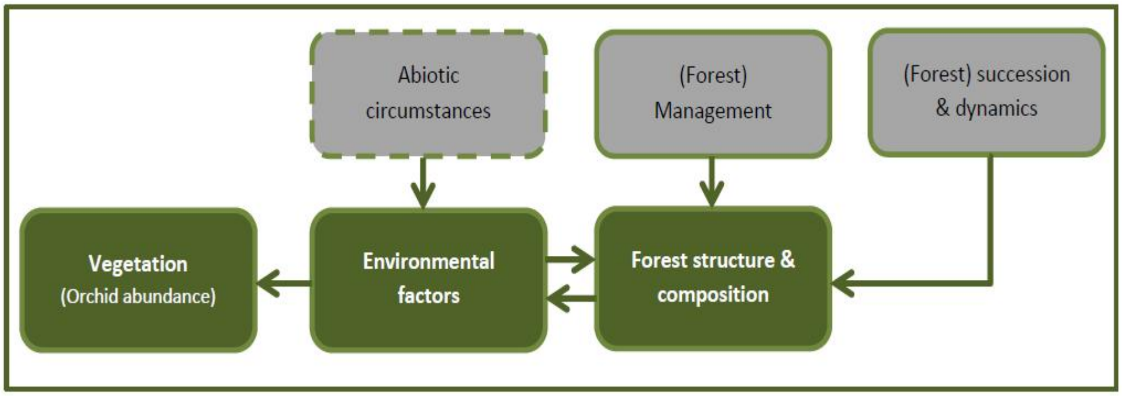

Fig. 1. Theoretical framework (Simplified) relation between forest structure and composition, environmental factors and the vegetation and the effect of abiotic circumstances, forest management and forest succession and dynamics

The research question was consists of three parts: the vegetation (abundance of *G. repens* and *N. cordata*), the environmental factors determining the vegetation and the forest structure and composition. This is visualized in Fig. 1; the relationships in this figure are simplified to make the theoretical framework applicable in our research. In this theoretical framework, the vegetation and the abundance of orchids is a result of environmental factors. Dispersal is not taken into accounts because in general this is not considered as a problem for orchids [15,16]. However, the occurrence of orchid species indeed will be affected by the interaction of biotic factors (forest structure and composition) and abiotic factors (climate, soil, soil moisture and pH) (Fig. 1). More importantly, the forest structure is influencing the environmental factors by shading, recruitment and production of litter in certain quantities and of certain quality. The forest structure and composition is constantly changing due to succession, degradation, forest dynamics and recruitment of trees. These processes are again influenced by the environmental factors in the forest. The forest development can be accelerated, adjusted or slowed down by forest management.

2. MATERIALS AND METHODS

2.1 Study Site and Sample Plot Design

The study were conducted in three forest areas located in the ""Hoornse bos" on Terschelling (Centre of the "Hoornse bos": 53 .406181 N, 5 .344591. These sites were selected based on the following criteria: pure coniferous forest (Basal area *Pinus nigra* > 85%*)*, broadleaved forest (e.g. *Quercus robur* and some *Fagus sylvatica*> *70%*) and mixed coniferous and broadleaved forest (e.g. *Pinus nigra* and *Quercus robur*). Out of these selected three forest types, 31 releveé were systematically selected which further divided to 10 x 10 m and 4 x 4 m releveé size. A 10 X 10 m plots were used to inspect woody vegetation, while 4 X 4 m plot were used to inspect Orchids species. At each sites, elevation (valley, slope or hill) were noted to exclude it as a confounding factor. In each releveés, we recorded vegetation composition (including orchid abundance), forest structure and composition, humus and litter layer and light environment. Also we took soil samples and transported to Wageningen UR laboratory and analyzed the soil organic matter, soil moisture content and electric conductivity.

2.2 Vegetation Inventory, Forest Structure and Composition

In the 4 x 4 m releveé, the presence and abundance of vascular plants and mosses were recorded using the ordinal scale of Braun-Blanquet [17]. The abundance of *G. repens* and *N. cordata* was recorded and classified according to numbers of individuals, such as 0= 0, 1= 1-10, 2= 11-50, 3= 51-100, 4= 101-300, 5= >301. Apart from the presence and abundance per species the total cover of vegetation, moss, litter and bare soil was recorded. The vegetation data was used to classify the relevées into vegetation communities. The orchids were counted to relate the environmental factors in a relevée to the abundance of orchids.

The forest structure in the plots was measured to relate the environmental factors to the forest structure and composition. Also the forests composition in a plot was used to classify a plot as coniferous, mixed or broadleaved forest. The forest structure and composition was measured by measuring the basal area (m²/ha/species), seedlings and shrubs in 10 x 10 m plot and canopy openness (%). Basal area was measured by the Bitterlich method using a relascope [4]. The Bitterlich method is a commonly used method in forestry to measure forest density and composition. Also it helps to gain insight into the openness of a stand although it does not take the shrub layer into account (which actually is very relevant for the orchids). The method is not very precise, especially when stands become more mixed and uneven aged, but the uncertainty in the method is acceptable in the homogeneous pine forests because they are very even aged. The basal area was used to classify the plots as coniferous, mixed or broadleaved and to relate the occurrence of vegetation to the forest composition.

A 10 x 10 m plots were used to study species composition of the study forest. The presence and abundance of trees, shrubs and tree seedlings (> 0.5 m tall) was recorded using the ordinal scale of Braun-Blanquet (1951). It was also recorded whether trees where present in the canopy. The data was used, together with the data from the 4 x 4 releveés, to classify the releveés into plant communities. Also the data was used to gain insight into the spontaneous development of the forest and to relate environmental factors and orchid presence with forest composition in a plot.

The canopy openness was assessed using photos of the canopy of every plot (see Fig. 2). Pictures were taken at 30 cm height with a Canon D70 using a Sigma 4.5 mm circular fish-eye lens. Canopy openness was used to relate forest structure to forest composition and light availability for the vegetation under the canopy.

2.3 Humus and Litter Layer

In all 31 plots, the thickness of the humus and litter layer was measured. Also the composition of the litter with respect to presence of needles was assessed. Samples of the humus were taken to measure moisture content (%), pH, electronic conductivity (ɥs/cm) and organic matter content (%). The focus on the litter and humus layer instead of the soil as a whole is because both *G. repens* and *N. cordata* root exclusively in the litter and humus layer [7].

Samples were taken at the same day in a short moment of time. Moisture content was measured by comparing the fresh weight of the samples with the dry weight. The humus pH was measured by mixing (shaken for an hour) 20 gr of the sample with 50 gr of distilled water and measuring the pH and electronic conductivity of the mixture. Organic matter content was measured using the loss on ignition method [18].

The data was used to relate the forest structure and composition to the litter and humus traits. Also the effect of the litter and humus traits on the vegetation and the abundance of *G. repens* and *N. cordata* was assessed.

2.4 Light Environment

The Photosynthetic Active Radiation (PAR) under the canopy was measured using a SunScan Canopy Analysis System [19] (Fig. 2). The SunScan, with the one meter long probe, was used two times for every plot. Pointing is done alternatively one time pointing to north and one time east. The time of measurement was noted for every plot. Control values where measured in the open dunes every five minutes to correct for light intensity changes over day. The unit we used was remaining light under the canopy (in comparison to the control). The light measurements were used to relate light environment to the forest structure and composition and the vegetation and abundance of *G. repens* and *N. cordata*.

2.5 Data Analysis

The collected data was analyzed using Microsoft Excel, IBM SPSS Statistics 19, TWINSPAN (Hill 1979) and TurboVeg [20].

2.6 Vegetation Composition

The vegetation data was classified using TWINSPAN [21] and determined with the help of 'Veldgids Plantengemeenschappen' [22]. The distribution of the plant communities and the orchid abundance classes over the forest types was tested using a Pearson Chi-square test. The Ellenberg indicator values [23] per plant community were calculated using TurboVeg [20].

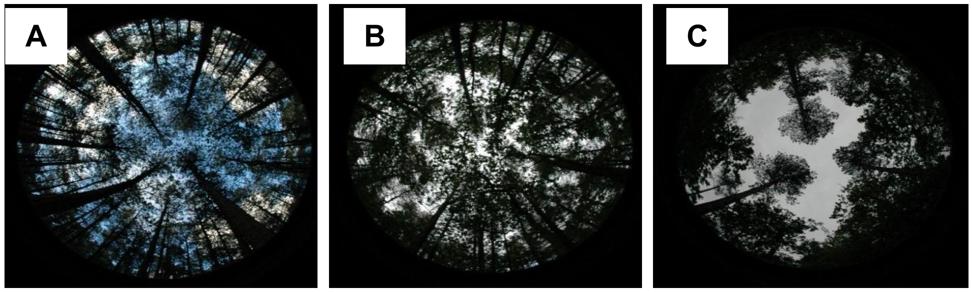

Fig. 2. Canopy pictures of (a) coniferous forest, (b) broadleaved forest and (c) an open mixed forest

2.7 Forest Structure and Composition

The result from basal area measurements, the 100 m² plot and the canopy openness were analyzed and statistically tested using IBM SPSS Statistics 19 and Microsoft Excel. Since most of the data was multivariable and consisted of more than two groups we used Two-Way-ANOVA. The abundance data was tested using a Pearson Chi-square test. The Canopy photos were analyzed using Gap Light Analyzer software [24] to calculate the canopy openness. The effects of canopy openness on the vegetation composition were analyzed using logistic regression function. To do this analysis, the abundance data of the two orchids were transformed into absence/presence data.

2.7.1 Humus and litter layer

To statistically test the litter traits, we used a Two-Way ANOVA with the forest types and the plant communities as groups and the different traits as independent variables. To test the effects of different litter traits on the presence of the orchid species, we performed a logistic regression using the presence/absence data of the orchids.

2.7.2 Light environment

Regression analyses were used to test the relationship between canopy openness, available light under the canopy and basal area. We also test the relationship between remaining light different forest types and plant communities, using Two-Way ANOVA.

3. RESULTS

3.1 Vegetation Composition

We found two plant communities, i.e. *Vaccinio-Piceetalia and Quercetalia-roboris* (Fig. 3). *Vaccinio-Piceetalia* (41A) plant community was found in 14 releveés and it is a typical plant community for coniferous forests. Both *G. repens* and *N. cordata* occurs in 92.86% of the total releve's and classified as Vaccinio-Piceetalia community (Fig. 3). *G. repens* is limited to this plant community and does not occur in the other releveés. *Pinus nigra* is in general the dominant tree species in the canopy above the relevee's containing the Vaccinio-Piceetalia, but some relevee's also have broadleaved *Quercus robur* or *Fagus sylvatica* in the canopy (Fig 3). A further distinction of the Vaccinio-Piceetalia is not possible because we have not found enough differential species. The vegetation in the other 17 relevee's was classified as *Quercetalia-roboris* (42Aa), which is a typical plant community for broadleaved forests (Fig. 3). Also here further classification was not possible because of the low amount of differencing species.

The Ellenberg indicator values [23] between the two plant communities are similar. Both plant communities are bound to the forest and grow in more or less the same abiotic circumstances. This suggesting that the existing environmental condition is conducive to both plant community and partially explain why both plant community score similar Ellenberg values. When we look to the distribution of the plant communities over the forest types in Fig. 3, we see that the Vaccinio-Piceetalia community, where both *G. repens* and *N. cordata* occur, occurs in both coniferous forests as in mixed forest but does not occur in broadleaved forest. Vaccinio-Piceetalia does mostly occur in coniferous forest (x^2=28.4, df=2, p<0.01). The two releveés of Vaccinio-Piceetalia in the mixed forest both contain *G. repens* as well as *N. cordata*.

Fig. 4 shows the abundance of *G. repens* and *N. cordata* distributed over the forest types. We can see that *N. cordata* occurs both in coniferous forests as in mixed forests, but reaches the highest abundances in coniferous forests (x^2=21.23, df=10, p=.03). *G. repens* does not reach the same abundances as *N. cordata* does and is even more bound to the coniferous forest (x^2=64.9, df=10, p=.01).

3.2 Forest Structure and Composition

The average basal area in the relevee's is 26.8 m²/ha (± 7.8 m²/ha), this is low in comparison with Dutch yield tables where the basal area in comparable forests is set around 32 m²/ha [25]. These tables are although based on even-aged monocultures where basal area is higher because of the higher tree density. There is no significant (p > 0.05) difference in basal area between forest type as well as plant community.

In general the trees that are found in the canopy in the 100 m² releveé have been planted, except for some of the mixed forests where broadleaves have spontaneously reached the canopy (i.e. sometimes also by liberation through thinning). Seedlings under the canopy have although spontaneously colonized the area and are a good indicator for the suitability for different tree species. In the 16 m² releveés we have recorded all seedlings under 0.5 m present in the releveé. Fig. 5 shows the results of the tree seedlings in these plots. We can see that *Q. robur* is colonizing most of the plots irrespective whether *Q. robur* is present in the canopy or not (x^2=41.6, df=6, p=.01). *P. nigra*, *B. pendula* and *F. sylvatica* are barely found in the releveés. The result of Fig. 5 shows that, without management, the forest is developing in the direction of a broadleaved oak forest.

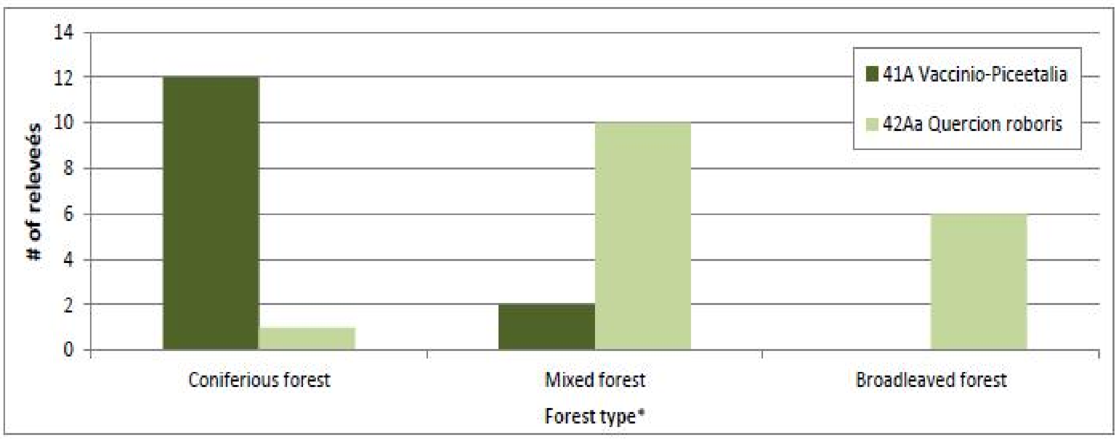

Fig. 3. Distribution of plant communities over forest types (x^2=28.4, df=2, p=.01)

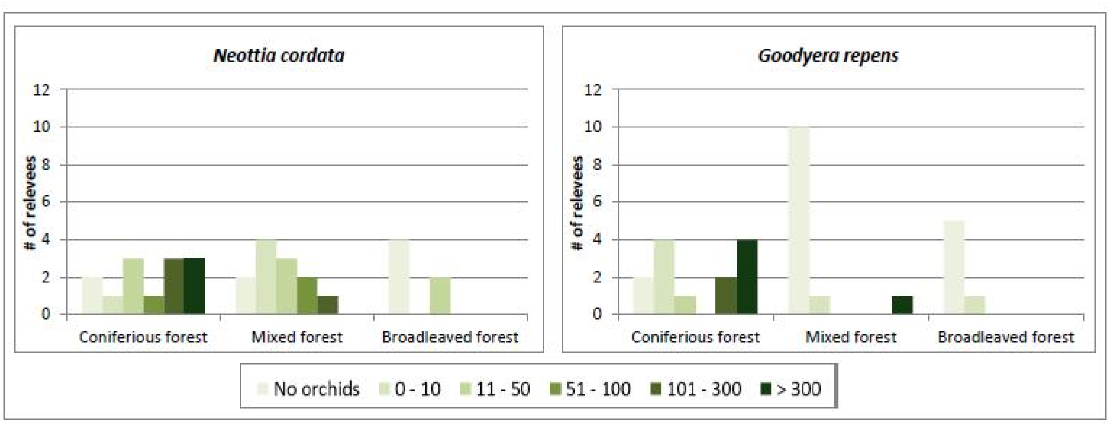

Fig. 4. Distribution of abundance classes over forest type for *N. cordata* (x^2=21.23, df=10, p=.03) and *G. repens* (x^2=64.9, df=10, p=.03)

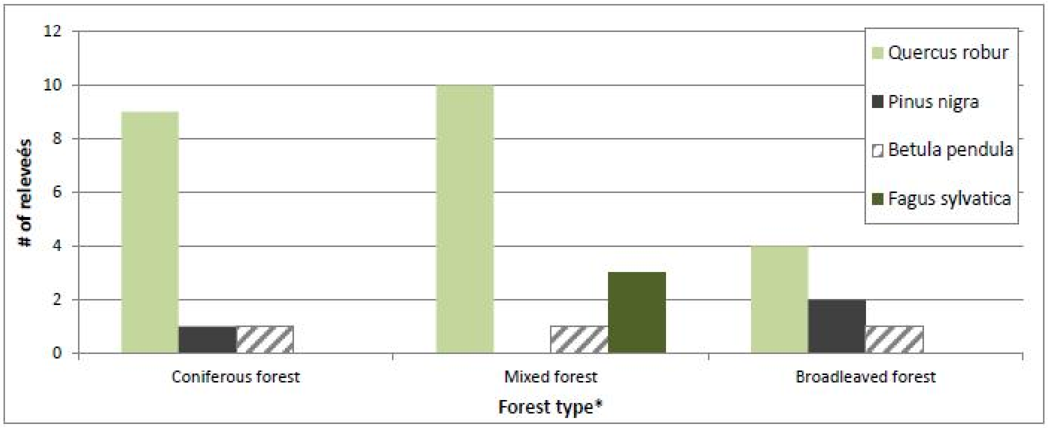

Fig. 5. Occurrence of tree seedlings over the 16 m² releveés over forest types (x^2=41.6, df=6, p=.01)

The canopy openness of the plots was on average 21% (± 7.0 %). The comparison between forest types suggests that coniferous forests are more open and broadleaved forests have the most closed canopy. After testing this was not significant ($F_{2,\ 28}$ =3.314, P=.051) but the difference between coniferous and broadleaved, without mixed forest was. The measurement of canopy openness was taken at 30 cm height and therefore takes the shrub layer into account, the shrub layer seems to explain the more closed canopy in broadleaved forests partly (see also results of light environment). When we look at the occurrence of *G. repens* and *N. cordata* as a result of the canopy openness we do not find any significant effect of canopy openness on *G. repens*. *N. cordata* is although more probable to grow when the canopy is more open (Wald= 6.975, df =1, p =.03). Fig. 6 shows this effect.

3.3 Humus and Litter Layer

On average the humus thickness is 6.7 cm (± 1.9 cm). The thickness is significantly lower in coniferous forests in comparison to broadleaved and mixed forests ($F_{2,\ 28}$ =7.88, P<0.05), but there is no difference in the litter layer (average litter thickness 1.7 cm ± 1.1 cm). In the coniferous forest type, we observed a sharp gradient between the litter layer, the thin humus layer and the mineral soil. In the broadleaved forests we found this gradient was much more gradual. Although we did not find a significant difference in organic matter concentration of the humus, we assume that there is more organic matter available for plants in the broadleaved forest because the thickness of the humus layer is bigger. We did not find a significant difference in litter layer, suggesting the lower litter production in coniferous forest compared to broadleaf forest.

Despite the higher decomposition rates in the broadleaved forest, the pH of the humus was slightly lower in the broadleaved forest ($F_{2,\ 28}$ =15.634, p=.01).

The moisture content of the humus in broadleaved forest was although also higher ($F_{2,\ 28}$=4.362, p=.02), which can have a positive influence on decomposition rates.

When we compare the litter from the two plant communities we see the same patterns. The Quercetalia-roboris plant community, which is more or less correlated with the occurrence of broadleaved forest, has higher moisture content ($F_{1,29}$=4.565,p=.03), lower pH ($F_{1,29}$=37.378, p=.01) and a thicker humus layer ($F_{1,28}$=8.8416, P=.03).

When we test for the effect of the different litter traits on the presence or absence of *G. repens* and *N. cordata* we do not find any significant effect on the presence of *N. cordata*. *G. repens,* although is affected by the litter, the results of this are visualized in Fig. 7a and 7b. When the thickness of the litter increases, the probability of *G. repens* occurrence decreases (Wald= 6.975, df=1, p=.02). Also with an increase of the percentage of needles in the litter, we have found an increase in the probability of *G. repens* occurring (Wald= 6.975, df=1, p=.03). We did not find any significant differences between forest types, orchid presence nor plant communities with respect to electronic conductivity.

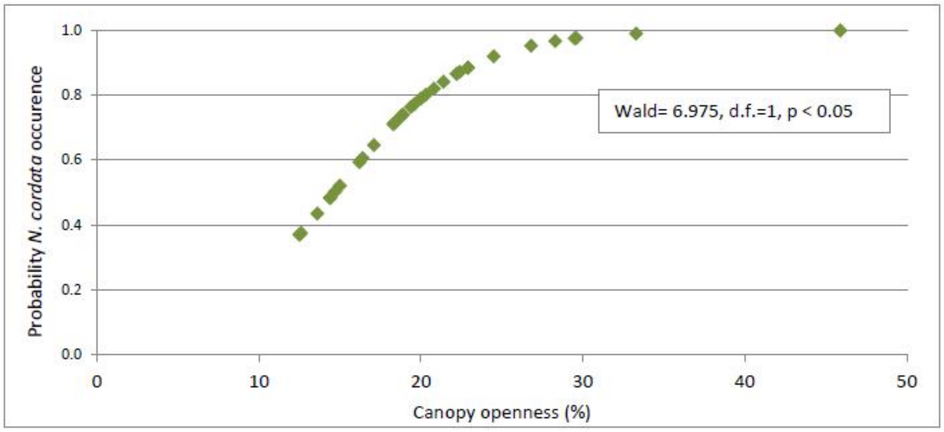

Fig. 6. Probability of occurrence of *N. cordata* as a result of canopy openness (Wald= 6.975, d.f.=1, p = .02)

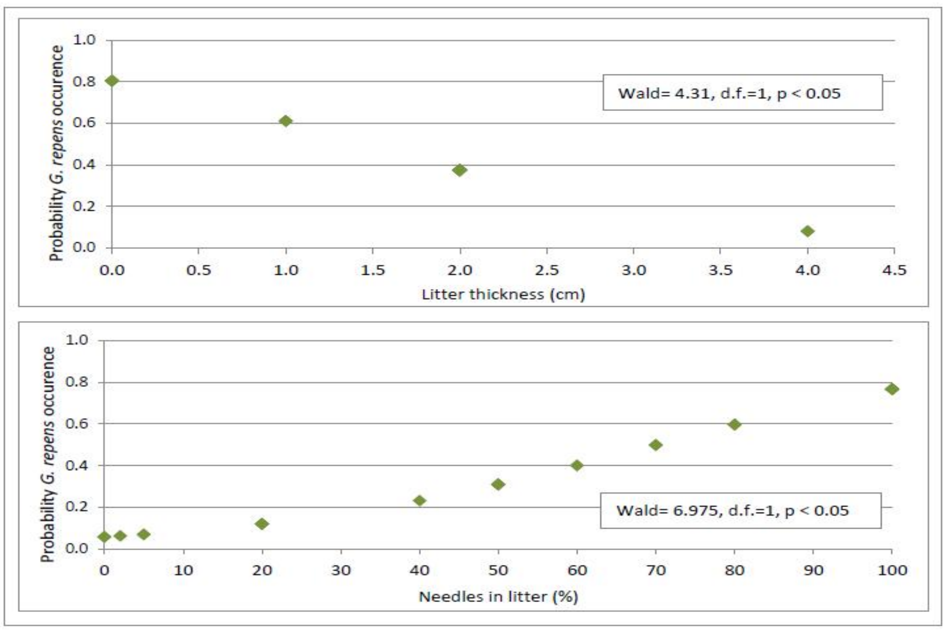

Fig. 7. (a) Probability of occurrence of *G. repens* as a result of litter thickness (Wald= 4.31, d.f.=1, p=.02). (b) Probability of occurrence of *G. repens* as a result of % needles in litter (Wald= 6.975, d.f.=1, p= .02)

3.4 Light Environment

The remaining light under the canopy was very closely related to canopy openness (R^2=0.79; y = 1.0012x - 0.1042). The relation between remaining light and basal area is poor, partly because basal area does not take the shrub layer into account. The canopy of the broadleaved forest intercepts more light than mixed and coniferous forests. The most light can penetrate through the coniferous canopy ($F_{2, 28}$=6.589, p=.03).

4. DISCUSSION

Both N. cordata and *G. repens* are differential species for the Vaccinio-Piceetalia plant community, indicating that orchid species disappearance is not always linked to the absence of plant community, because the community can simply no longer be identified because of the absence of *G. repens* and *N. cordata*. Apart from that the Ellenberg values of the Quercetalia-roboris, the replacing plant community has similar Ellenberg values with that of Vaccinio-Piceetalia, hence it is hard to draw conclusions only based on the plant communities. Therefore, it is better to take into consideration the environmental factors in different forest types into consideration to explain the absence, presence, and abundance of *G. repens* and *N. cordata* in the forest. Our results suggest that the mechanisms explaining the occurrence of the species differ partly for *G. repens* and *N. cordata.*

N. cordata is affected by canopy openness and highly abundances in coniferous forest (Figs. 4 and 6). It is difficult to disentangle the effect of broadleaved forest and light/canopy openness on the *N. cordata* occurrence. However, a moderate light availability/ canopy openness at each site partly explain the occurrence of *N. cordata* and. Although not statistically tested, we did not find *N. cordata* under big forest gaps, indicating that there might be an optimum level of canopy openness for *N. cordata* to survive and beyond that optimum level of light might create a conducive microenvironment for other plant communities and outcompete *N. cordata* from its ecological range [26]. On the other hand, we have found no effects of canopy openness on *G. repens*.

The abundance of *N. cordata* is higher in broadleaved forests, compared to the abundance in coniferous forest. This might be minimum light availability in broadleaved forests. *G. repens* does not occur in broadleaved forest indicating that *G. repens* requires high level of light for their establishment and survival. The difference in light environment can be explained by the shape of Pine trees which are more vertically organized and therefore more light gets through the canopy [27]. Other factors than light that are changing in broadleaved forests that might explain the decline of *N. cordata* and absence of *G. repens* are lack of pine needles and increased moisture and organic matter availability in broadleaved forests. Also pH differs between coniferous and broadleaved forest, but the difference is although considered to be too small to have any significant effect on the orchid species and both forest types have acidic humus layer.

The humus in the broadleaved forests has higher moisture content and there is more organic matter available for plants. The higher moisture and organic matter availability in broadleaved forests create more chances for different plant species [28,29] and will lead to the *N. cordata* being outcompeted.

Other studies indicated that the occurrence of needles in the litter is also considered to be important for *N. cordata* and *G. repens* [1,2,3]. Our results suggested that the presence of *G. repens* and *N. cordata* is most explained by the forests type and needles in the litter. We haven't found any explanation in the literature for the importance of the litter for *N. cordata* and *G. repens*, but we do see that abundance of the species is highest in pine forests. We hypothesize that the physical traits of needles containing litter might be important for the orchid to successfully germinate, grow and/or reproduce. Litter of broadleaves is more horizontally oriented in comparison to needle litter and forming a sort of physical barrier [30]. This might be problematic for the orchids to penetrate with their roots through the litter layer. Also this will have an effect on the moisture availability and light penetration to the humus layer, making the broadleaved forest more suitable for other plants and increasing the competition on these sites. As soon as, the needle litter becomes less mixed this problem will be less important. This might explain the abundance gradient from coniferous forest and mixed forest to broadleaf forest.

Some orchids need specific mycorrhiza for their germination [31,32], and they also have an obligate relationship with mycorrhiza for germination [26,33]. In general, there is different mycorrhiza in coniferous forests [34], which make this hypothesis more plausible. We cannot prove this hypothesis based on our results and further research on the relation between the mycorrhiza and the orchids is needed. The current management of the State Forestry Service does not seem to be effective to preserve the circumstances suitable for the occurrence of the orchids. No management in the monocultures of *Pinus nigra* will lead to a gradual development to more broadleaved forest with *Quercus robur* and on the long term *Pinus nigra* will disappear out of the forest system. The results from the vegetation recordings showed that there might be a shift from the Vaccinio-Piceetalia plant community to the Quercetalia-roboris plant community and a disappearance of the orchids *G. repens* and *N. cordata*. Also without regeneration of broadleaf forest the environment will become unsuitable for *G.*

repens since in forests of *P. nigra* there will be a build-up of the litter layer [35]. Our results show that the probability of *G. repens* to found decreases when the litter layer increases. So when these *P. nigra* forests age they will become less suitable for *G. repens*. We haven't found evidence that this will also count for *N. cordata*.

The successful colonization of *Q. robur* might be explained by the closed canopy and the thick humus layer in the forest. *Q. robur*, in contrary to *P. nigra* and *B. pendula*, has big seeds which have enough reserves to get through the litter layer and is dispersed in a forest easy by birds and rodents [26,36]. Also *Q. robur* is more shade tolerant than *P. nigra* and *B. pendula* and can cope (longer) with the shaded circumstances under the forest canopy [37]. The lack of regeneration of *F. sylvatica* is probably due to seed limitation due to lack of reproductive trees. For successful regeneration of *P. nigra* forest gaps should exists where the forest climate is interrupted and no litter layer is present. In these pioneer situations *P. nigra* can germinate and maintain itself [26].

5. CONCLUSION AND RECOMMENDATIONS

Both orchid species are dependent on the occurrence of needle litter and grow in a low productive environment where they are not outcompeted by other plants. The presence of mycorrhiza is likely to be important for the species. For *N. cordata* it is also important that there is enough light available. For *G. repens* the litter layer should not be too thick. In general the development to more broadleaved forest has a negative effect on the environmental factors that stimulate occurrence of *G. repens* and *N. cordata*. The development of a shrub layer leads to increased shading and canopy closure and will decrease *N. cordata* abundance. Aging of coniferous forests will also lead to a further build-up of litter what eventually will lead to the disappearance of *G. repens*. In line with this the two mechanisms that eventually will lead to the decrease of *G. repens* and *N. cordata* are succession from planted coniferous forest with *P. nigra* to a mixed broadleaf forest with *Q. robur, F. sylvatica and B. pendula* and the build-up of the litter layer in the present *P. nigra* stands. For preservation of the suitable conditions for *G. repens* and *N. cordata* there needs to be a focus on regeneration of (patches of) unmixed *P. nigra* forests, without management the suitable conditions will slowly disappear and active management is required for sustainable conservation of the orchid species.

The recommendations are written aimed at forest management focused on a sustainable conservation of *G. repens* and *N. cordata*, attaining a mixed, species rich forest attractive for recreants. These recommendations will possibly lead to a decrease of the total amount of individuals of *G. repens* and *N. cordata* in the forest, but will lead to a sustainable conservation of the species.

- Spread in space and time patches of forest should be removed (min. 2 x dominant tree heights) to create regeneration gaps specifically for *Pinus nigra* recruitment.
- The soil in the regeneration gaps should be scarified to make it possible for *P. nigra* to germinate and settle.
- When regeneration of *P. nigra* in the gaps lags behind or is outcompeted by other species (e.g. *Betula pendula*) additional planting and removal of undesired species can be considered.
- Thinning should be focused on obtaining a mixed forest with a diverse forest structure, but should preserve a certain minimum amount of *P. nigra*, preferably in groups.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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