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Master Thesis

Assessment of habitat restoration for the conservation of *Phengaris teleius* butterfly

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Assessment of habitat restoration for the conservation of Phengaris teleius butterfly

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Objectives of thesis

Master thesis included in the LIFE+ Project "Blues in the Marshes" (LIFE 11 AT/NL/000770/ Action C1 19 and D1) that is carrying out in the areas of Vlijmens Ven, Moerputten and Bossche Broek, south of Netherlands. The LIFE+ Project aims to restore the habitat of a one threatened butterfly species: the scarce large blue, Phengaris (Maculinea) teleius. Objectives are focus on this species because included a great complex ecological relation and the scarce large blue is listed in Annexes II and IV of the Habitat Directive. The butterfly has only one host plant, Sanguisorba officinalis and one myrmecophilous relation with Myrmica scabrinodis ants. When caterpillar get the larva instar L1-L3, ants move the larva to the ant nest where they will be feed on and hibernate. Species of related ant and host plant should be in stable population to improve the P. teleius status in the area being the main purpose of LIFE+ Project. The target zone is a threatened N2000 habitat type, Junco-Molinion, consists in fen and damps meadows that were intensively damaged by agriculture and almost disappeared in Netherlands.
Some experimentation was set up during two years to determine the vegetation structure and the process of ants colonization in the purpose restored area. The LIFE+ project has not finished yet and measurements should continue in the zone. In this master thesis the main objective is to monitor the process of colonization by Myrmica scabrinodis and other ant species, that can competitive with the target ant such as Lasius niger, in the Natura 2000 site "Vlijmens Ven, Moerputten and Bossche Broek". Secondly, monitor the structure vegetation and its succession in the restore area thus it is highly dependent of success of establishment M. scabrinodis.

Methodology

The study area is present in the south-west of 's-Hertogenbosch, Nature 2000 place "Vlijmens Ven, Moerputten and Bossche Broek" (Netherlands). This area suffered a great intensively agricultural pressure producing the disappearance of the wetland ecosystems; just one nature reserve (Moerputten) presents peatbogs and meadows with the vegetation of the alliance Junco-Molinion, association Cirsio dissecti-Molinietum.

Since 2007 some restoration activities have been applied with the purpose to enhance the habitat for P. teleius. Some agricultural land were excavated and become new wet meadows. Vegetation was transplanted in 2013, exactly 63 sods of one square meter were collected from Moerputten meadows and were located randomly in four restoration areas. 9 sods were localized in each area, in a grid of three by three and separated three meters between them. In 1,5 m from each sods translocated were made control plots among inside (4) and outside (8) of translocated area to test better the colonization process (see picture below).

Ant baits were put inside of the translocated plot and outside control, to attract ants presented in surrounding areas. This method designed is used during whole process of the restore project, where include recording some experiments to test the presence of Myrmica scabrinodis and other ants species and to investigate the vegetation cover and height.

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Lepidoptera, Phengaris, ecology, oviposition

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Elmes GW, Thomas JA, Wardlaw JC, Hochberg ME, Clarke RT & Simcox DJ (1998) The ecology of Myrmica ants in relation to the conservation of Maculinea butterflies. Journal of Insect Conservation 2: 67-78.

Van Swaay CAM, Cuttelod A, Collins S, Maes D, Munguira ML, Sasic M, Settele J, Verovnik R, Verstrael T, Warren MS, Wiemers M & Wynhoff I (2010) European Red List of Butterflies. Publications Office of the European Union, Luxembourg.

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Assessment of habitat restoration for the conservation of *Phengaris teleius* butterfly

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Prague 2017

Declaration

I hereby declare that I am the sole author of the thesis entitled: “Assessment of habitat restoration for preserving *Phengaris teleius* butterfly”. I duly marked out all quotations. The used literature and sources are stated in the attached list of references.

In Prague on 1.12.2017 **Cristina González Sevilleja**

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Abstract

This master thesis is part of the LIFE+ Project "Blues in the Marshes" that is carried out in a N2000 nature reserve in the South of the Netherlands. The project aims to restore the habitat of a threatened butterfly species: *Phengaris teleius*. This butterfly is threatened in Europe and its recovery is difficult due to its complex ecological relations with other species. The butterfly has only one host plant, *Sanguisorba officinalis,* and a myrmecophilous parasitic relation with *Myrmica scabrinodis* ants. Females lay eggs on the host plant and after hatching and developing into the 4^{th} larval instar within only four to five weeks, the caterpillar is adopted by *Myrmica* ants, where it will develop inside the ant nest until the adult phase. The populations of both, ant and host plant, should be in stable conditions to carry the burden of the parasitic *P. teleius* population in the area. In the Netherlands, the target vegetation is a threatened N2000 habitat type belonging to the *Cirsio-Molinietum* community, occurring in fens and damp meadows. This ecosystem has declined in Europe; therefore conservation efforts are addressed to enlarge the area, also in the Netherlands. Within the LIFE+ Project, an experiment has been conducted to improve the ecosystem and consequently the ecological relations of *P. teleius*. In restoration areas, the soil was excavated to achieve nutrient-poor conditions and hay cut from wet meadows with climax vegetation communities was spread to re-establish the seed bank. In addition, to enhance the probabilities of success, sod translocations were done from climax vegetation to extend and restore wetland plants in the area and to attract *Myrmica* ants. Data of vegetation and ant occurrence from three consecutive years are analysed in this study to determine the short-term effects of restoration. Some signals of recuperation are observed within two years of research; vegetation composition is changing and several characteristic species of wetlands appeared in the restored areas. Vegetation coverage is increasing and soil conditions are going towards wetland situation. With respect to the ant population, *M. scabrinodis* is slowly spreading from the translocated sods and appearing in further areas. The target ant coexists with the competitor *Lasius niger* in some plots, where habitat conditions allow *M. scabrinodis* to colonize. The restoration process presents some positive signals of recuperation just after three years from soil excavation, but it still needs time for the colonization of ants and total establishment of wetland plants.

Keywords: *Phengaris* butterfly; *Myrmica* ant; conservation; restoration methods; ecology; fen meadows.

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1. Introduction

The huge amount of lost species and its speed determined during the Anthropocene extinctions is unusual (Barnosky *et al.*, 2011; Ceballos *et al.*, 2015; Tilman *et al.*, 2017; Williams *et al.*, 2015). Anthropogenic disturbances produce a threat to biodiversity with unknown enormous consequences, where displacement of indigenous by invasive species and habitat destruction are the most mentioned causes of extinction. An additional one is the lack of knowledge about coextinctions derived from narrow ecological relations. This driver has not been studied deeply and it is considered one of the most important producing biodiversity loss (Diamond *et al.*, 1989; Koh *et al.*, 2004; Wynhoff *et al.*, 2015). Parasites and mutualists react differently to changes with unexpected consequences (Dunn *et al.*, 2009). Also, the plant-insect interaction is described as an important loss of functions in ecosystems; a cascade of extinctions in arthropods will be produced by the loss of vascular plants, leading to irremediable coextinctions (Haddad *et al.*, 2009; Krauss *et al.*, 2010).

1.1 Threatened habitat: European grasslands

The grassland ecosystem is threatened in Europe and it has lost a huge percent of its coverage, of which 90% due to the conversion to other land uses (WallisDeVries *et al.*, 2002). These ecosystems are biodiversity hotspots and lodge a big number of endangered fauna and flora. Extinction debt exists currently for specialist plants of European grasslands affecting all organism related to them (Matus *et al.*, 2003; WallisDeVries *et al.*, 2002). Wetlands provide 40% of ecosystem services of the world, despite of their small distribution (3,5%) (Costanza *et al.*, 1997; Zedler & Kercher, 2005). Pollinators and herbivores from these environments may be used as an indicator to determine the conservation status of grasslands, because of their ecological narrow relation to the plants. The short-term response of insects, such as butterflies, can exacerbate by more threats to their population when grassland plants are affected (Krauss *et al.*, 2010; Thomas *et al.*, 2004; Van Swaay *et al.*, 2015; WallisDeVries *et al.*, 2002).

One example of threatened grassland is the vegetation of *Cirsio dissecti-Molinietum* present in lowland North-Western Europe (Blackstock *et al.*, 1998; Matus *et al.*, 2003). This community is characterized by a high number of plant species growing on basic soil, poor in nutrients, under wet soil conditions, due to the seasonal flood of water that run off the minerals and promote deposition of calcium. Plant communities of fen meadows are controlled by the hydrologic regime that provides the pH and moisture conditions (Cronk & Fennessy, 2016; Jansen *et al.*, 2000; Van der Hoek & Heijmans, 2007). In the Netherlands, this vegetation occurred in many regions, but currently, it is reduced to 30 ha, isolated in nature reserves (Schaminée *et al.*, 1996). Fen meadows occurring on low productive soil, are being isolated while carrying a high number of species among those many endangered Red List species

(Grootjans *et al.*, 2002). There are two main reasons for semi-natural grassland loss: conversion to intensive agricultural lands with possibility to cultivate or abandonment of managing farmland in mountains or wet areas (Grootjans *et al.*, 2002; Matus *et al.*, 2003; Öckinger *et al.*, 2006; Walker *et al.*, 2004). Nowadays, agricultural swards account for high levels of nutrients from fertilizer application, sunk groundwater and variation in precipitation regimes (Buishand *et al.*, 2013; Daniels *et al.*, 2013), which are resulting in an impoverishment of species (Jansen *et al.*, 1996; Matus *et al.*, 2003).

1.2 Special case of Phengaris butterflies

Grassland butterflies are one of the most affected species of grassland loss, declining during the last decades in Europe, mainly due to the disappearance of their habitats (Munguira & Martín, 1999; Van Swaay *et al.*, 2015). Monitoring of butterflies throughout Europe shows a negative trend of 30% of butterfly species since 1990 (Van Swaay *et al.*, 2015). Meadow butterflies have been used as an indicator of grassland status, analysing at the same time the agricultural policy efficiency, but also, butterflies can be used to find out more about restoration processes (Musters *et al.*, 2013; Thomas, 1994; Van Swaay *et al.*, 2015). A review in England (Thomas *et al.*, 2004) reports about the huge decline of butterflies (70% in about two decades) being a great difference compared with the other indicators birds and vascular plants. Monitoring of butterflies may portend the status of other taxa, based on the direct environmental conditions that influence them. The narrow plant relation and the short life, make butterflies good indicators of the environment, showing a fast response to habitat changes as an essential part of conservation projects (Thomas *et al.*, 2004; Van Swaay *et al.*, 2015).

Inside of the order *Lepidoptera,* a special relation with ants exists that is called myrmecophily. About 70% species of the family *Lycaenidae* are associated with ants (Fiedler, 1991; Hinton, 1949; Lach *et al.*, 2010; Pierce, 1987). All known European *Phengaris* species are parasites, in general, of one (sometimes a few) specific *Myrmica* species at local scale, showing the narrow coevolution process between these two taxa (Elmes *et al.*, 1998; Thomas *et al.*, 1989; Witek *et al.*, 2006). Therefore, to conserve these myrmecophilous butterflies, ant ecology should also being taken into account (Elmes, 1991b; Nowicki *et al.*, 2005; Thomas *et al.*, 1989; Witek *et al.*, 2010).

Grassland conservation managements require active actions for their maintenance (Elmes & Thomas, 1992; Van Swaay *et al.*, 2015). The current fragmented status of grassland butterfly habitats is clamouring for conservation efforts (Van Swaay *et al.*, 2015). *Phengaris* butterflies are just examples for grassland species, destruction and attenuation of their habitats for land use changes are the main reasons for the decrease in number of populations, especially in Western Europe. *Phengaris* species show a negative trend in distribution and population size, which is even intensified in fragmented and isolated populations (Elmes & Thomas, 1992; Van Swaay *et al.*, 2015; Wynhoff, 1998b).

1.2.1 Life cycle of Phengaris teleius

Phengaris (= Maculinea) teleius is categorized as *Vulnerable* in Europe and it is listed in Annexes II and IV of the Habitat Directive (Van Swaay *et al.*, 2010). Studies about the phylogeny of this butterfly treated *Maculinea* as junior synonym of *Phengaris* because of the narrow phylogenetic relation of shared species (Fric *et al.*, 2007). In the Netherland*, P. teleius* became extinct in 1976, disappearing from fen meadows (Tax *et al.*, 1989). After several years, in 1990, *P. teleius* was reintroduced in the Moerputten Nature Reserve in the region of North-Brabant, the only place with the required conditions for the butterfly reestablishment (Wynhoff, 1998a). 33 males and 53 females were collected from a stable Polish population and they were released in the suitable meadow "Bijenwei" inside the nature reserve (Wynhoff, 2001). Currently, *P. teleius* continues living in the reserve, thus it might be concluded a successful reintroduction (Wynhoff, 1998a). Nevertheless, the butterfly is still restricted to the releasing area, Moerputten. In 2013, the LIFE+ Project "Blues in the marshes" started to address the problem of isolated population of *P. teleius* by enlarging the wet grassland habitat in the south of the Netherlands.

P. teleius, the Scarce Large Blue, has a specialised life cycle, being an obligate parasite of *Myrmica scabrinodis* ants (Figure 1). The butterfly species used to live in moist habitats as wetlands and marshes where *Sanguisorba officinalis,* its host plant, is present in closed populations (Thomas, 1984b; Wynhoff, 1998a). *S. officinalis* is also a food plant for butterflies and it is distributed along river systems, in particular in the Netherlands, sharing habitat with *Cirsium officinale, Succisa pratensis* and *Molinia caerulea* among others (Wynhoff, 2001). The flight period of *P. teleius* is between end-June to mid-August (Wynhoff, 1998a). The eggs are laid on the flower buds of *S. officinalis*, when caterpillar hatch this plant will provide food during the first weeks (Elmes & Thomas, 1992). After several weeks of larval development, the caterpillar of *P. teleius* leave the plant. They are specialised to attract *M. scabrinodis* ants by producing sounds to be adopted. Analysed stridulations produced from host ants and *Phengaris* caterpillar calls prove the great similarity between these two species. However, caterpillar sounds are not totally equal to ant calls, therefore other characteristics are also used for mimicry. During the underground period, caterpillars use chemical compounds to camouflage completely in the ant nest (DeVries *et al.*, 1993; Elmes, 1991b). In addition, caterpillars of *Phengaris* species in general evolved other characteristics, such as a thicker cuticle to be safe for ant bites (Elmes *et al.*, 1998; Thomas & Wardlaw, 1992).

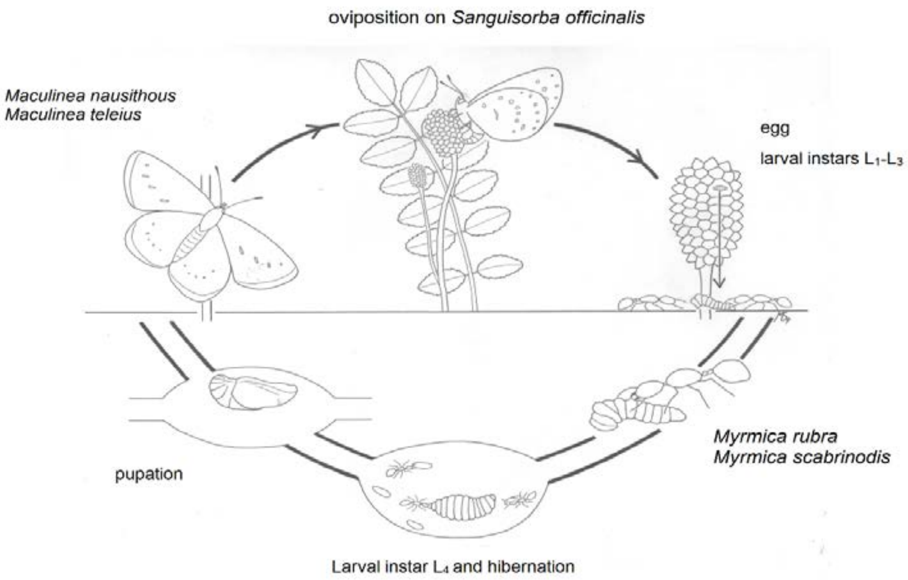

Figure 1. Life cycle of *Phengaris teleius* and *Phengaris nausithous* (After Wynhoff, 2001)

Then, when a *M. scabrinodis* worker ant is in the direct surrounding of a *P. teleius* caterpillar, the ant may recognise its sounds as one of a lost ant larva and takes the caterpillar to the nest (Figure 1). Caterpillars spend around 10 months inside of the *M. scabrinodis* nest, where they prey on ant brood. Also the pupal period passes inside of the chambers (Hinton, 1949; Thomas, 1984a). The species is tolerated inside the ant nest, where they can find protection from the environment and large resources to complete caterpillar and pupal development (Elmes, 1991b).

Early studies concluded that this butterfly species uses just one specific host ant, *M. scabrinodis* (Elmes *et al.*, 1998). However, recent researches found that *M. scabrinodis* is the most important and frequent host ant for *P. teleius* but not the only one. In Eastern and part of Central areas of Europe, it was found that *P. teleius* caterpillar can also infest ant nests of *Myrmica ruginodis* and *M. rubra* when *M. scabrinodis* population density is low (Figure 2) (Stankiewicz & Sielezniew, 2002; Witek *et al.*, 2010; Witek *et al.*, 2008). For the population of *P. teleius* of the target study area it is known that *M. scabrinodis* is the only host ant.

1.2.2 Ecology of Myrmica ants

Myrmica ants are important for the target butterfly species of this study, therefore for restoration purposes, we need to know the ecology of *Myrmica* ants. Ants play an important role in ecosystems, due to the impacts generated in the soil with their activities producing aeration, heterogeneity and increasing decomposition (Elmes, 1991a; Gabet *et al.*, 2003; Lach *et al.*, 2010). Other essential activities of ants are related to the food chain and their interaction with other species. *Myrmica* ants share quite similar life styles, stable colonies differ in size between

200-5000 workers and one to several functional queens (Elmes, 1973; Elmes *et al.*, 1998; Wynhoff, 2001). New fresh queens spend around six weeks in the parent colony to increase their fat reserves. After mating, they are prepared for colonization. For mating and the establishment of new colonies nuptial flights of the new queens are required, occurring between mid-August to mid-September (Elmes *et al.*, 1998). Nuptial flights occur mainly at the end of the summer to give time to the new queens to lay eggs and have some workers before winter starts. The acquisition of fat reserves of *Myrmica* ants is a crucial condition for *Phengaris* caterpillar survival because they need plenty of ant larvae in the ant nest during winter. Non-*Myrmica* species cannot be host ant for these butterflies, because they don´t have ant brood during hibernation (Elmes *et al.*, 1998; Thomas & Wardlaw, 1992; Wynhoff, 2001). Colonization by young queens takes place at a local scale of few metres (around 100 m) from the source nests, the number of long distance colonizers is only low. Competition between ants is strong and formation of new colonies becomes difficult when habitats are already colonised by competitors with similar size, such as *Lasius niger* or *Tetramorium caespitum.* Generally, new habitats are settled by a new colony that is a fragment of the old colony (Elmes *et al.*, 1998). The splitting of an ant colony is called budding. After budding the distance between old and new colony are always short (around 10 m).

M. scabrinodis prefers high values of soil moisture, medium temperatures and insolation (Elmes & Wardlaw, 1982; Wynhoff, 2001). *Myrmica* ant's activity is concentrated at the beginning of the day and from the afternoon to the early evening under influence of insolation, matching with the *Phengaris* caterpillar's activity (Wynhoff, 2001). Ant species have different strategies of colonization, among others dependent on the vegetation structure of the area. Competitor species, such as *L. niger*, are able to colonize areas with a high percentage of bare soil at early stages of succession (Dauber & Wolters, 2005), while *M. scabrinodis* requires a lot of vegetation cover and height of herbs in its niche (Elmes *et al.*, 1998; Wynhoff *et al.*, 2016). *Myrmica* ants are more sensitive to disturbances compared to their main competitor, making it more difficult for these species to colonize disturbed areas (Wynhoff *et al.*, 2001).

1.3 Challenges for Phengaris Conservation

Complex ecological relations of *P. teleius* (Figure 2) turn restoration projects to support the species into challenging projects, where several stipulations should be taken into account for the creations of suitable habitat (Hochberg *et al.*, 1994; Maes *et al.*, 2004; Wynhoff, 2001). In general, butterflies from European grasslands need high coverage and height of the vegetation (Pöyry *et al.*, 2004). According to the habitat, *P. teleius* occurs in areas with high values of vegetation coverage and moisture, appearing close to stream valleys or water bodies because of the presence of wetland plant species (Wynhoff, 2001).

Especially high abundance of *S. officinalis* is required for the oviposition and of other plants as nectar resource (Nowicki *et al.*, 2005; Thomas, 1984b; Wynhoff, 2001). *P. teleius* migrates low distances among areas, where population survival is affected by habitat fragmentation (Nowicki *et al.*, 2005). Then, connectivity between habitats is required for butterfly dispersal (Öckinger & Smith, 2007).

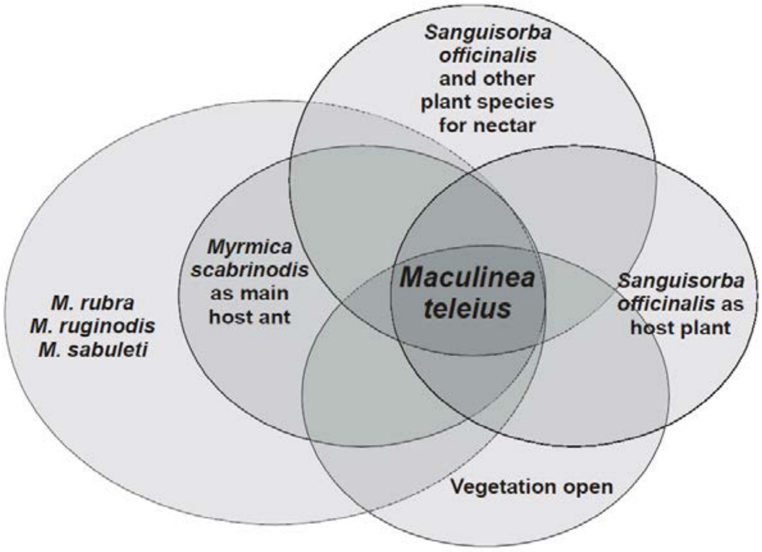

Figure 2. Diagram of complex relationships and narrow niche of Ph*engaris teleius (*After Wynhoff, 2001)

To enhance and keep high species richness in plants and butterflies, it is necessary to manage grasslands avoiding negative effects from abandonment (Öckinger *et al.*, 2006; Wenzel *et al.*, 2006). Meadow cutting should be controlled for the negative influence on butterfly populations; eggs and larvae present in the vegetation are destroyed with this management (Saarinen & Jantunen, 2005). Nevertheless, there is a trade-off between the two sources of *Phengaris*; longer mowing intervals affect *M. scabrinodis* in a negative way and *S. officinalis* plants may be displaced by succession (Johst *et al.*, 2006; Wynhoff, 2001). Therefore, the vegetation should be mown once or twice per year, before the flying period or after the caterpillars have left their host plant and stay safe in the ant nests (Grill *et al.*, 2008; Morris, 2000). Medium disturbances of mowing will balance the butterfly presence and it resources (Dover *et al.*, 2010; Johst *et al.*, 2006; Wynhoff, 2001).

For a small population of *P. teleius*, a high density of *M. scabrinodis* nests of around 500 nests in the target area is needed (Elmes *et al.*, 1998; Thomas, 1984b). The foraging walks of worker ants are estimated around two meters around the nest. Butterflies neither move large distances and there is a reduced exchanges of individuals among populations (Elmes & Wardlaw, 1982; Wynhoff, 2008). Therefore, limitations of small foraging areas determine the necessity of high densities of ant nests for *P. teleius* caterpillars to be found by workers. Reasonable actions addressed to ant populations will affect the butterfly presence in a positive way (Elmes *et al.*, 1998; Wynhoff *et al.*, 2016). About 85% of the butterfly´s life takes place inside the ant nest and 98% of its biomass comes directly from ants. In the opposite direction, *P. teleius* is considered a parasite in the ant colony consuming ant larva and limiting the defence of the colony for the reduced number of workers (Elmes *et al.*, 1991). Nevertheless, when the average of caterpillars is one per ant nest, the ant colony is supposed not to be damaged seriously, only a small impact in the host ants colony is done (Elmes *et al.*, 1998).

Proper knowledge about ecological relations of *P. teleius* are crucial for conservation and restoration projects for this species, where providing suitable habitats for *Myrmica* should be a priority (Thomas *et al.*, 1989; Witek *et al.*, 2010).

1.4 Projects and management prior to this study

After *P. teleius'* reintroduction, several activities were generated in the nature reserve Moerputten and its surroundings to enhance and enlarge the habitat of this butterfly. Local small scale sod cuttings and sod translocations were applied to assess the effect of *Myrmica* ant colonization after the creation of a small microhabitat. In one area of Moerputten, this action had a beneficial effect on ant nests increasing its density and consequently, an increment in *P. teleius* population size (Wynhoff, 2008). However, in other areas of the nature reserve, local sod cuts have not shown any influence in ant nest density after several years (Ollivier, 2013). Therefore, the local characteristics and situation will determine the success. In 2013, sods were translocated to the soil removal areas to enhance the colonization success of *Myrmica* ants. During the LIFE+ Project, the development of vegetation and ant colonization have been monitored.

Before starting the LIFE+ Project, various actions with restoration purposes have been done in the area. In 2007, these activities started with different years of soil removal (See table 1) in the surrounding farmland areas of Moerputten (See figure 2). Soil excavations were applied to remove the nutrient content in the upper soil layer (Janssens *et al.*, 1998). To avoid acidification of the terrain liming was applied after excavation and then, hay cuttings from mature meadows with wetland plant species were distributed over the sandy soils. These managements are assumed to provide favorable conditions for the establishment of target plant species in the restoration areas, accelerating the succession process and avoiding other pioneer species that might counteract (Klimkowska *et al.*, 2007; Kolvoort, 2015; Lamers *et al.*, 2015; Wynhoff, 2013).

2. Aims of the project

In this study, our main objective is to determine the effect of sod translocations (management done with restoration purposes in 2013) on the ant community in the newly created habitat, in order to enhance the *P. teleius* status in the target area.

With the data collected in three consecutive years since the LIFE+ project started, the aim is to investigate the changes in the ant community and in the habitat throughout these years to assess the success of this part of the restoration project.

Two different traits are researched for this year of monitoring (2016) and during the whole LIFE+ project. Firstly, in the source zone (Moerputten), where the sods were taken, we investigate how the vegetation succession changes species occurrences and their densities. Secondly, where the sod transplantations were placed, in the restored areas, we analyze vegetation variations in the vegetation structure and composition. In addition, we monitor the (re)colonization process of *M. scabrinodis* and other ant species to evaluate whether the characteristics of the new habitat influences the presence/absence of *Myrmica* and other ant species. We investigate which factors determine the success of colonization, such as structure of vegetation and composition in the plots.

Given the results in the past year, we expect that the project will show success as a result of the management actions done in 2013. Analysis of the vegetation in 2016 will show, what factors and parameters determine variations in vegetation structure and composition in three treatments (sods, c-controls and o-controls) in source and restored areas (hypothesis 1). Using vegetation data from 2014 and 2016, it can be assessed whether the experiment generated benefits for the restoration of the ecosystem. We expect several changes in vegetation structure and composition during this short-term experiment and find out which environmental variables define more mature plots developing towards wetland conditions (hypothesis 2). On the other hand, ant occurrence will vary during three years of the project due to vegetation variations: in the restored areas, more vegetation coverage and height in the plots will determine faster colonization of *M. scabrinodis* than on plots with high percentage of bare soil and short vegetation, as preferred by pioneer ants such as *Lasius niger* (hypothesis 3).

3. Materials and Methods

3.1 Study Area

The study area, Natura 2000 nature reserve "Vlijmens Ven, Moerputten and Bossche Broek" (932 hectares) is located in the southwest of the city of 's-Hertogenbosch (The Netherlands). The reserve Moerputten (115 ha) (51°41'N, 5°15'E, 2 m.a.s.l.) still hosts most of the biodiversity typical for traditional hay making meadows and wet forests while the surroundings used to be dominated by modern agriculture (Fig. 3). It is famous for its moist meadows with vegetation of the alliance *Junco-Molinion,* association *Cirsio dissecti-Molinietum* and other vegetation types (Wynhoff, 1998a). In the past, it was part of the floodplain of the rivers Maas, Dommel, and Dieze. A loamy sand layer, locally covered by peat or sand deposits from past floods characterize the soil composition in the study area (Wynhoff, 2001). The climate in this region is Atlantic with wet conditions (737 mm annual precipitation) and warm temperature (10ºC mean annual temperature) (Volkel Meteorological Station, KNMI 2016).

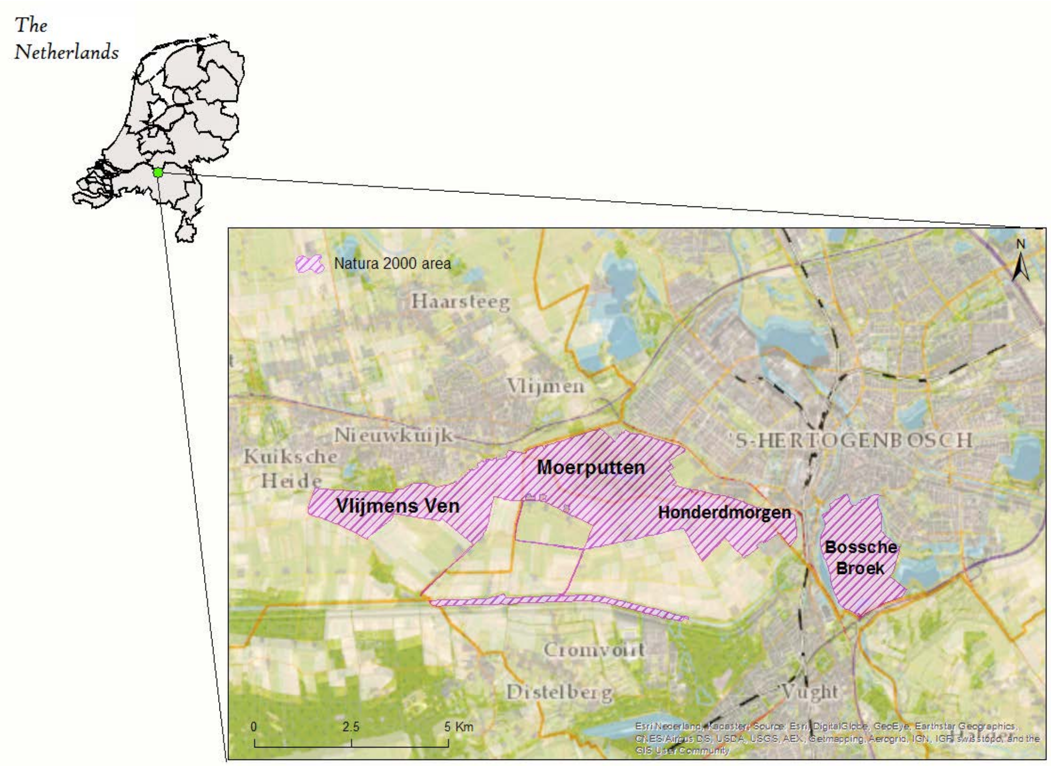

Figure 3. Natura 2000 site shown in purple, Vlijmens Ven, Moerputten, Honderdmorgen and Bossche Broek.

In this Natura 2000 area, the LIFE+ Project "Blues in the Marshes" (LIFE+ 11 NAT/NL/000770/ Action C1, C6 and D1) has been carried out to restore the agricultural areas surrounding Moerputten to a wetland habitat. In addition, a water retention area has been constructed in parts of Natura2000 to avoid flooding of the city of s'-Hertogenbosch.

Several Dutch organizations participate in this project, Staatsbosbeheer, Natuurmonumenten, De Vlinderstichting, waterboard Aa and Maas and the municipality of Heusden. The main purpose of the LIFE project is the extension of the habitat of the butterfly *Phengaris teleius*, which occurs with just one population in moist meadows of Moerputten after a reintroduction performed in 1990 (Van Langevelde & Wynhoff, 2009; Wynhoff, 1998a; Wynhoff, 2001).

3.2 Experiment

The LIFE+ project “Blues in the Marshes” started in 2013, recovering the fen meadows around nature reserve Moerputten as its main objective. Intensive agriculture had been a normal practice during the past 50 years where once part of the fen meadows used to be suitable as the habitat of *P. teleius*.

Several actions were needed to prepare the terrain for the recovery of wetland habitat. Firstly, 250 ha of the Natura 2000 site were excavated, removing the enriched topsoil (40cm) of former cornfields and cattle pastures, decreasing the level of phosphate in the top soil to create nutrient poor conditions. At the same time, a retention area surrounded by dikes was created partly overlapping Natura2000. To prevent acidification after soil removal, 1000 kg per hectare lime was dispersed (Dorland *et al.*, 2004). Establishment and development of the target wetland vegetation were facilitated by spreading of freshly cut clippings in the restored area. Clipping was collected from the mowing of the fen meadows in the nature reserves Moerputten, Langstraat and Bruuk (Donath *et al.*, 2007; Hölzel & Otte, 2003; Matus *et al.*, 2003; Török *et al.*, 2011). The time these managements were done in each location is shown in Table 1.

In October 2013, 54 sods of one square meter each, with the target wetland vegetation, were collected from three different meadows, Hooiland van Bijnen (HvB), Punthooiland Zuid (PHZ) and Westelijk hooiland (WH) (Fig. 4). These sods were taken from the nature reserve Moerputten in order to create suitable habitat islands with appropriate vegetation for *Myrmica scabrinodis*. In October 2013, sods were removed in long narrow strips to generate more edges in the vegetation (Wynhoff, 2013) that is favorable for *M. scabrinodis*. From each strip, nine sods were collected, with exception of two lines at PHZ, where a strip of 18 sods and a strip of three sods were collected.

Sods collected were transplanted to the soil removed areas in Honderdmorgen to generate an appropriate environment for *Myrmica* ant colonization. They were distributed over six different places to investigate the effect of sod translocations: Honderdmorgensedijk Driehoek (HMD) Honderdmorgen Middenperceel (HOM), Tegenover Compensatiegebied1 (TCG1), Tegenover Compensatiegebied2 (TCG2), Compensatiegebied1 (CG1), Compensatiegebied2 (CG2) (Fig. 4).

Table 1. Years of different managements applied in the area.

Location	Year of excavation	Manipulation
HonderdmorgensedijkDriehoekje, HMD	2011	Liming in fall 2011 Hay transplantation in 2011, 2012
HonderdmorgenMiddenperceel, HOM	2013	Liming in 2013 Hay transplantation in 2013
Compensatiegebied 1, CG1	2007	No liming Hay transplantation in 2010
Compensatiegebied2, CG2	2007	Liming part of the area and hay translocation in 2011
Tegenover Compensatiegebied 1, TCG1	2013	Liming and hay transplantation in 2013
Tegenover Compensatiegebied 2, TCG2	2013	Liming and hay transplantation in 2013

In the source area Moerputten, on Hooiland van Bijnen (HvB) 35 sods were removed from 4 strips, on Punthooiland Zuid (PHZ) 20 sods from 2 strips and on Westelijk Hooiland (WH) 8 sods from 1 strip (See figure 4). Relevé's were located in three different categories: on the bare soil, along with the edge and in the climax vegetation around (Fig. 3). In all of these relevés, we measured the vegetation structure and composition. Also, ant baits were placed for 24 hours to determine the presence of *M. scabrinodis* and other ant species.

In the restoration area, the sods were transplanted in a specific position creating one monitoring patch. Nine sods of climax vegetation were located in a 3 x 3 grid separating each sod from others by 3-meter distance to avoid interferences between sods (Wynhoff, 2013). Four control plots are placed between sods with a distance of 1.5 meter and eight controls around the sods with a distance of 3 meters being the inside-controls (codified c-control). The control plots between the sods are only used for vegetation analyses.

A second series of eight control plots was positioned in 2015 randomly of at least ten meters distance from the grid (codified o-controls) to better test the colonization process (See Fig. 6). Figure 6 shows where the ant baits were placed by black dots in the middle of the plots: in the sods, c-control and in the o-controls. Ant baits were not set in the controls between sods because the distance is reduced to 1,5 meters, which does not prevent interference of ants.

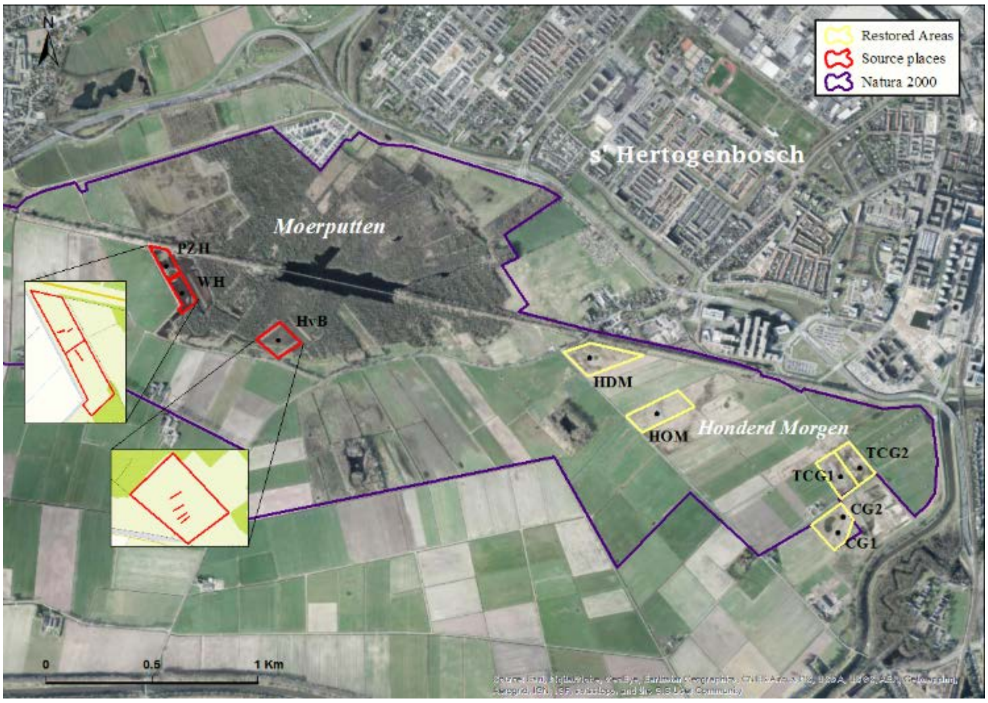

Figure 4. Locations where the sods were collected (orange) and installed (yellow). At the left side, Moerputten nature reserve with three meadows, Hooiland van Bijnen (HvB), Punthooiland Zuid (PHZ) and Westelijk hooiland (WH). At the right side the restoration area.

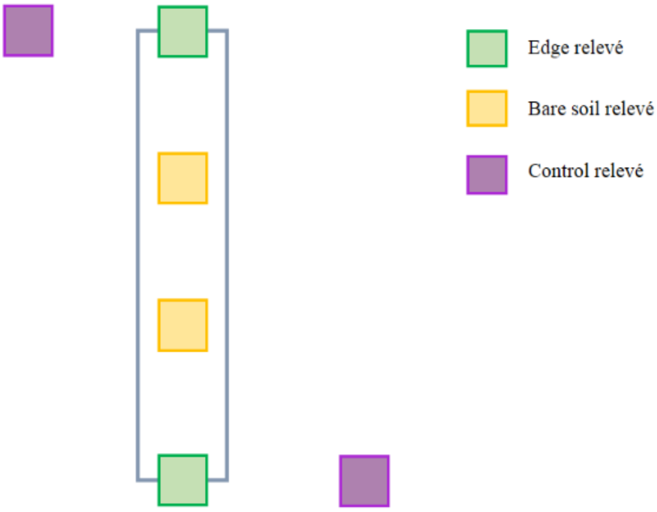

Figure 5. Scheme of the relevés position in the sod cut strips. Two relevés are located in the edge, two in the bare ground and two in the climax vegetation at a distance of 3m from the sod cut strip.

3.3 Data Collection

We studied vegetation composition and structure and the (re) colonization by *M. scabrinodis* ants both in the source area and the target area. Special attention was paid to the colonization of *S. officinalis*, the host plant of *P. teleius* and whether the vegetation composition moves towards wetland vegetation types.

Firstly, we determined the vegetation composition with the method of Braun-Blanquet measuring the abundance of all plant species in one square meter (Meijden & Bruinsma, 2007). We measured the total vegetation cover, moss cover, tree cover, shrub cover, herb cover, DOM cover (dead organic matter) and bare soil cover in each relevé. The vegetation height was measured by the Barkman stick method. This method consists of a Styrofoam disk of 10 cm diameter placed around a stick, which is placed inside the vegetation relevé. The disk is dropped on the vegetation to measure the height on the scale of the stick. The Styrofoam disk is pretty light, therefore it does not press the vegetation. On each relevé, the height of the vegetation was measured at five random spots and averaged. Using the program Turboveg we could calculate the weighted average Ellenberg values of nitrogen, moisture, and pH for each relevé (Hennekens & Schaminée, 2001).

The colonization by *M. scabrinodis* and other ant species was investigated capturing ants with fruit wine baits. Ant baits were placed in the plot and stayed there for 24 hours only in order to prevent damage to the ant population. Plastic tubes (15 ml, Ø 1.7 cm, 12 cm long) filled with 8 ml of fruit wine were positioned in the soil in the middle of the sods. The upper part of the tube is situated equally to the ground surface.

The alcohol helps to spread the smell and attracts ants to the bait. The fruit wine baits are collected after 24 hours of their positioning to be sure that all periods of the ant's daily activity are covered. All insects collected are washed and ants are separated from the other insects. The identification of ants is carried out using Boer (Boer, 2010). The collection of ants was done twice; one collection in the middle of July and another collection at the end of August because in the first capture only a low number of ants was captured.

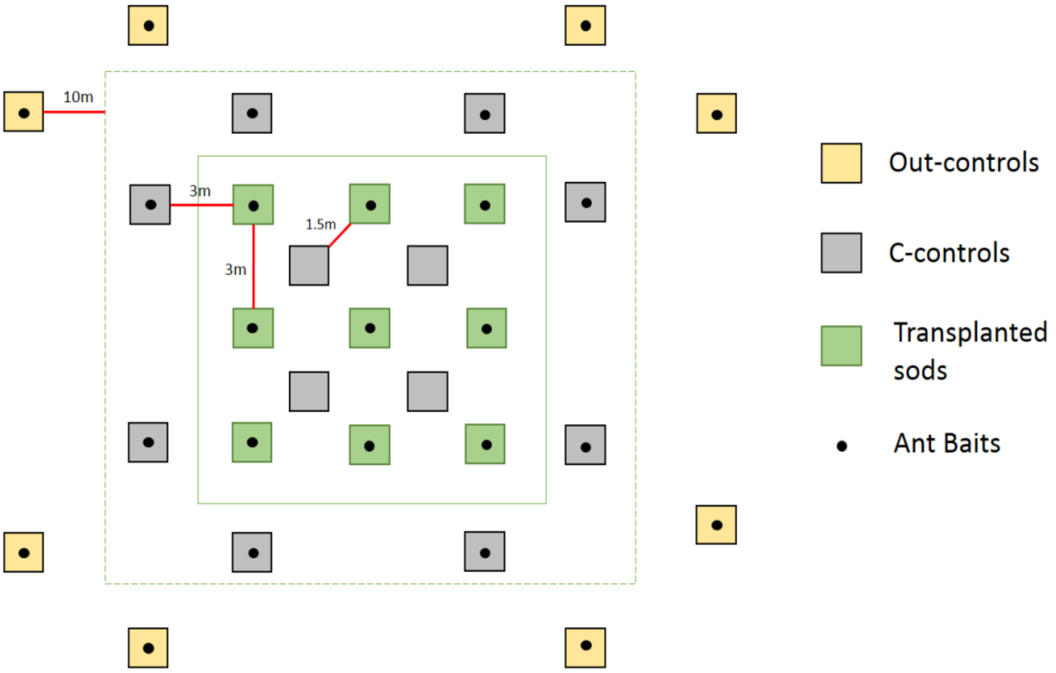

Figure 6. Monitoring patch of the sod transplantation experiment in the soil removed areas, consisting of 29 different plots. Shown in green are the transplanted sods, c-control in grey and out-control in yellow. In the plot with a black dot inside an ant bait was placed.

3.4 *Statistic Analysis*

The section dealing with the statistical analysis is divided into three parts. The first one deals with the vegetation analysis in 2016, where the ecological situation generated in the area for the recuperation of the *Cirsio-Molinietum* vegetation is checked; the second part analyses the development of the vegetation throughout the years and the third part is focused on the analysis of the occurrence of ants in order to find out which factors determine the presence/absence of ants and their colonization of the area during the years of research.

3.4.1 *Vegetation Analysis*

The vegetation and its relation to environmental variables was studied using a multivariate statistical analysis. I applied a Principal Component Analysis (PCA) using the software R (Core Team, 2008). This analysis organizes plots and species in a multidimensional space based on their similarities with the function *rda()* provided by package Vegan (Oksanen *et al.*, 2015). With the abundances of species in each plot, the analysis generates a scatterplot in which each relevé is projected along the axes of the PCA. The output of the PCA shows the eigenvalues, which explains the variation by each axis in the plot organization. The first axis shows the most variation explained, followed by the second axis. Usually with two axes most of the variation based on the environmental variables and similarities of species in the plots can be explained. The environmental variables included are the total cover of vegetation, shrub cover, herb cover, moss cover, DOM cover (dead organic material), bare soil cover, the number of *Sanguisorba officinalis,* average vegetation height and Ellenberg values (nitrogen, pH, and moisture). These environmental factors have been included afterwards in the analysis with the function *enfvit()* of the package Vegan. Environmental factors are shown as a vector with arrows and their direction explains the increase of the environmental gradient in the surrounding plots (Kent, 2011), while the length shows the correlation presented with the axis. Important environmental factors were defined using Pearson correlation tests between the score of PCA1 and all environmental factors.

A linear model is calculated between the PCA1 score and the environmental variables with the function *lm()* in R, to analyse which variables and in which proportion explain the variation in the data. First of all, correlations between environmental variables were checked before formulating the model. Two PCAs were done for only the 2016 data: the first one for the source areas´ plots to show the differences with respect to the vegetation composition between the three meadows. The second ordination was performed to analyse the differences between sods and control plots (c-controls and o-controls) of the six restoration areas, where some differences of vegetation composition can be distinguished.

Another multivariate analysis was performed with the data of plant species composition and environmental factors in each relevé (source and restored areas) collected in 2014 and 2016. The objective, in this case, is to visualize and test how each relevé changed in vegetation composition and structure during two years after the sod translocation. Two different Detrended Correspondence Analyses (DCA) were performed: one for the source areas and another for the restauration areas. The basis of this analysis is the same as PCA, however now the command used in R is *decorana()* from the package Vegan. Species similarities between relevés are used to project them in the space and visualize distinctions between 2014 and 2016. Several environmental factors , i.e. total cover of vegetation (within restored areas), shrub cover, herb cover, moss cover, DOM cover (dead organic matter), bare soil cover, vegetation height and Ellenberg values for nitrogen, pH and moisture were included afterwards in the DCA analysis with the function *enfvit*() of the package Vegan. Another linear model was created for the first DCA axis and several environmental factors.

A t-test analysis was performed to detect differences between the DCA1 results in the two different years (2014 and 2016), both variables present normal distribution.

3.4.2 Ant Analysis

The presence of ants is explained by diverse factors related to the vegetation characteristics (Wynhoff *et al.*, 2016). Which specific factors of the vegetation determine the ants´ occurrence throughout three consecutive years of data (from 2014 till 2016) is analyzed using Generalized linear mixed models (GLMM). All the analyses are done for the occurrence of *M. scabrinodis,* all *Myrmica* species lumped together (our target group of ants)*, L. niger* and all ants together.

First, we tested whether the presence/absence of ants is determined by the variables treatment and year using GLMM with a binomial distribution and logit link function, where *M. scabrinodis, Myrmica species, L. niger* and all ant species are the dependent variable (one GLMM analysis was done for each). As independent variables, we included the treatment with three different categories, 54 sods and 48 c-controls (not included plots inside sods) for the three years (2014, 2015, 2016) and 48 o-controls for the last two years (2015, 2016). Patch ID of the plots was included as random factor. We included the year of excavation as independent variable, as it might determine the current vegetation structure. In all GLMMs, differences between the treatments and years were tested with a post hoc sequential Sidak test (Sokal & Rohlf, 1969).

Finally, it was tested which independent variables are related to the presence of ants using another general linear mixed model analysis. Four different models of GLMMs are produced, one for each group of ants as dependent variable. Every independent variable was included one-by-one generating different models. The best variables that explained the ants presence were selected based on the lowest Akaike´s Information Criterion (AIC). Binomial error distribution with logit link function was used for generating GLMMs. Repeated covariance type ARMA 11 (Autoregressive moving average 11) and the Variance Components covariance structure were used to calculate the models.

In addition, we tested if the presence of opposite ants influence the results: for the *M. scabrinodis* model we included the presence of its competitor *Lasius niger* as independent variable and, in the opposite way, *M. scabrinodis* was included as independent variable in the model of *L. niger*. Then, all environmental variables that describe the vegetation structure are incorporated (Total vegetation cover, herb cover, shrub cover, moss cover and DOM, mean vegetation height and standard deviation of the height). Finally, all Ellenberg values were tested for the correlation with the ant presence and the DCA axis 1 and 2 that explain differences in the vegetation composition of the plots. All GLMM analyses were produced with IBM SPSS Statistic program version 23.

4. Results

4.1 Vegetation Analysis in 2016

Two different results are produced by the Principal Component Analysis (PCA) for the two separated areas of the study area, investigating the vegetation composition and structure during the collected data of 2016.

4.1.2 PCA Source areas

The ordination in the PCA of the source's relevés in space is based on the species and their abundance, where each relevé has a PCA value determined by the species similarity (See fig. 7). The environmental variables included afterwards show the environmental preferences of each plot. The first axis of PCA divides PHZ meadow from WH meadow, while the second axis of PCA divides, in turn, HvB meadow from the other two source meadows. The eigenvalue of PCA1 explains 23.95% and the PCA2 15.86% of the variation in the ordination, with both axes together, the multivariate ordination analysis represent 39.81% of the total variation in the source relevées.

All the plots from the same meadow are close to each other in the ordination when environmental conditions and species composition are similar. Focussing more on the different meadows, WH5 meadow is characterized by the large cover of moss in its plots. PHZ plots have high values of Ellenberg nitrogen, vegetation height, total vegetation cover and a large number of *S. officinalis* (Sang). The relevés of HvB meadow in the left part of the graph, have a high Ellenberg moisture value. In less proportion, HvB relevé in the right part are influenced by DOM (Death Organic Matter) and bare soil cover. Some environmental factors were highly correlated with the first PCA axis: total cover explains the axis PCA1 for 47% (Spearman Correlation, rho= 0.4737, p-value =2.3 e^{-03}), value of Ellenberg moisture is correlated with PCA1 axis for 54% (Spearman Correlation, rho= -0.5433, p-value =3.5 e^{-03}), bare soil can explain the PCA1 axis for 53% (Spearman Correlation, rho= -0.5319, p-value =4.9 e^{-04}) and herb cover can also explain 57% of the PCA1 axis (Spearman Correlation, rho= 0.5759, p-value =1.2 e^{-04}).

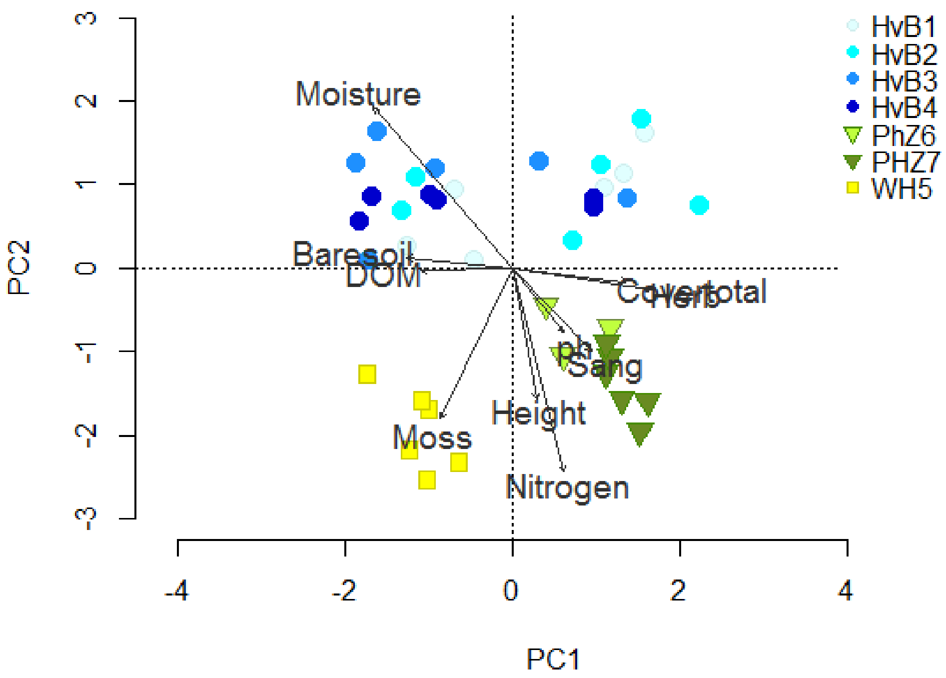

Figure 7. PCA analysis of the source relevé. The ordination shows the difference between source areas along two axes. There are three colours and different figures to distinguish the meadows: green triangles for Punthooiland Zuid (PHZ), yellow squares for Westelijk Hooiland (WH5) and blue circles for Hooiland van Bijnen (HvB). The arrows represent the gradient of different environmental factors.

One model was performed to test the influence of each variable in the plot variation. The combination of factors: Ellenberg nitrogen and moisture value, total vegetation cover and moss cover have a significant influence on the source plots (Linear model PCA1= 4.871 + 0.065·Nitrogen – 0.892·Moisture – 0.030·Moss + 0.023·Total cover, R^2= 0.47, p-value < 0.00). This model can explain 47% of the variance in the first axis of PCA.

4.1.2 PCA Restored areas

The second ordination analysis was performed for the six patches on the meadows in the restored areas. In the graph given in figure 8, only the sods are shown but not the control plots. All sods are found in the left part of the graph and all of them cluster in a cloud, showing a great similarity of species between them. The environmental variables that influence the ordination are the high number of *S. officinalis* and values of Ellenberg moisture in the upper left part, the majority for the meadows CG, HMD, and HOM. High values of vegetation height, total cover and herb cover appear in the bottom left part mainly for TCG meadow; more vegetation cover explain a higher level of maturation (Zedler, 2000). Meanwhile, shrub cover, bare soil cover and moss cover are in the opposite part of the graph, showing low values of these factors for the translocated sods.

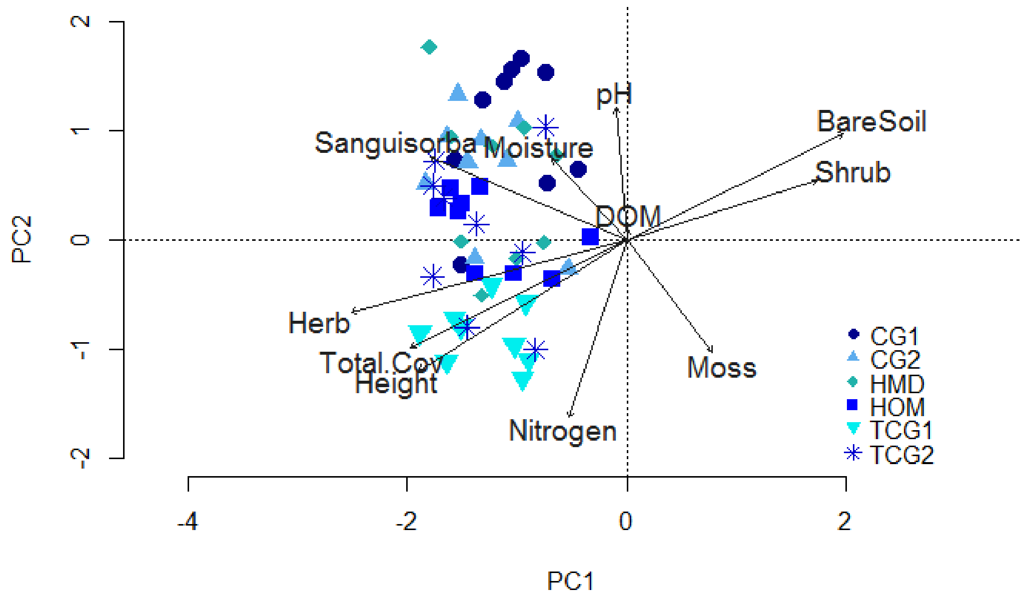

Figure 8. PCA ordination analysis of sods in the restored areas. Different figures are shown for each area with diverse tone color of blue. Environmental variables are represented with arrows and they increase from the origin.

The result of the Principal Component Analysis of the transplanted sods and controls in the restauration area is shown in figure 9. Transplanted sods are represented in orange in the left part of the graph around the PCA axis1, determined by variables such as vegetation height, total vegetation cover, herb cover, the value of Ellenberg moisture and number of *Sanguisorba*. Two kinds of control plots are included in this study. Blue figures are the c-controls and green figures correspond with the out-controls (plots separated more than 10 meters from the sods). The pH value, shrub cover, moss cover and bare soil cover influence the ordination of the c-control plots. Controls are more scattered and separated from the sods in the graph, they present high values of Ellenberg nitrogen, bare soil cover, moss cover and shrub cover. Plots of the area TCG1 (upside down triangles) are placed further from the rest of the plots, being less similar. Two different axes ordinate the plots in the graph, where PCA axis 1 present an eigenvalue of 23.72% and PCA axis 2 12.43% (in total 36.15% of the explained variation with these two axes).

The result of the Spearman correlation shows which environmental factors define the vegetation composition in the sods. Some variables were intensely correlated with the PCA1 axis, such as bare soil for 73% (Spearman Correlation, rho= 0.72, p-value < 0.00), total cover for 71% (Spearman Correlation, rho= -0.71, p-value < 0.00), herb cover for 78% (Spearman Correlation, rho= -0.78, p-value < 0.00) and height cover for 65% (Spearman Correlation, rho= -0.65, p-value < 0.00). A linear model was performed to check the variation explained by specific environmental variables more in depth. The best model produced included five environmental factors such as the cover of herbs and shrubs, values of Ellenberg moisture and nitrogen and the number of *Sanguisorba officinalis* that explain 72% of the explained variation in the sod´s vegetation composition (Linear model PCA1= 2.941 – 0.009·Sanguisorba – 0.132·Nitrogen – 0.193·Moisture – 0.017·Herb + 0.025·Shrub, R^2= 0.72, p-value < 0.00).

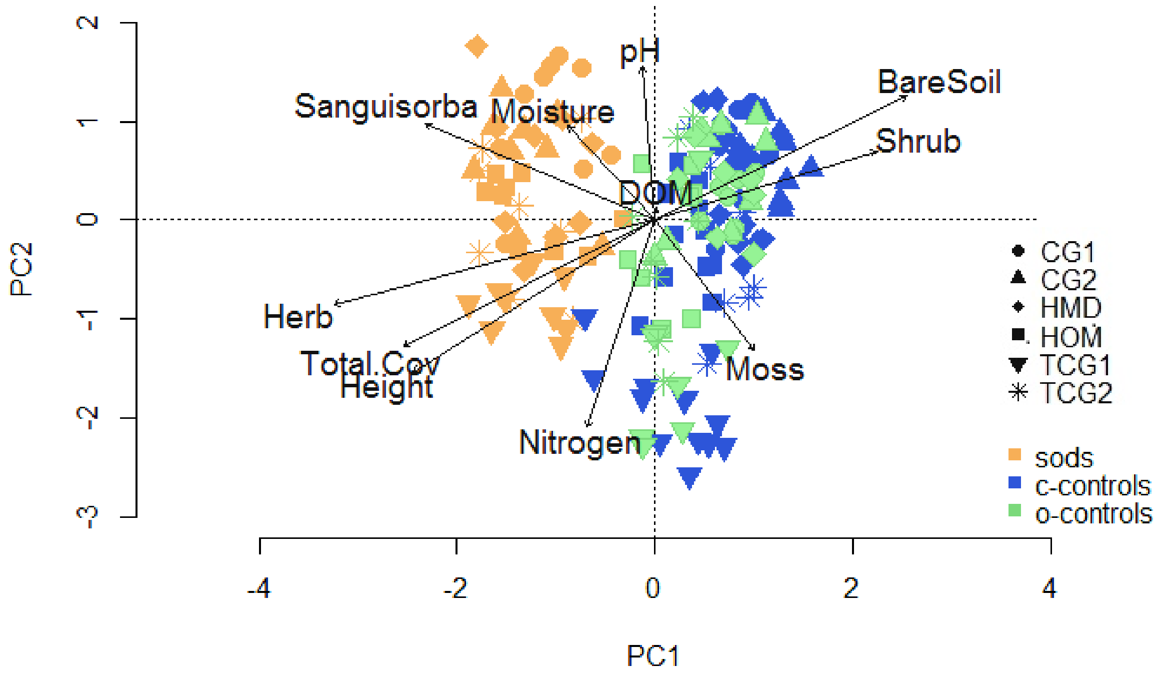

Figure 9. PCA ordination analysis of all relevés in the restored areas. Different figures are shown for each area explained in the legend. Orange figures represent the transplanted sods, blue figures are the c-controls and green figures are the out-controls. Environmental variables are represented with arrows and they increase from the origin.

4.2 Vegetation Comparison of 2014-2016

In this section, results from the vegetation comparison between both years of the study are given. Two DCAs (Detrended Correspondence Analysis) were performed to show the differences between the vegetation structure and composition in 2014 and 2016. In the source area, the effect of removing the first layer of vegetation in strips and its development over time is investigated, while in the restored area, different factors are analyzed to visualize the ecological succession (Refers always to secondary ecological succession). Environmental factors, from both areas, are shown in tables and in graphs to point out the changes produced in two years of restoration.

4.2.1 Conditions in Source area

Environmental factors in source area changed between two years; herb cover and height of vegetation increased in 2016, presenting signification (Table 2; Fig.10). Moss and bare soil cover decreased in 2016, last one with significant differences (Fig.10). Ellenberg values kept constant over time with small rises in moisture and nitrogen; pH moved towards more neutral-basic values (See appendix II).

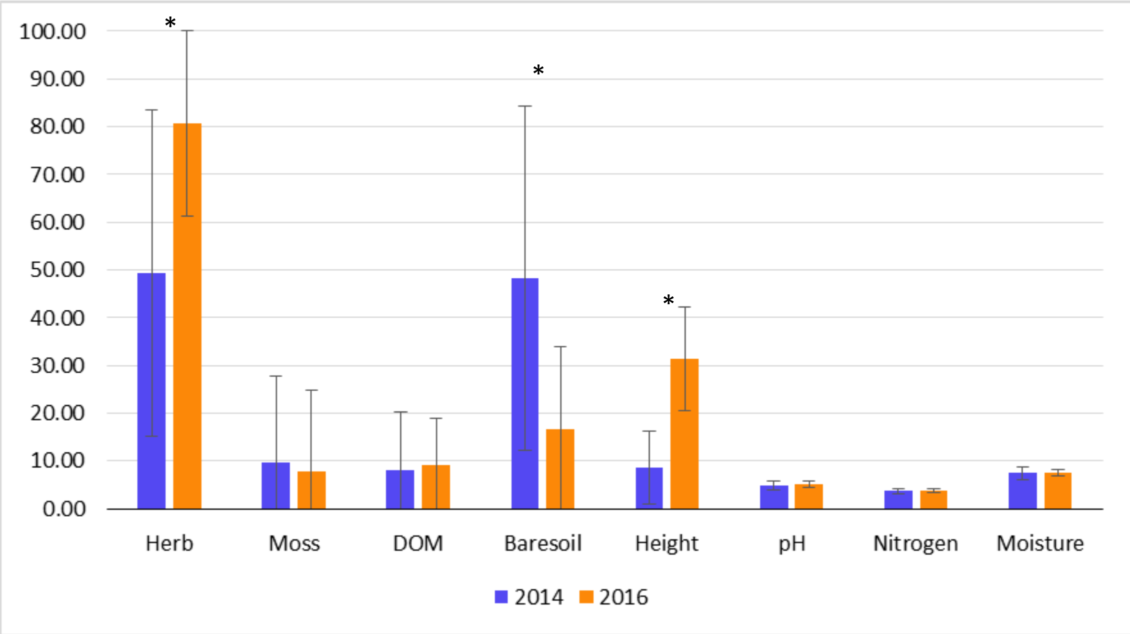

Figure 10. Graph of environmental factors average in source areas during 2014 and 2016.Stars indicate significant differences with $p<0.05$ in paired t-test. Environmental factors described in appendix I.

4.2.2 DCA Source areas

In figure 11, the development of the vegetation on the c-controls (eight plots, C1 until C8, see appendices) and sods in the source area during 2014 and 2016 is given. Two different clouds of points can be distinguished; the right-upper part (blue) represents the ordination for the relevés found in 2014 in the source area and in the left-bottom part (orange) the data collected in the same plots in 2016 are given. This ordination shows a clear difference between both years with respect to species composition and environmental factors having changed during two years. Figure 11 represents the organization of the plots, separated by year, and highlights the environmental factors that characterize them. DOM, values of Ellenberg nitrogen and, to a lesser extent, bare soil cover are the conditions that made out the relevé in 2014 (blue part). Meanwhile, for plots in 2016, the vegetation height, pH, the number of *S. officinalis* and shrub cover determined these relevés. Figure 11 represents the same DCA than figure 12, but in the latter case, different symbols are included to distinguish the source meadows. All meadows moved towards left-bottom part with different distances; for example, PHZ7 (diamond) did not move much compared to WH5 (circles), throughout the DCA ordination.

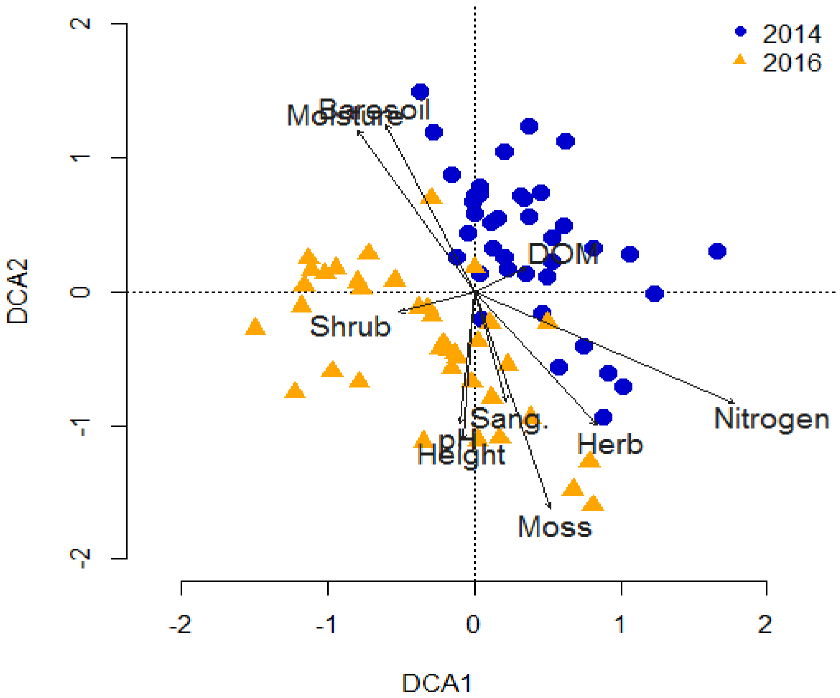

Figure 11. Result of DCA for source areas in 2014 and 2016. It shows the relevés of each year (blue 2014, orange 2016) together with environmental factors. DCA axes for source areas explain 58.56% of the variance for the plots in both years (DCA1 Eigenvalue= 0.2977 and DCA2 Eigenvalue= 0.2879).

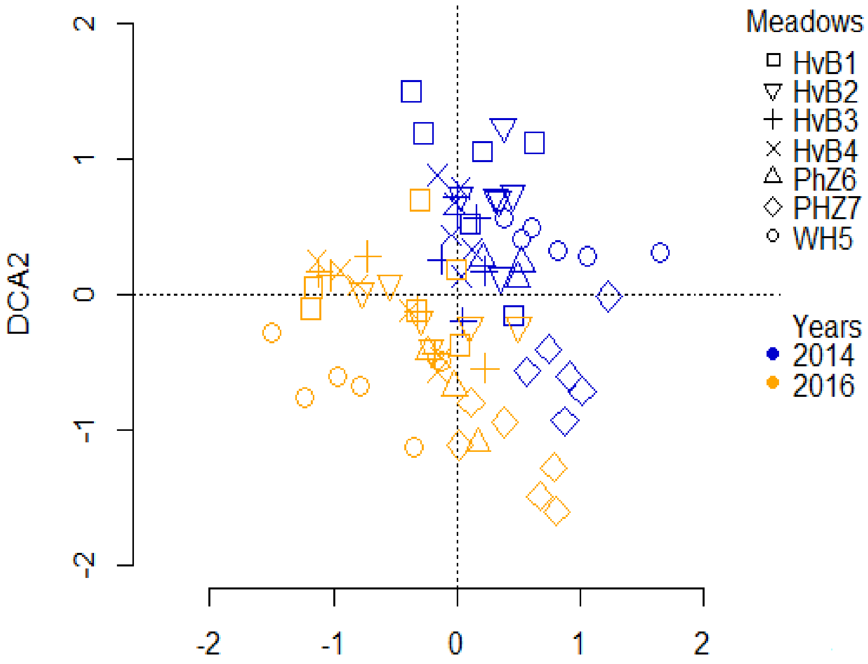

Figure 12. Result of DCA for source areas in 2014 and 2016. Graph displays a DCA ordination with different visualization than figure 11; diverse symbols, presented in the legend, differentiate seven source meadows in two different years. Meadow abbreviations show in table 1.

The results of a Spearman correlation show the relation between the DCA1 axis and each investigated environmental variable. Bare soil is negatively related with DCA1 for 63% (Spearman Correlation, rho= -0.6354, p-value =4.11 e^{-10}); herb cover can explain a variation of 66% for the DCA1 in source areas in both years (Spearman Correlation, rho= -0.6650, p-value =3.12 e^{-11}), vegetation height and DCA1 are related for 66% (Spearman Correlation, rho= -0.6649, p-value =3.15 e^{-11}) and the Ellenberg moisture value presents 35% of DCA1 ´s coordinates (Spearman Correlation, rho= -0.3581, p-value =1.28 e^{-03}).

The outcome of the t-test shows a significant difference between the DCA 1 axis in 2014 and in 2016 (t = -8.0567, df = 38, p-value = 9.6e^{-10}), explaining that the species compositions (which the ordination was done) are significantly different after two years in the source areas.

4.2.3 Conditions in Restored areas

The composition of the species on sods and controls in the restoration area changed from 2014 to 2016. A total of 100 species are in the 2014 data, while 151 species were found in 2016, making a difference of 51 new species that appeared in the restored areas. There are several differences in environmental factors in the restored areas; total cover on the sods increased slightly in 2016, herb cover and height of vegetation increased more, both in sods and c-controls (Fig 13). Moss and bare soil cover decreased in 2016, being a significant difference compared to 2014. Shrub cover increased over time and more intensely in c-

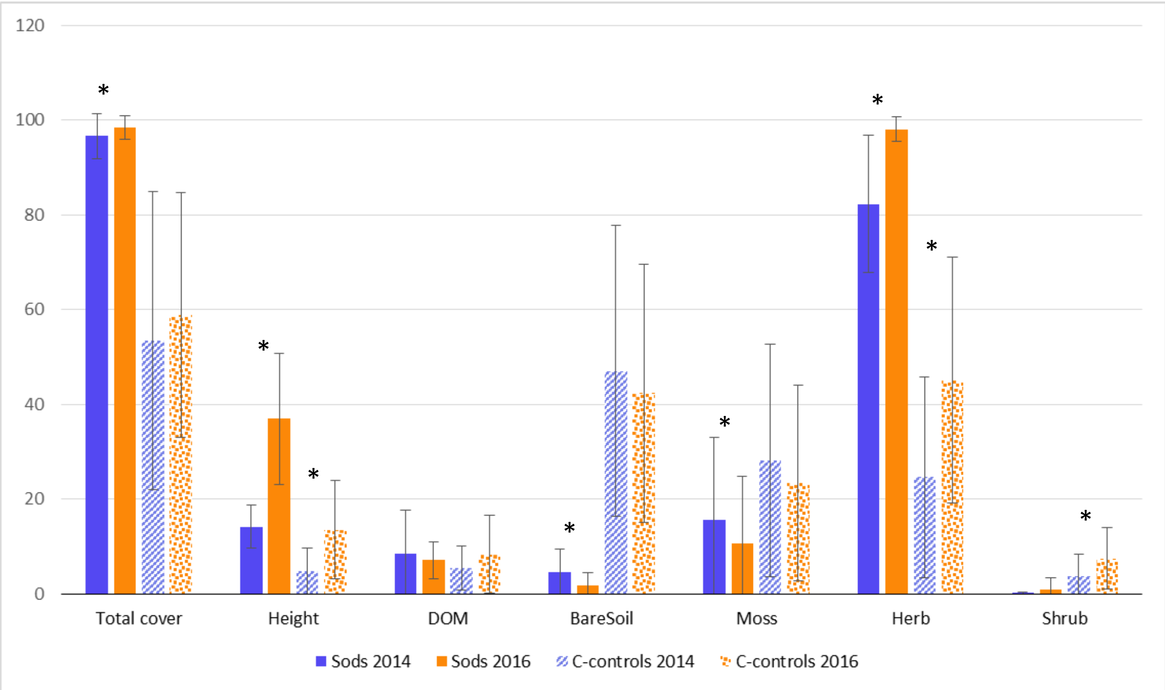

Figure 13. Average values of environmental factors in sods and controls in 2014 and 2016 on restored areas. Stars indicate significant differences with p<0.05 in paired t-test.

controls. Ellenberg values kept constant over time (Fig. 14). For more details see appendices I.

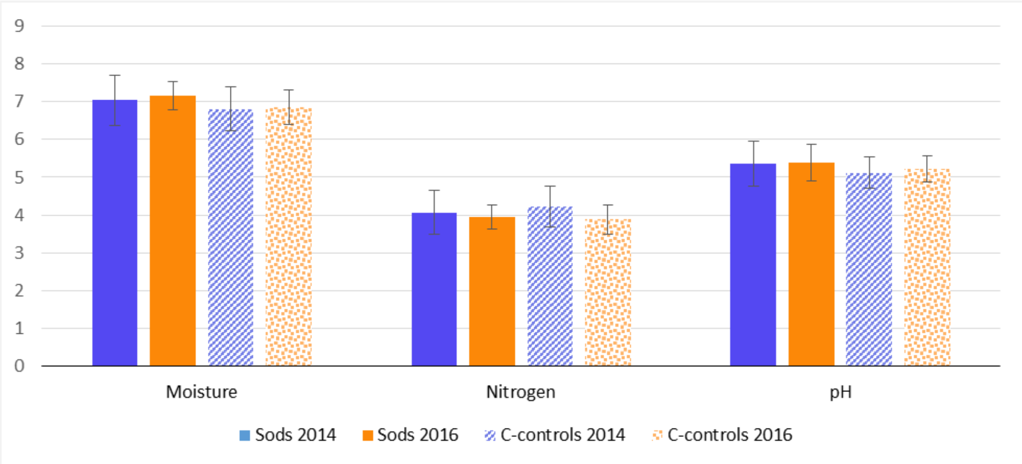

Figure 14. Average values of Ellenberg values in sods and controls in 2014 and 2016 on restored areas.

4.2.4 DCA Restored areas

The DCA analysis of the vegetation in 2014 and 2016 in the restored areas is displayed in figure 15. Blue circles represent the relevés of 2014 and orange triangles of 2016. There are two areas divided by the first axis of DCA, highlighted with circles: on the left side, the sods are displayed (yellow circle), influenced by herb cover, vegetation height, Ellenberg moisture value and total vegetation cover. On the right side of the graph, the c-controls (purple circle) appear more scattered due to the lack of similarity between plots. The translocation of the sods has not yet resulted in great changes in their vegetation composition from 2014 to 2016.

Control plots are influenced by different environmental factors such as the cover of moss, shrub and bare soil. At the same time, the second axis of DCA slightly separates the plots between the different years, 2014 plots appear in the upper part while 2016 plots are displayed in the bottom part. The vegetation development of two years results in a movement towards the bottom part of the ordination, increasing in pH value, nitrogen, and more moisture.

In the DCA analysis, Eigenvalues have been calculated for the different axes: the first axis of DCA can explain 46% of the variance and the second axis 24%. Together the first two axes explained 70.7% of the total variance in the plots present in the restored areas.

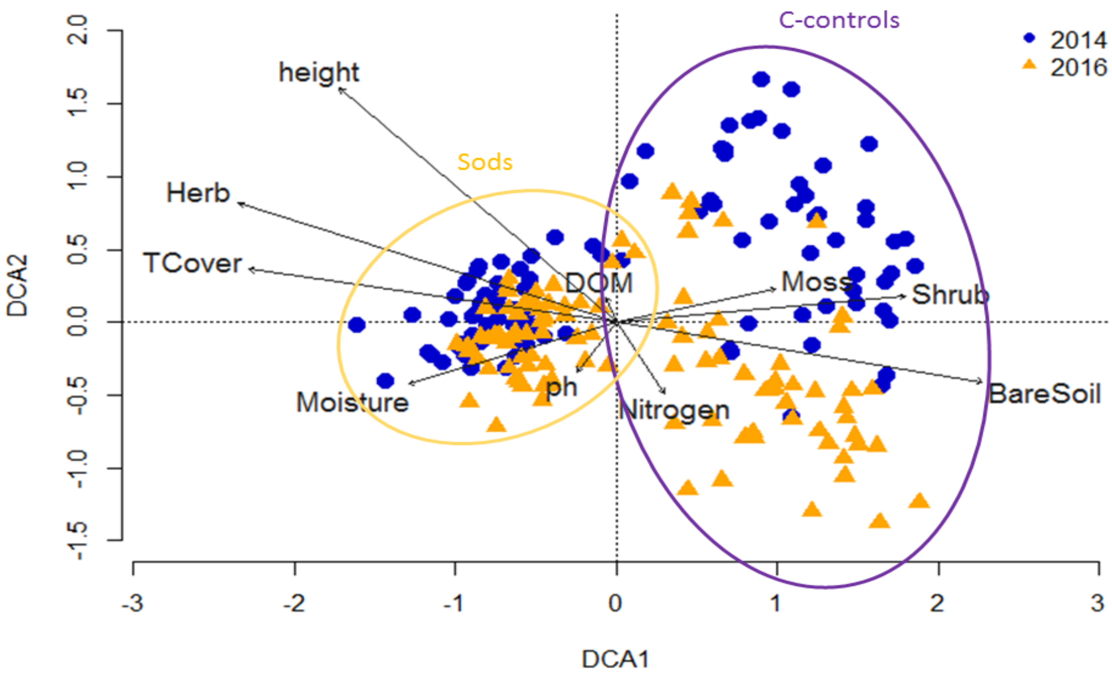

Figure 15. DCA of restored areas comparing 2014 and 2016. Blue circles are plots in 2014 and orange triangles in 2016; yellow circle assembles the sods and purple circle group the c-control plots in both years. Environmental factors are represented with arrows expressing their gradient with the length

Several environmental factors explain more variation in the ordination in the last DCA than others. A Spearman correlation between the DCA1 axis and each environmental factor shows which are the most important ones. Bare soil presents a correlation of 66% with the DCA1 axis (Spearman Correlation, rho= 0.6628, p-value =2.2 e^{-16}); the total vegetation cover correlates negatively with the DCA1 for 67% (Spearman Correlation, rho= -0.6783, p-value =2.2 e^{-16}); herb cover can explain a variation of 74% for the DCA1 in restored areas in both years (Spearman Correlation, rho= -0.7457, p-value =3.12 e^{-11}) and finally, the average vegetation height and DCA1 present a correlation of 60% (Spearman Correlation, rho= -0.6093, p-value =4.31e^{-07}).

A paired t-test was done to show the significant differences. For the DCA1 axis, the variables show no normal distribution or significant differences between years. However, for the analysis of the DCA2 axis, a significant difference between 2014 and 2016 was found (t =10.704, df = 101, p-value = 2.2e-^{16}). Finding significant differences in the DCA2 axis scores is more logical because in the graph of the ordination for the restored area (See figure 15) this axis determines the division between data from 2014 and 2016.

4.3 Ants statistic analysis

4.3.1 Examination of ant community

Over the three years of investigations in this project, eleven ant species were captured but only some species were present every year: *Lasius niger, Myrmica scabrinodis* and *M. gallienii*. In 2014, we found three species in addition to the constant species, *M.rugulosa, M.sabuleti and Lasius umbratus*. For 2015, six species were found, including *M.sabuleti, M. ruginodis, and M. rubra*. Last year, 2016, only five species were encountered, the common species plus *M. rugulosa and M. schencki.* The species richness in the baits changed from 2014 until 2016.

Examining the number of sods occupied by ants for each meadows and for the three consecutive years, several different trends can be observed (See figures 16, 17, 18). During the first two years of the project, the number of ants increased in the restored areas, first in the translocated sods, followed by the c-controls.

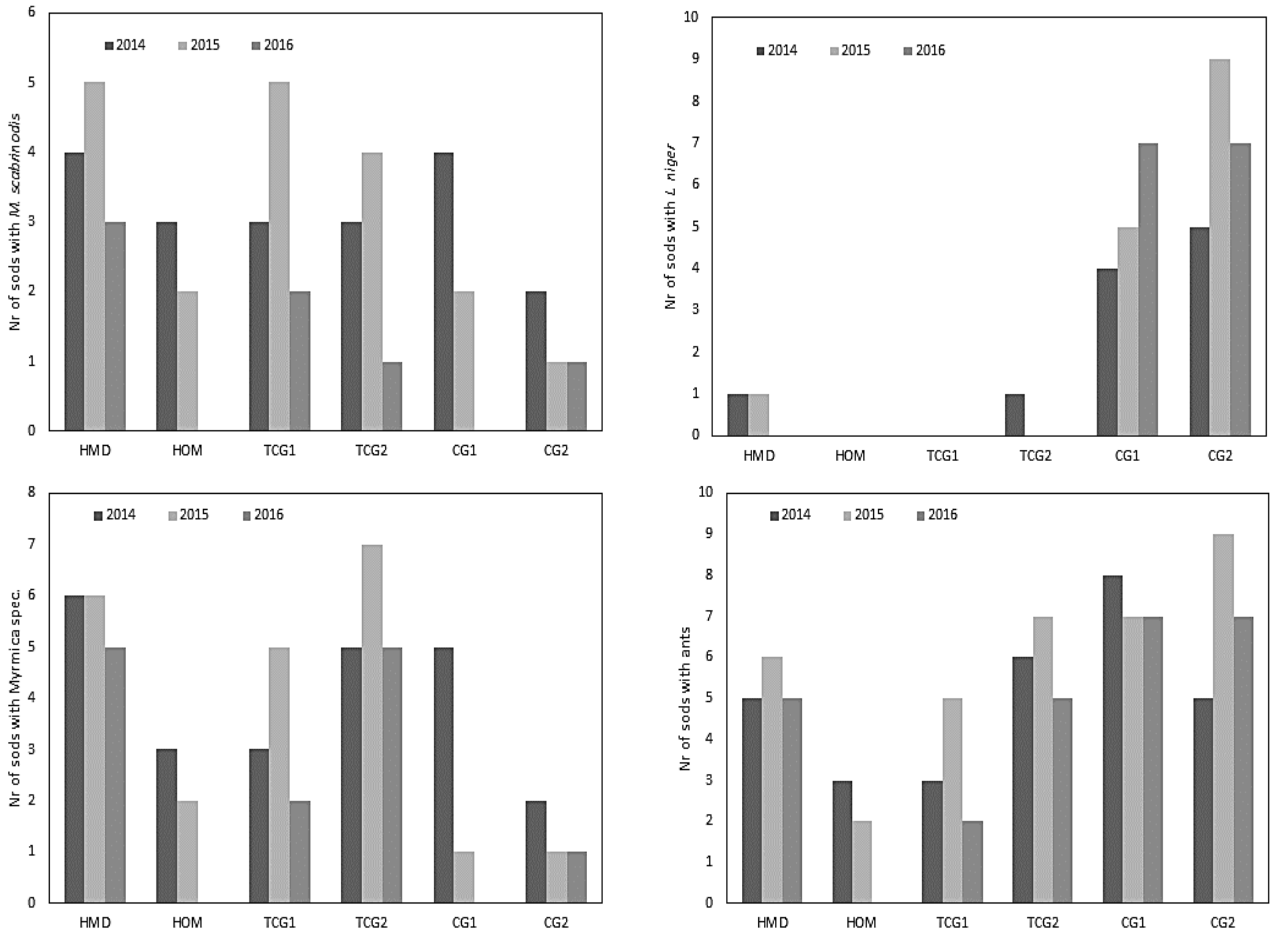

Figure 16. Number of occupied sods by ants in six patches on meadows in the restoration area over three years. The maximum number of occupied sods is nine. Left upper graphs for *Myrmica scabrinodis,* left bottom graph for all *Myrmica* species¸ right upper graph for *Lasius niger* and right bottom graph for all ant species. Abbreviations of meadows show in table 1.

However, the results from 2016 show a decrease of sods being occupied by ants. Sometimes no ants are found at all, such as on HOM meadow. *M. scabrinodis* occurred in the majority of the sods from 2014 to 2015, in HMD, HOM and TCG1 together with other *Myrmica* species, and it reduced its occurrence from 2015 to 2016 in the sods in all patches (Fig. 16). It even disappears on CG1 and HOM meadows. A similar trend is shown for all *Myrmica* species lumped, too. For *L. niger*, meadow CG is its favorite region, increasing its presence throughout the years.

In 2014 in the year after the start of the experiment, the majority of the c-control plots are not occupied by ants, apart from TCG2 and CG meadows (See fig. 17). One year later, in 2015, many c-control plots have ants, but in 2016 a depletion in the number of occupied plots is found, though in this last year, most of the c-control plots still have ants present, with the exception of HOM. *M. scabrinodis* keeps its presence in four meadows but disappears from HOM meadow and never colonized in CG2 (maybe due to the presence of *L. niger*). *L. niger* increases its occurrence in CG1 during 2016 and decreases a little in CG2 in the c-control plots. Examining all species of *Myrmica* and all ants, different trends appear apparently independent from each other, however, there is a general reduction throughout the years with some deviations.

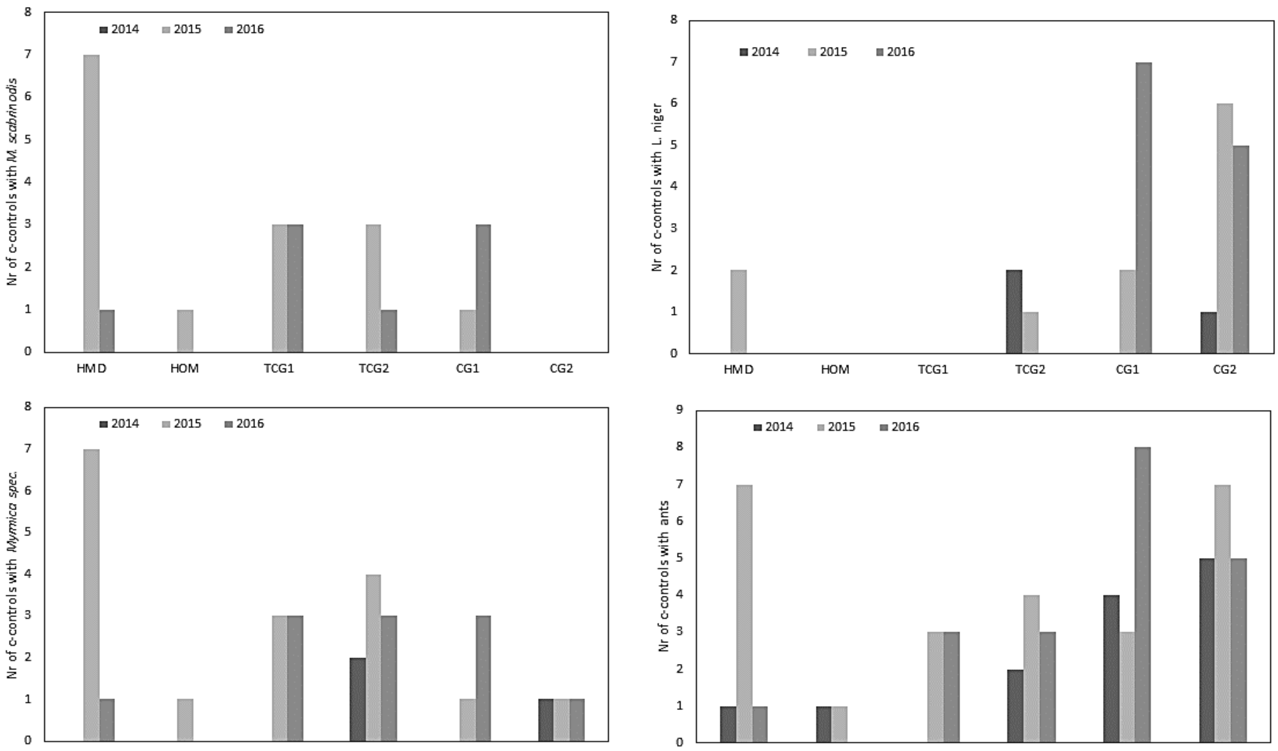

Figure 17. Number of occupied c-controls by ants in six patches on meadows in the restoration area over three years. Left upper graphs for *Myrmica scabrinodis*, left bottom graph for *Myrmica* species¸ right upper graph for *Lasius niger* and right bottom graph for all ant species. Abbreviations of meadows show in table 1.

On the o-control plots, ant colonization from the surrounding areas has taken place from 2015 to 2016 (see figure 18). In all patches, the ant's presence has increased, with the exception of TCG1 meadow where no ant occurrence could be detected during the study period. In the o-controls, *L. niger* is totally restricted to CG meadow. The target ant species*, M. scabrinodis* was found in many meadows being a good signal for the restoration purposes; appearing in four new meadows and increasing in the one colonized before. Other *Myrmica* species were detected in the same meadows too.

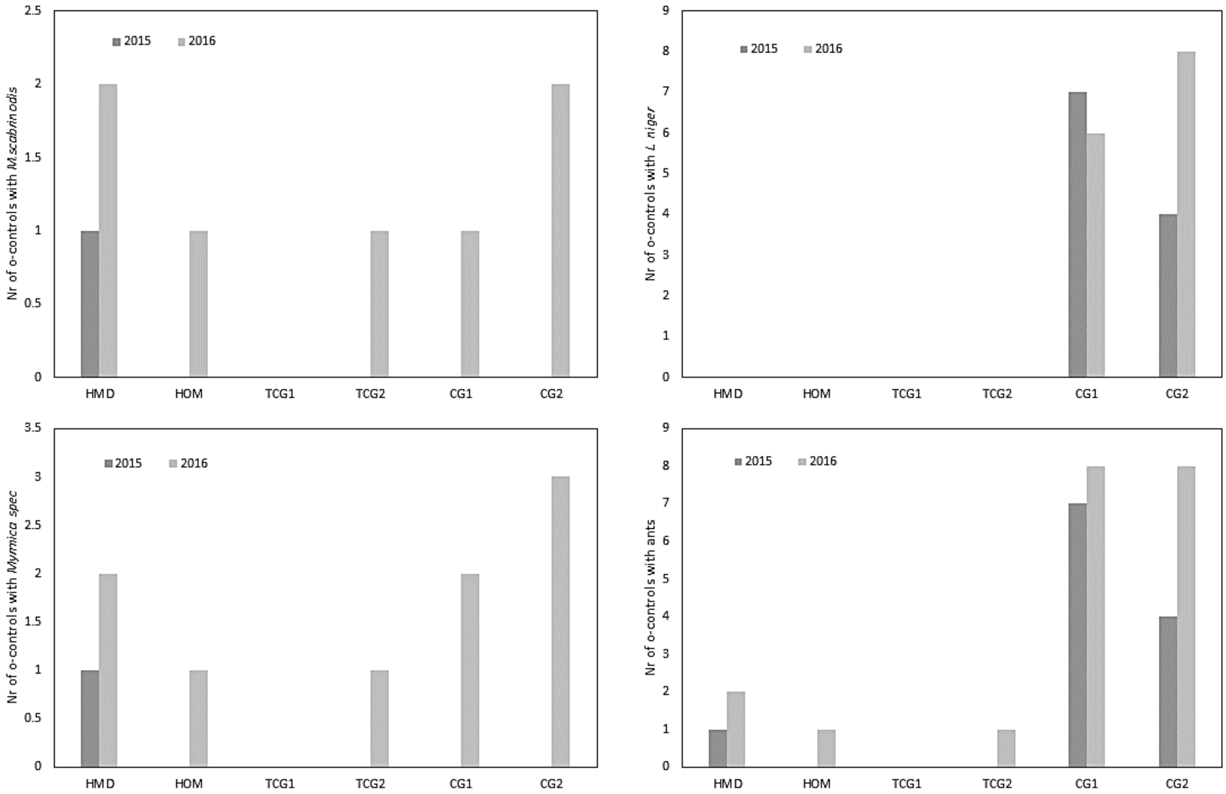

Figure 18. Number of occupied sods by ants in six patches on meadows in the restoration area over three years. Left upper graphs for *Myrmica scabrinodis,* left bottom graph for *Myrmica* species¸ right upper graph for *Lasius niger a*nd right bottom graph for all ants. Meadow abbreviation shown in table 1.

4.3.2 Generalized Linear Mixed Model: Year and Treatment

Environmental factors, which influence the presence of ants, were investigated with the Generalized Linear Mixed Model (GLMM) for each group of ants (*M. scabrinodis, all Myrmica spec, Lasius niger*, all ants). The first analysis was performed to detect the importance of the year, treatment and the interaction between them (see table 4).

Table 2. Results of the four Generalized Linear Mixed Models for each group of ants as dependent variables.

Model	F	df1	df12	p-value
Myrmica scabrinodis				
Year	4.115	7	417	<0.001**
Treatment	1.337	2	417	0.264
Year x Treatment	6.854	2	417	0.001*
Myrmica spec				
Year	4.546	7	417	<0.001**
Treatment	1.95	2	417	0.144
Year x Treatment	9.906	2	417	<0.001**
All ant species				
Year	0.989	2	417	0.373
Treatment	8.346	2	417	<0.001**
Year x Treatment	3.689	3	417	0.012*
Lasius niger				
Year	0.575	2	417	0.563
Treatment	0.759	2	417	0.469
Year x Treatment	0.936	3	417	0.423

Year, treatment and interaction between them were included as independent variables. Values of the table present in the columns: coefficient F (F), degrees of freedom 1 (df1), degrees of freedom 2 (df2) and p-value (* <0.05, ** <0.001).

The two tested variables affect the presence/absence of the groups of ants differently. For *M. scabrinodis* as well as for all *Myrmica species*, a significant effect of the variable year and the interaction between year and treatment was found. Different years produce modifications in the presence/absence of *Myrmica* ants. The GLMM for all ants shows a significant effect of the treatment and for the interaction. Finally, *L. niger* was not affected neither by year nor by treatment as it is a primary colonizer and generalist species with a wide range of environmental niches (Hölldobler & Wilson, 1990). Throughout the years, the presence of all ants (see fig. 19-4) changes based on the modification of environmental conditions in the area (see chapter 4.3.1). In a general overview, in the first year of the investigation after the translocation in 2013, *Myrmica* ants were mainly found in the sods while *L. niger* was found both on sods and controls. After one year, in 2015, the *Myrmica* ants moved towards surrounding c-controls, which are at a short distance to the sods. Lastly, in 2016 many *Myrmica* ants appeared in the o-control plots as well while their occurrence in the sods and the c-controls decrease in comparison to the year before. At least for some groups of ants, the year of the investigation and the treatment are not independent. Depending on the treatment, or the starting conditions of the plot, the vegetation composition and structure change during the years, producing changes in the meadows as habitat of the ants.

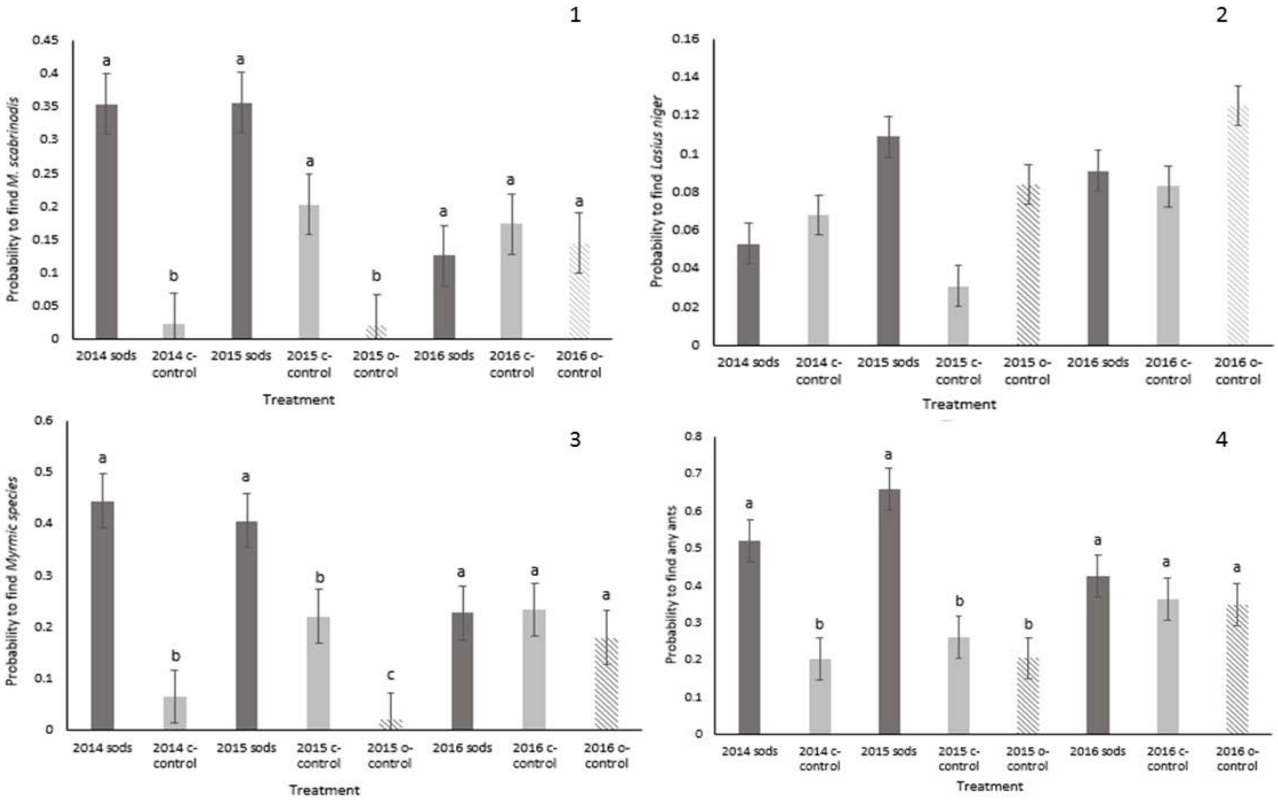

Figure 19. Mean of predicted probabilities of each group of ants (y axis) against treatments per years (x axis). Letters represent significance between treatments within years (after sequential Sidak posthoc test). Each group of ants are: *Myrmica scabrinodis* graph number 1, *Lasius niger* graph number 2, all *Myrmica* species graph number 3, and all ants graph number 4. Treatments are indicated by different shades of grey. For statistics, see text

In 2014, the probability to find any ant or *Myrmica* in sods was significantly different from c-controls, except for *L. niger* that does not show differences in any treatment or year. For 2015, different changes for each group were found; *M. scabrinodis* shows two clusters, sods and c-controls differ from o-controls; all *Myrmica* species presents three groups, one for each treatment, and all ant species together show significant differences between sods from c-controls and o-controls which appear clustered (see Figure 19). According to the 2016 results, there are no variances for years and treatments, due to, probably, the lower ant numbers collected in that year. However, there is an expansion of the ants to the surrounding areas (c-controls and o-controls) from the sods.

4.3.3 Generalized Linear Mixed Model: Environmental factors

A second series of GLMM's was performed to investigate the effect of environmental factors explaining the presence of each group of ants. The following tables 5, 6, 7 and 8, show the results of the effect of various parameters that can determine the occurrence of each group of ants.

The total vegetation cover has an important effect on the presence or absence of ants because it appears significant for all groups of ants ($p<0.05$). For the case of *M. scabrinodis,* it is a decisive variable for its presence. Other parameters that affect ant occurrence are the herb cover and bare soil cover. Herb cover has a significant effect in all groups of ants except for *L. niger.* Bare soil cover has a significant effect for all investigated groups with the exception of all *Myrmica* species lumped. The amount of bare soil has a negative effect on the ants: the more bare soil the lower the probability of finding any of the *Myrmica* species. Even for *L. niger* a significant negative effect has been found.

L. niger presence was included in the GLMM of *Myrmica* species to investigate a possible negative effect, but no effect was found in the models. Another important effect arises from the variable Year of excavation for *L. niger*. The more time has passed since the excavation, the more likely it is to find *L. niger* in the area. This means that with more years passed from the excavation more opportunities are available for *L. niger* to colonize the area. However, *L. niger* does not influence the presence or absence of other *Myrmica* species even though it is said to be a strong competitive species (Hölldobler & Wilson, 1990). Last significant variable for all group of ants is the DCA axis 1 from the ordination of 2014 and 2016 vegetation composition. This variable is produced from the similarities of species between plots and it is closely related to the other rest of environmental factors. Therefore, four variables could be extracted as important to explain the presence and absence of *M. scabrinodis* and *L. niger*. These are total vegetation cover, bare soil cover, herb cover and DCA axis 1.

Finally, four different graphs were generated to show the influence of the most important environmental factors; DCA1, total vegetation, herb and bare soil cover, into the predicted probabilities of *L. niger* and *M. scabrinodis* (See figure 20). The effect of the total cover of vegetation is different for both ant species. For our target species, *M. scabrinodis*¸ more cover of vegetation on the ground offers more possibilities of colonization of the area. For *L. niger* the effect is diverse; its occurrence is reduced slightly with more vegetation cover. With respect to the effect of herb cover, we observe a similar relation. *M. scabrinodis* is affected positively while *L. niger* is set back. However, this trend changes slowly for both ants and the predicted probabilities are different for herb cover than for total vegetation cover.

Table 3. Generalized linear mixed model result of *Myrmica scabrinodis´* presence based on various factors after the translocation in 2013

Model	AICs	Coeff	StE	t	p-value
Year excavation	2051.799	-	-	-	0.221
L. niger presence	2036.288	0.363	0.044	0.367	0.367
Total Vegetation Cover	2066.337	0.013	0.006	2.321	0.021*
Shrub cover	2041.825	-0.013	0.016	-0.812	0.417
Herb cover	2081.571	0.014	0.005	2.983	0.003*
Moss cover	2046.601	-0.001	0.005	-0.176	0.86
DOM cover	2046.496	0.009	0.009	1.089	0.281
S. officinalis cover	1582.249	0	0.006	0.026	0.979
Bare soil cover	2073.037	-0.017	0.006	-2.727	0.007*
Vegetation height	2046.012	-0.004	0.01	-0.452	0.652
StD vegetation height	2045.436	-0.029	0.021	-1.394	0.164
Ellenberg nitrogen	1673.971	-0.078	0.322	-0.241	0.81
Ellenberg moisture	1688.662	0.023	0.264	0.086	0.931
Ellenberg pH	1677.003	0.353	0.291	1.215	0.225
DCA1	778.61	-1.395	0.429	-3.25	0.001**
DCA2	743.393	0.456	0.443	1.029	0.305

Each row refers to one independent variables (one model per row). In the columns appear AICs, Coefficient of F-, StE (standard error) and p-value. All variables have 1 degree of freedom, except year of excavation with 2 degrees of freedom. * less than 0.05 and ** less than 0.001.

Table 4. Generalized linear mixed model result of *Lasius niger´* presence based on various factors after the translocation in 2013

Model	AICs	Coeff.F	St E	t	p-value
Year excavation	2525.127	-	0.026	-	<0.001**
M. scabrinodis presence	2462.77	0.181	0.428	0.423	0.672
Total Vegetation Cover	2543.915	0.015	0.006	2.71	0.007*
Shrub cover	2454.361	0.026	0.016	1.66	0.098
Herb cover	2501.391	0.008	0.005	1.727	0.085
Moss Cover	2474.721	0.005	0.006	0.874	0.383
DOM	2502.912	0.015	0.012	1.294	0.196
S. officinalis cover	1978.992	0	0.005	-0.072	0.943
Bare soil cover	2558.754	-0.017	0.006	-2.854	0.005*
Vegetation height	2524.979	0.023	0.013	1.779	0.076
StD vegetation height	2498.558	0.041	0.025	1.654	0.099
Ellenberg nitrogen	2053.777	0.407	0.432	0.944	0.346
Ellenberg moisture	2059.693	-0.303	0.307	-0.985	0.325
Ellenberg pH	2099.526	0.534	0.354	1.509	0.132
DCA1	911.572	0.191	0.248	0.769	0.443
DCA2	909.037	-0.127	0.659	-0.193	0.847

Each row refers to one independent variables (one model per row). In the columns appear AICs, Coefficient of F-, StE (standard error) and p-value. All variables have 1 degree of freedom, except year of excavation with 2 degrees of freedom. * $p< 0.05$ and ** $p< 0.001$.

Table 5. Generalized linear mixed models explaining *Myrmica* species´presence based on various factors after the translocation in 2013

Model	AICs	Coeff.F	StE	t	p-value
Year excavation	1970.735	-	0.086	-	0.559
L. niger presence	1956.191	0.348	0.37	0.94	0.348
Total Vegetation Cover	1975.6	0.012	0.005	2.291	0.022*
Shrub cover	1961.71	-0.012	0.014	-0.848	0.397
Herb cover	1981.643	0.012	0.004	2.838	0.005*
Moss Cover	1970.094	-0.003	0.005	-0.578	0.563
DOM	1965.247	0.006	0.008	0.817	0.415
S. officinalis cover	1530.728	0.004	0.005	0.822	0.412
Bare soil cover	1973.688	-0.013	0.005	-2.379	0.018
Vegetation height	1966.841	-0.008	0.009	-0.861	0.39
StD vegetation height	1967.854	-0.037	0.019	-1.957	0.051
Ellenberg nitrogen	1617.939	-0.44	0.313	-1.407	0.16
Ellenberg moisture	1603.144	0.197	0.251	0.783	0.434
Ellenberg pH	1600.118	0.176	0.269	0.654	0.513
DCA1	740.107	0.935	0.314	2.981	0.003*
DCA2	706.303	0.02	0.409	0.048	0.962

Each row refers to one independent variables (one model per row). In the columns appear AICs, Coefficient of F-, StE (standard error) and p-value. All variables have 1 degree of freedom, except year of excavation with 2 degrees of freedom. * less than 0.05 and ** less than 0.001.

Table 6. Generalized linear mixed models explaining all ant species presence based on various factors after the translocation in 2013

Model	AICs	Coeff.F	StE	t	p-value
Year excavation	1901.34	-		0.11	0.079
Total Vegetation Cover	1943.285	0.018	0.005	3.773	<0.001**
Shrub cover	1902.85	0.004	0.012	0.324	0.746
Herb cover	1940.71	0.014	0.004	3.456	0.001*
Moss Cover	1908.972	-0.004	0.004	-0.827	0.409
DOM	1905.673	0.011	0.008	1.389	0.165
S. officinalis cover	1486.947	0.007	0.005	1.315	0.19
Bare soil cover	1950.162	-0.019	0.005	-3.828	<0.001**
Vegetation height	1904.912	0.004	0.008	0.508	0.612
StD vegetation height	1900.053	-0.012	0.016	-0.779	0.436
Ellenberg nitrogen	1593.087	-0.407	0.292	-1.393	0.165
Ellenberg moisture	1585.767	-0.079	0.235	-0.336	0.737
Ellenberg pH	1589.309	0.248	0.256	0.97	0.333
DCA1	701.539	0.487	0.236	2.067	0.04*
DCA2	694.437	0.483	0.396	1.219	0.225

Each row refers to one independent variables (one model per row). In the columns appear AICs, Coefficient of F-, StE (standard error) and p-value. All variables have 1 degree of freedom, except year of excavation with 2 degrees of freedom. * less than 0.05 and ** less than 0.001.

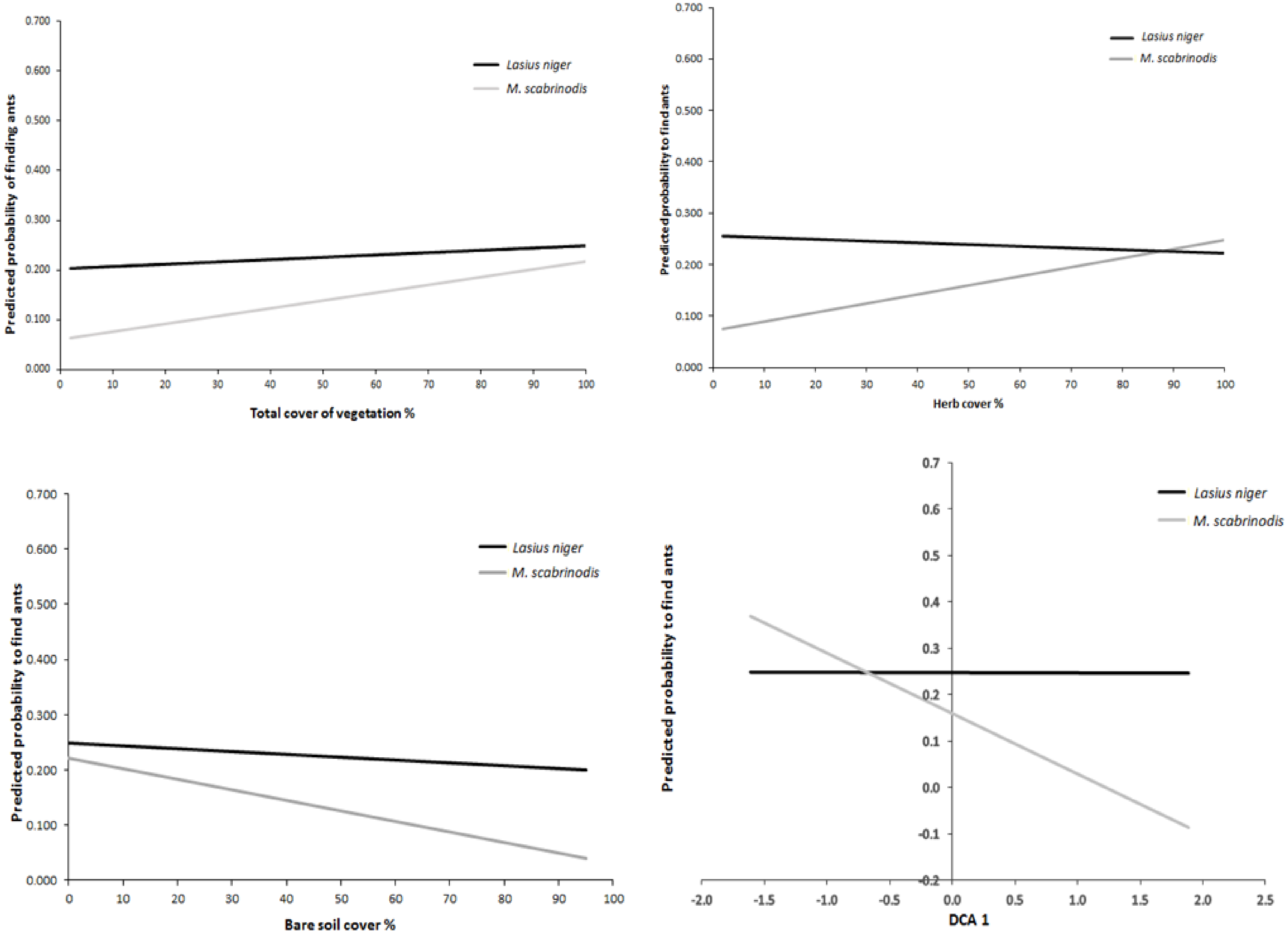

Figure 20. Graphs of predicted probabilities to find ants (y axis) with four different environmental factors (x axis). Graphs of predicted probabilities to find ants (y axis) with four different environmental factors (x axis). First graph refers to total cover of vegetation %, second graph is for the herb cover %, third graph for bare soil cover % and fourth graph is the DCA1. Black line represent predicted probability of *Lasius niger* and grey line predicted probability of *Myrmica scabrinodis*.

The effect of bare soil cover on the ant presence is inverse compared to the effect of vegetation cover. For the strong species *L. niger*, the lack of vegetation does not strongly affect its occurrence. Bare soil cover negatively affects *M. scabrinodis* presence. The last factor in the graphs is the first DCA axis, showing interesting results. In the left part of the DCA graph the probability of finding *M. scabrinodis* is higher (Fig.20.4), due to negative values of DCA corresponding to more coverage of vegetation. Maximum values of total vegetation, herb cover and height of vegetation are reflected in this part of the graph. The generalist *L. niger* is not affected by DCA axis 1 scores at all. Therefore, with these results we can conclude that the ecology of both ant species is different and these parameters perfectly show the conditions that each species needs for the colonization of new areas.

5. Discussion

Recovery of species is a demanding challenge for nature conservation actions, and the difficulty of achieving positive results increases with special ecological requirements of the target species. An example for this is the case of *Phengaris teleius*, a parasitic butterfly with complex associations with its environment. Enough host plants should be present in the future habitat of this butterfly (Elmes, 1991b; Witek *et al.*, 2010; Wynhoff, 2001). In addition, the obligate myrmecophilous relation with *M. scabrinodis* ants requests more efforts, enough ant nests should be available in the area for the butterfly survival as well (Elmes, 1991a; Jansen, 2009; Thomas, 1984a; Wynhoff *et al.*, 2011). For the compliance of these conditions, a stable habitat is necessary. The loss of wetland habitats has been reduced in Europe during the last century and currently, they are threatened by abandonment and land use changes (Joyce, 2014; WallisDeVries *et al.*, 2002). All over Europe, projects to restore wetland habitats have started. This study is part of such a restoration project. Its objective is to determine changes occurring due to the application of different managements for the restoration of *Phengaris teleius´* habitat. In particular, this report describes the relationship between the ecological succession taking place and the expansion of wetland plants followed by *Myrmica* colonization.

Since 2007, several management actions have been applied in the area to recover the environmental conditions of fen meadows. Plant colonization is not possible without good soil conditions, which should be achieved first (Eviner & Hawkes, 2008; Kardol & Wardle, 2010; Klimkowska *et al.*, 2007). In the past, the study area suffered from intensive agriculture (high amount of fertilizers), making it more complicated for a conversion to mesophilic grasslands (Hirst *et al.*, 2005; Joyce, 2014). Soil fertility and seed-limitation determine a decisive constraint to the restoration of swards (Bakker, 1989; Janssens *et al.*, 1998; Walker *et al.*, 2004). Since the restoration project started, the input of fertilizers has been terminated, cutting regimes have changed and the upper 40 cm of the top soil has been removed on an area seized 250 ha. The purpose was to achieve low levels of nutrients in the soil that are associated with a decline of dominant grasses and a wide range of co-existing species typical for moist grasslands (Bakker, 1989; Hayes & Sackville Hamilton, 2001; Janssens *et al.*, 1998; Tallowin & Smith, 2001; Verhagen *et al.*, 2001; Walker *et al.*, 2004). The adaptation of the hydrological regime takes an important part in the restoration of the target vegetation (Cronk & Fennessy, 2016; Jansen *et al.*, 2000; Van der Hoek & Heijmans, 2007); constructions created a high water level of seepage water, providing the required basic conditions on the surface.

Another restricting aspect is the limited dispersal capacity of seeds from plant species belonging to the target vegetation in the grasslands. The seed bank present in the ground will determine the success possibility to restore a fen meadow (Bakker *et al.*, 1996). The majority of *Cirsio dissecti-Molinietum* species have short-term seed persistence being absent in the seed bank (Jansen *et al.*, 2000) due to the poor dispersion and inefficient colonization by seed (Matus *et al.*, 2003). Spread of fresh hay from meadows is the best restauration method for the increment of plant species richness (Hölzel & Otte, 2003; Klimkowska *et al.*, 2007; Mortimer *et al.*, 2002; Walker *et al.*, 2004). Freshly cut clippings were spread from the meadows of Moerputten nature reserve with a stable community of *Cirsio-Molinietum*. Sod translocation as efficient action plus the presence of seed bank by hay translocation will impulse the habitat restoration and establish the target plant community in the area (Klimkowska *et al.*, 2007). Translocating pieces of climax vegetation produces the dispersion of *Cirsio-Molinietum* vegetation and helps to restore the seed bank in the restored areas (Jansen *et al.*, 2000; Matus *et al.*, 2003). The most benefitting species will be *S. officinalis* and other characteristic wetland species will appear over time. In addition, by means of translocating sods, not only the wetland species but also the habitat structure is translocated to the restored sites, offering colonization possibilities for insect species, amongst them ants.

5.1 Variations between 2014 to 2016: vegetation and environmental factors

5.1.1 Source areas

In the source area of Moerputten, open sod cut strips were created after collecting the sods in 2013. Sod cutting results in taking away nutrients of the topsoil in order to restore different ecosystems and creation of local suitable sites for germination of seeds from target species around (Bakker, 1989; Bootsma, 2002). This alteration stimulates the plant colonization (Dorland *et al.*, 2004; Jansen & Roelofs, 1996). Modifications in the vegetation composition are visible in the DCA of source areas comparing data from both years, where there is movement in the ordination (fig. 10). This difference is statistically significant due to the different species compositions in these two years. One year after translocation (2014), also, the removal of vegetation is expressed in environmental factors characterized by early stages of secondary ecological succession, such as bare soil, DOM cover and Ellenberg value of nitrogen and moisture (Odum, 1969; Persson, 1984; Sammul *et al.*, 2012).

Several studies define five to ten years for the recovery of the *Cirsio-Molinietum* community after disturbances by sod cuts (Jansen *et al.*, 1996; Jansen *et al.*, 2000; Van der Hoek & Heijmans, 2007). After two years (2016), sod cut strips are being covered by the expansion of vegetation. Environmental factors such as herb, moss and shrub cover increased in 2016, as well as Ellenberg pH value, number of *S. officinalis* and height of vegetation These factors are logically changing and increasing over the years (Odum, 1969; Zedler, 2000), because the source areas are recovering from the disturbances and the composition of the vegetation is moving towards the initial situation; in this case the maturation stage. Sod cut strips in 2016 present more plant colonization than 2014, as can be seen in the increase in the herb and moss cover as well as in the increased number of plant species found. Shrub cover increases due to ecological succession (Rosenthal, 2010).The expansion of shrubs should be limited by regular cuts every year to keep suitable wetland ecosystem conditions.

The method of sod cutting stimulates the colonization of plant species that disappeared from the source area, increasing the richness of species (Dorland *et al.*, 2004; Jansen & Roelofs, 1996; Van der Hoek & Heijmans, 2007). However, sod cut management can produce unsuccessful results in fen meadows, if prolonged inundations occur. Long floods reduce the oxygen in the soil and can produce acidification or eutrophication in the soil, generating undesirable circumstances (Jansen & Roelofs, 1996). Future research ought to focus on the control of environmental changes and species composition in the sod cut strips in order to assess the conservation status of the area for *P. teleius.*

5.1.2 Restoration areas

The two axes of the DCA ordination distinguish clearly between year and treatment (fig. 15). The first DCA axis divides into sod and control plots, where any significant difference was found. Sods are clustered because they are similar; this is expected because sods are coming from the source area that are already covered with vegetation belonging to the mature phase. There is a difference in the environmental conditions between treatments; for the sod plots these characteristics reflect a high degree in the ecological succession due to vegetation maturation and specific factors. Releve's from sods from both years are lumped together due to similar conditions while c-controls are shifting towards them, acquiring climax conditions over time (Odum, 1969; Persson, 1984; Sammul *et al.*, 2012; Zedler, 2000). Meanwhile, controls are more scattered in the ordination due to the ecological succession happening, plant colonization takes time until climax conditions are achieved (Odum, 1969; Prach *et al.*, 2007).

Controls have had only little time for plant colonization that is supported by these environmental factors. Lack of vegetation coverage and high levels of nutrients, as nitrogen, are related to primary phases of swards restoration, avoiding the presence of characteristic grassland species (Bakker, 1989; Smith *et al.*, 2002; Zedler, 2000).

Assessing the conditions that changed between these two years, still a high percentage of the soil is void of vegetation in 2016 controls. For the restoration of the vegetation, more coverage of bare soil is not a limitation, *S. officinalis* produces short rhizomes in a vegetative expansion needing bare soil, the spread by seed is often diminished (Musche *et al.*, 2008; Wynhoff, 2001). It is known, that grasses colonize areas with root systems or stolons in a faster way (Ingrouille & Eddie, 2006). Therefore, other grasses of wetland ecosystems might continue spreading in the area. Other factors, as moss and DOM cover have reduced their expansion; these factors should reduce their impact over time in the mature plots.

Ellenberg values of moisture and pH rose barely over time in 2016 sods, being regulated by the hydrologic regime of the area. *Cirsio-Molinietum* communities demand specific regimes of water conditions (Van der Hoek & Heijmans, 2007; Wilcox *et al.*, 1986), where the upward discharge of water from the ground (mainly calcareous) produces the deposition of basic salts in the ground, keeping a high pH (Jansen *et al.*, 2000; Wilcox *et al.*, 1986). In the Natura2000 area the hydrology was modified to control the water levels and discharges. Thanks to this engineering the hydrological conditions for *Cirsio-Molinietum* can be achieved reaching the optimal pH and moisture of fen meadows over time (Jansen *et al.*, 2000; Persson, 1984). The Ellenberg value of nitrogen decreased slightly in 2016 compared to 2014. The removal of top soil was applied to achieve nutrient-poor conditions for the *Cirsio-Molinietum* community (Jansen & Roelofs, 1996; Klimkowska *et al.*, 2007). Low values of nutrients in the soil (low content of nitrogen) allow wetland species to develop and set up. If the nitrogen increases, more competitive species will appear reducing the richness (Zedler, 2000) and the ecosystem will come back to early stages of ecological succession (Bobbink *et al.*, 1998; Kiehl & Wagner, 2006; Odum, 1969; Verhagen *et al.*, 2001). The reduction of nitrogen is slow in the study area but nutrient-poor conditions are accomplishing over time.

The second DCA axis presents a significant difference between data of both years. A separation between upper part (2014 data) and bottom part (2016 data) is distinguished by the second DCA axis. The movement of c-controls is the highest in the ordination, they are more spread and the area of dispersion is bigger than sods. The species composition changed between years expressed in this downward movement. Based on the average values from the data, environmental conditions are changing towards maturation stage of the fen meadows in the restoration areas. From 2014 to 2016, there is an increase in total vegetation and herb cover as well as in the vegetation height, these last two more pronounced. I expected a rise in these vegetation conditions because the proximity enhances the dissemination of plants and the ground would be covered over time (Van der Heijden & Moutoglis, 1998). The simple proximity of target vegetation community might help for the plant propagation and increase the likelihood of success (Jansen *et al.*, 2000; Matus *et al.*, 2003). With sod translocations, the probability of dispersion has been enhanced. After three years of transplantation, currently several sods are surrounded by vegetation expanding from the sods and even in some of them, it is difficult to recognize the original square shape. Nowadays, plant expansion continues towards control plots and none of the plants on the sods are dead or dried out.

Cirsio dissecti-Molinietum species require several years for their restoration, depending on the previous perturbations, the total restoration of fen meadows takes between 10 to 40 years (Hirst *et al.*, 2005; Joyce, 2014). Fifty new plant species appeared after just two years of research, classifying the difference as substantial. The primary phase of *Cirsio-Molinietum* community is formed by competitive and pioneer species more tolerant to ammonium, as *Carex oederi, Juncus bulbosus* and *Filipendula ulmaria*, already present in 2014 and again in 2016, and *Scirpus setaceus* shows up in 2016 for the first time (Jansen & Roelofs, 1996; Van der Hoek & Heijmans, 2007). The occurrence of these species indicates that nutrients are still high for a wetland ecosystem and the study area needs more time to reduce them. However, there are characteristic wetland plants in the restored area as well, such as *Carex panicea, Cirsium dissectum, Succisa pratensis* and *Viola persicifolia* that were already present in 2014, surely coming from the source areas by translocation of clippings. Other representative species of this community have not been found within this experiment, as *Carex hostiana, Molinia caerulea, Valeriana dioica*, *Gentiana pneumonanthe* and *Viola stagnina* (Grootjans *et al.*, 2002; Jansen & Roelofs, 1996), however they occur on other meadows in the restoration area and will hopefully expand over time.

Juncus conglomeratus appears in 2016 after the restoration efforts (Jansen & Roelofs, 1996), being a species of the mature stage of *Cirsio-Molinietum*. Other species of the mature phase are *Carex panicea* and *Leontodon hispidus*, present since 2014; the last one indicates basic conditions in the ground (Blackstock *et al.*, 1998), showing that pH is recovering in the study area. In 2016, other new species appeared as *Lysimachia thyrsiflora* and *Potentilla palustris,* which are characteristic of natural mesotrophic meadows with sub-neutral pH conditions (Grootjans *et al.*, 2002). As the consequence of the recovery of environmental conditions in the restore area, a new species appears, *Dactylorhiza majalis subsp. praetermissa*. This species belongs to the old phase of the ecological succession in *Cirsio-Molinietum* being a great discovery in 2016 (Jansen & Roelofs, 1996). This orchid is considered one of the most vulnerable endemic species in Europe by IUCN (Wotavová *et al.*, 2004). Another important appearance is the species *Drosera intermedia* in the surrounding of TCG2 meadow, not included inside a plot. This species has specific narrow conditions in its habitat, belonging to the old phase of ecological succession, where the probability of threats increases (Jennings & Rohr, 2011) and it expresses nutrient-poor conditions where it grows (Ingrouille & Eddie, 2006).

There are still signals of primary phases of ecological succession expressed in several plant species but the appearance of plants that belong to old phases give positive indications of recovery of the area. Future investigations could focus on concrete exchanges of vegetation during the years to evaluate in detail the ecological succession phases towards *Phengaris teleius´* fen meadow habitat.

5.2 Ant population

Lasius niger, M. scabrinodis and *M. gallienii* appeared every year in the research and dependent on the year other ant species were found (mainly *Myrmica* species). Just one species, *M. schencki* was new for 2016, being a late colonizer and potentially parasitized by *Phengaris alcon* mainly. This ant species prefers warm and dry microclimatic conditions for nest founding (Elmes *et al.*, 1998; Sielezniew *et al.*, 2010).

5.2.1 Ants' occurrence

One year after sod translocations (2014), *M. scabrinodis* already colonized the sods in the restored areas. In the research of Wynhoff et al. (2016), it was clarified from where they could come. The probability to transport ants with the sods is reduced because ants hibernate deep in the ground during winter and only 10 cm of soil was translocated from the source areas in October the year before. Therefore, the most likely possibility of colonization is from vicinity sources close to restored areas and colonization after nuptial flights (Dauber & Wolters, 2005). Nuptial flights of ants have occurred early in the summer induced by high temperatures in 2014, explaining their appearance just one year later (Wynhoff *et al.*, 2016). In 2015, *M. scabrinodis* was expected to be found more often than in 2014; ants increased mainly in many sods and appeared for the first time in c-controls. However, for 2016, there is a general trend of reduction in ant occurrence, especially for *M. scabrinodis* decreasing or even disappearing from some meadows. In one meadow, CG, the low colonization of *M. scabrinodis* can be explained by the high presence of *L. niger.* In the restored areas this species is mainly found in CG, increasing its occupancy over years. Long periods of re-establishment after the excavations of CG can explain the preference of *L. niger*; since year of excavation is a significant variable for its presence in the GLMM (Table 6); when the time increased from the year of excavation the probability of colonization for *L. niger* rose and CG is the first meadow that was excavated.

Utterly different results were found for the o-control plots; in 2015 ants occupied just a few o-control plots (one with *M. scabrinodis*). For 2016, almost all meadows were occupied by *M. scabrinodis* and a few plots with other *Myrmica* species. The maximum number of colonized o-control plots is low (two for *M. scabrinodis*), but this colonization delivers information about the restoration process. Ants are spreading from the sods or other sources in the close vicinity of monitoring patches to areas where the habitat is suitable for them (Dauber & Wolters, 2005). Vegetation is changing in the restored areas and wetland conditions are achieved over time due to ecological succession.

Normally, a stable colony of *L. niger* obstructs the colonization of *M. scabrinodis* (Elmes *et al.*, 1998) but sometimes, they co-occur in the same area. In 2015, *L. niger* and *M. scabrinodis* were found in the c-controls of CG1 and one year later, they were found in the o-controls of CG1 and CG2. GLMM analysis reveals no influences in the presence of one species to the other and vice-versa. The numbers of occupations are small, 3 plots of *M. scabrinodis* vs 7 plots of *L. niger* in c-controls for instance, but this event gives good indications about the habitat. Environmental conditions are probably turning to suitable habitats for *M. scabrinodis* allowing both species to stay in the same area (Elmes *et al.*, 1998), however over time the habitat conditions would deteriorate for *L. niger*. In the climax vegetation in Moerputten nature reserve this ant species does not occur any more, while *M. scabrinodis* is dominant.

During 2016, ant occurrence was less than was expected for sod and c-control plots. Several reasons may explain this decrease of ants' occupation in the restored areas. The first possibility refers to the inefficient method of monitoring. It might have been difficult to detect the ants with ant baits in the study areas; however, two different ant monitoring occasions were done (July-August) due to the low number of captures and in order to be sure of finding them. However, the number of ant workers and colonies was confirmed to have been reduced from one year to the next. Another possibility may be an insufficient amount of food resources; ecological succession takes time in the control plots and not all environmental factors are fully developed to provide required amount of resources for a further increase in ant numbers or colonies. A final explanation is related to the heavy rains during summer of 2016 (own observations). *M. scabrinodis* is a species tolerant to cool and wet conditions (Elmes *et al.*, 1998). It is known that it can survive inundations in the winter during diapause or otherwise in the altitude of *Festuca* or *Sphagnum* mounds at the edges and the lag zone of bogs (Markó *et al.*, 2004). Colonies on *Sphagnum* mounds float on the bog thus staying comparatively dry, even after severe rains. Persistent floods during summer when the ants are not in diapause could result in drowning of ant populations. In addition, currently, the Dutch calcareous groundwater is decreasing (Van der Hoek & Heijmans, 2007) and some studies corroborate a general increment in precipitations (Buishand *et al.*, 2013; Daniels *et al.*, 2013) with a rise of

11% in extreme precipitations in the Netherlands (Attema *et al.*, 2014). These situations might produce acidification on the ground due to the replacement of groundwater by rainwater and hydrology conditions would modify totally with consequent changes in vegetation (Van der Hoek & Heijmans, 2007; Wheeler & Shaw, 1995). If these anomalies continue, it could produce a negative effect in the recovery of ant populations in our restored area and slow the whole restoration process.

The probability to find *M. scabrinodis* or other *Myrmica* species is affected by the year of excavation and the interaction between year and treatment of the study (Table 4). The year of excavation is an important variable and the interaction between year and treatment both result in significance for these species. Therefore, changes occurred in different years affect these ants significantly, and also, the combination of year and treatment are different scenarios with changing conditions for *M. scabrinodis* and other *Myrmica* species. During the years, ecological succession modifies the study area producing variations both in the composition of ant communities and the numbers of ants and ant colonies. The probability to find ants in 2014 and 2015 is higher in the sods compared to the controls. These differences are related to the translocation of the sods done in 2013. The vegetation conditions on the sods are a good starting point for the colonization of ants which is followed by spreading over the area in the next years. Optimal habitat islands were created with the sods, where on the excavated soil ants found a suitable place and moved towards c-controls over time during 2014 and 2015. For 2016, the expansion continues, ants appearing in the three treatments but there is no significant difference between treatments in 2016 anymore; low values of ants in this year could explain the lack of significance.

5.2.2 Environmental factors for ants

At local scale, the specific environmental conditions of the area play an important role for the niches of the different ant species (Elmes *et al.*, 1998). Among the sixteen environmental variables collected in the study, four were most relevant for the presence of ant species explaining their presence in the habitat (Total vegetation cover, Herb cover, Bare soil cover and DCA1) (Tables 5-8). An increase of the presence of *M. scabrinodis* is related to an increase in the cover of the total vegetation or the herbs (Elmes *et al.*, 1998; Wynhoff *et al.*, 2016), while the occurrence of *L. niger* keeps almost constant. During ecological succession more extension of vegetation is generated approaching mature stages (Zedler, 2000), being an appropriate situation for *M. scabrinodis* (Elmes *et al.*, 1998; Wynhoff *et al.*, 2016). Bare soil affects the ants in an opposite way, being more pronounced in *M. scabrinodis* even in low values. Bare soil induces more insolation resulting in high temperatures and dry conditions in the top soil, influencing *M. scabrinodis* negatively (Elmes *et al.*, 1998; Elmes & Wardlaw, 1982; Steiner *et al.*, 2010; Wynhoff *et al.*, 2016). The vegetation composition expressed as DCA axis

1 score affects probabilities to find *M. scabrinodis* as well. The more vegetation succession has developed climax vegetation with more cover the more likely it is to find *Myrmica* ant species (Zedler, 2000). Contrary, *L. niger* does not show any trend in DCA1. The results allow the conclusion that *L. niger* is not affected at all by the sod translocations or the years of vegetation development after soil removal. In addition, *L. niger* does not show any change dependent on any of the studied environmental factors. The results confirm that it is a generalist species, resistant to anthropogenic disturbances (Seifert, 1993) that prefers open areas with bare soil and short vegetation, characteristic of early stages in the ecosystem (Dauber & Wolters, 2005; Pontin, 1963). This investigation gives new information on the required environmental conditions for *M. scabrinodis.* This ant species is more sensitive to habitat disturbances, where high vegetation cover, moisture and medium temperature in the soil is essential (Elmes *et al.*, 1998; Wynhoff, 2001; Wynhoff *et al.*, 2016).

The presence of all *Myrmica* species is negatively influenced by the Ellenberg values of nitrogen and the coverage of moss. These factors give information about ant species requirements. Moss and nitrogen are characteristic of early stages of succession and decrease the success of wetland restoration (Beltman *et al.*, 1996; Jansen & Roelofs, 1996; Klimkowska *et al.*, 2007; Van der Hoek & Heijmans, 2007). Therefore, sensitive ant species require climax conditions for their presence and nitrogen values and moss cover should be reduced, as it is happening in our study area.

Further investigations will be crucial to determine the specific conditions for ant species and their colonization in fen meadows. Continuation of the monitoring of ants in restored areas is necessary to clarify the population conditions over years. The improvement of ant species due to the restoration of the habitat is expected to give more opportunities for the colonization of *Phengaris teleius*. Restoration efforts are focused in decisive ecological relations of this butterfly to enhance its situation and the stabilization of *Myrmica* species would increase the probabilities of *P. teleiu*s expansion in the restored areas.

6. Conclusion

This investigation is applied rather short after the start of the restoration process, just three years after the sod translocations, however, several species reacted to that in a very short time. The whole restoration of a fen meadow is considered to take many years, between 10 to 40 years (Hirst *et al.*, 2005; Jansen & Roelofs, 1996), until all soil conditions, hydrology, plant composition and others are accomplished (Jansen *et al.*, 2000; Lamers *et al.*, 2015; Tallowin & Smith, 2001). Hydrological engineering, topsoil removal, spreading of hay cutting together with the sod translocation appear to bet successful for the restoration of a fen meadow, producing good results in the environmental conditions of the study area (Jansen *et al.*, 2000; Klimkowska *et al.*, 2007). Within this short period of time, several conditions changed a lot thanks to restoration efforts while others require more time for their whole recovery. There are still signals of pioneer phases in the composition of species in the restored areas. However, other new species that belong to more mature phases are already established (Grootjans *et al.*, 2002; Jansen & Roelofs, 1996; Van der Hoek & Heijmans, 2007). Nutrients are reducing slowly, and the appearance of mature and characteristic species of *Cirsio-Molinietum* demonstrates changes in the environmental conditions in the restored areas. Unfortunately, after successful colonization and increase in occupation, the ant population suffered a reduction in 2016, nevertheless the expansion of ants around the monitoring patches continues giving positive prospects for the whole restoration*. M. scabrinodis* appears in plots further from sods, even in co-occurrence with *L. niger*. Vegetation coverage and bare soil cover are the main factors driving the presence of *M. scabrinodis* (Elmes *et al.*, 1998; Wynhoff *et al.*, 2016). Modifications in the habitat corroborate the efficiency of sod translocations at a local scale; target species are distributing in the area, due to enhances in site conditions. Over time, optimal conditions of fen meadows will be achieved. It is recommended to continue the study of the secondary ecological succession and ant colonization. Future climate changes and other scenarios should be taken into account to determine the management of fen meadows (Buishand *et al.*, 2013; Tainio *et al.*, 2016). Conservation of grassland is a trade-off between the ecological succession and opposing forces, where directed management plays a decisive role (Morris, 2000).

The population of ants and wetland conditions need to be stable for the *Phengaris teleius* population to increase. Different restoration efforts have been applied to achieve these objectives with positive results, therefore enhancing the short-term restoration situation of the *P. teleius* habitat. This stabilizes ecological interactions between ants, plants and *P. teleius* butterflies, and provides more opportunity for butterfly colonization in the restored areas.

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Appendices

Appendix I

- Average values (±Standard Deviation) of environmental factors collected in source areas during 2014 and 2016

	2014	2016
Herb	49.38 ± 34	80.76 ± 19.4
Moss	9.69 ± 18	7.74 ± 17
DOM	8.17 ± 12.7	9.10 ± 9.8
Baresoil	48.33 ± 35.9	16.56 ± 17.3
Height	8.63 ± 7.6	31.34 ± 10.9
pH	4.86 ± 0.8	5.11 ± 0.6
Nitrogen	3.68 ± 0.6	3.86 ± 0.5
Moisture	7.51 ± 1.3	7.62 ± 0.6

- Average values (±Standard Deviation) of environmental factors collected in restored areas during 2014 and 2016

	Sods		C-controls	
	2014	**2016**	**2014**	**2016**
Shrub	0.07 ± 0.26	1.04 ± 2.3	3.75 ± 4.5	7.50 ± 3.5
Moss	15.61 ± 17.4	10.74 ± 14.1	28.13 ± 24.5	23.52 ± 20.6
DOM	8.46 ± 9.2	7.19 ± 3.8	5.46 ± 4.7	8.38 ± 8.2
BareSoil	4.70 ± 4.8	1.81 ± 2.7	47.08 ± 30.7	42.40 ± 27.2
Moisture	7.04 ± 0.6	7.16 ± 0.3	6.81 ± 0.5	6.85 ± 0.4
Nitrogen	4.07 ± 0.5	3.95 ± 0.3	4.22 ± 0.5	3.88 ± 0.3
pH	5.36 ± 0.6	5.40 ± 0.4	5.12 ± 0.4	5.23 ± 0.3
Total cover	96.67 ± 4.8	98.44 ±	53.54 ±	58.90 ± 25.8
Herb	82.28 ± 14.4	98.11 ± 2.6	24.63 ± 21.2	45.10 ± 25.9
Height	14.14 ± 4.5	36.97 ± 13.8	4.78 ± 4.9	13.53 ± 10.3

Environmental factors are Total vegetation cover (Total cover), shrub cover (Shrub), herb cover (Herb), moss cover (Moss), DOM cover (Dead Organic matter), bare soil cover (Baresoil), heigh of vegetation (Heigh) and Ellenberg values of moisture, nitrogen and pH.

Appendix II

- Eigenvalues of Principal Component Analysis (PCA) in both areas for 2016

	SOURCE AREAS	RESTORED AREAS
PC1	*30.8166*	*23.724*
PC2	*14.1696*	*12.439*
PC3	*8.27259*	*8.329*
PC4	*6.05185*	*7.382*
PC5	*4.80252*	*5.896*
PC6	*3.14988*	*4.859*
PC7	*2.98726*	*4.608*
PC8	*2.39568*	*4.157*

- Eigenvalues of Detrended Correspondence Analyses (DCA) in both year for the comparison between 2014 and 2016.

	Source areas	Restored areas
DCA1	*0.2977*	*0.4612*
DCA2	*0.2879*	*0.2462*
DCA3	*0.2242*	*0.2186*
DCA4	*0.2038*	*0.1541*

Appendix III

- Number of sod plots with different ant species in restored areas:

Plot	Year	Msca	Lnig	Msch	Mrugi	Mgal	Msab	Mrub	Lumbr	Mrugu	Myrtot	Anttot
HMD	2014	4	1	0	0	2	1	0	1	1	6	5
	2015	5	1	0	0	1	1	0	0	0	6	6
	2016	3	0	0	0	2	0	0	0	0	5	5
HOM	2014	3	0	0	0	0	0	0	0	0	3	3
	2015	2	0	0	0	0	0	0	0	0	2	2
	2016	0	0	0	0	0	0	0	0	0	0	0
TCG1	2014	3	0	0	0	0	0	0	0	0	3	3
	2015	5	0	0	0	0	0	0	0	0	5	5
	2016	2	0	0	0	1	0	0	0	0	2	2
TCG2	2014	3	1	0	0	1	1	0	0	0	5	6
	2015	4	0	0	0	2	1	0	0	0	7	7
	2016	1	0	1	0	3	0	0	0	0	5	5
CG1	2014	4	4	0	0	1	0	0	0	0	5	8
	2015	2	5	0	0	0	0	0	0	0	1	7
	2016	0	7	0	0	0	0	0	0	0	0	7
CG2	2014	2	5	0	0	0	0	0	0	0	2	5
	2015	1	9	0	0	0	0	0	0	0	1	9
	2016	1	7	0	0	0	0	0	0	0	1	7

- Number of c-control plots with different ant species in restored areas:

Plot	Year	Msca	Lnig	Mrugi	Msch	Mgal	Msab	Mrub	Lumbr	Mrugu	Myrtot	Anttot
HMD	2014	0	1	0	0	0	0	0	0	0	0	1
	2015	7	2	0	0	0	0	0	0	0	7	7
	2016	1	0	0	0	0	0	0	0	0	1	1
HOM	2014	0	1	0	0	0	0	0	0	0	0	1
	2015	1	0	0	0	0	0	0	0	0	1	1
	2016	0	0	0	0	0	0	0	0	0	0	0
TCG1	2014	0	0	0	0	0	0	0	0	0	0	0
	2015	3	0	0	0	0	0	0	0	0	3	3
	2016	3	0	0	0	0	0	0	0	0	3	3
TCG2	2014	0	0	0	0	2	0	0	0	0	2	2
	2015	3	1	2	0	0	0	0	0	0	4	4
	2016	1	0	0	0	2	0	0	0	0	3	3
CG1	2014	0	4	0	0	0	0	0	0	0	0	4
	2015	1	2	0	0	0	0	0	0	0	1	3
	2016	3	7	0	0	0	0	0	0	0	3	8
CG2	2014	0	5	0	0	1	0	0	0	0	1	5
	2015	0	6	0	0	0	0	1	0	0	1	7
	2016	0	5	0	0	0	0	0	0	1	1	5

- Number of o-control plots with different ant species in restored areas:

Plot	Year	Msca	Lnig	Mrugi	Msch	Mgal	Msab	Mrub	Lumbr	Mrugu	Myrtot	Anttot
HMD	2015	1	0	0	0	0	0	0	0	0	1	1
	2016	2	0	0	0	0	0	0	0	0	2	2
HOM	2015	0	0	0	0	0	0	0	0	0	0	0
	2016	1	0	0	0	0	0	0	0	0	1	1
TCG1	2015	0	0	0	0	0	0	0	0	0	0	0
	2016	0	0	0	0	0	0	0	0	0	0	0
TCG2	2015	0	0	0	0	0	0	0	0	0	0	0
	2016	1	0	0	0	0	0	0	0	0	1	1
CG1	2015	0	7	0	0	0	0	0	0	0	0	7
	2016	1	6	0	0	0	0	0	0	1	2	8
CG2	2015	0	4	0	0	0	0	0	0	0	0	4
	2016	2	8	0	0	0	0	0	0	2	3	8

Nomenclature of species:

Msca- *Myrmica scabrinodis*

Lnig – *Lasius niger*

Msch – *Myrmica schencki*

Mrugi – *Myrmica ruginodis*

Mgal – *Myrmica gallienii*

Msab – *Myrmica sabuleti*

Mrub – *Myrmica rubra*

Lumbr – *Lasius umbratus*

Mrugu – *Myrmica rugulosa*

Myrtot - *Myrmica* totals

Anttot - Ant totals