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The role of Green Infrastructure in mediating plant species diversity in calcareous grassland fragments in Southern Belgium

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Summary

The biodiversity of the species rich herbaceous calcareous grasslands (CGs) have been suffering from habitat fragmentation, threatening their biodiversity. Remnant habitats in the intensively used landscape matrix might serve as refugia and stepping stones for dispersal of plant species, referred to as green infrastructure (GI). There is little empirical evidence on the actual ability of GI to enhance functional connectivity and biodiversity in the remaining calcareous grassland fragments.

Twelve CGs locations (six ancient ones and six recently restored) were selected in the Viroin Valley and floristic surveys were performed using ten quadrats of $1m^2$ and scoring plant abundances. Surrounding each CG, ten sites of GI were randomly selected at five different distances. Floristic surveys were performed and plant abundances were scored.

We found that the community composition of specialist plant species from ancient and restored CGs was different, but the species they do have in common are similar in numbers, pointing towards dispersal limitation as the main cause. Restored CGs contained less specialist and epizoochorous species, still having potential to be colonized by new species. Our results suggest that total GI and GI of road verges enhances the restoration process of recently restored CGs by a specialist species flow from the GI towards the recently restored CGs. The species rich ancient CGs were less affected by GI, even suggesting source-sink dynamics, with the ancient CGs acting as sources for the GI. GI seems a promising measure for enhancing connectivity in a landscape, serving as stepping stones for specialist species towards restored CGs.

Samenvatting

Kalkgraslanden zijn een van de meest soortenrijke omgevingen voor kruidachtige planten in Europa, hoewel habitat fragmentatie hun biodiversiteit bedreigt. Minder kwalitatieve stukjes resterend habitat in het landschap kunnen potentieel dienen als toevluchtsoord en stapstenen voor de verspreiding van plantensoorten. Deze toevluchtsoorden worden 'groene infrastructuur' (GI) genoemd. Tot op heden is er weinig empirisch bewijs dat GI weldegelijk functioneel is en de connectiviteit en biodiversiteit in resterende kalkgraslanden positief beïnvloedt.

In de Viroin Vallei werden twaalf kalkgraslanden geselecteerd (zes oude en zes recent herstelde). In elk kalkgrasland werden uit tien 1m^2 kwadranten de plantensoorten en hun abundantie genoteerd. Rond de kalkgraslanden lagen telkens tien willekeurig gekozen GI locaties op vijf verschillende afstanden. De plantensoorten en hun abundantie werden geregistreerd.

We zagen dat de gemeenschapscompositie van de specialisten plantensoorten verschilde tussen oude en herstellende kalkgraslanden. De specialistensoorten die ze gemeen hadden, verschilden niet in aantal. Daardoor bleek limitatie in soortverspreiding een belangrijke oorzaak. Herstellende kalkgraslanden bevatten minder specialistensoorten en soorten verspreid door epizoöchorie, waardoor ze nog potentieel hebben om gekoloniseerd te worden door nieuwe plantensoorten. Het blijkt dat de totale oppervlakte GI en GI in de wegberm een positief effect hebben op het restoratieproces van herstellende kalkgraslanden door een stroom van specialistensoorten vanuit de GI naar herstellende graslanden. De extreem soortenrijke oude kalkgraslanden worden minder beïnvloed door GI, potentieel zijn *souce-sink dynamics* aanwezig. GI lijkt een veelbelovende manier om de connectiviteit in een landschap te versterken. Specialisten plantensoorten gebruiken ze als stapstenen om te verspreiden naar herstellende kalkgraslanden.

List of abbreviations

NMDS	Non Metric Multidimensional Scaling
SNG	Semi-natural grassland
AES	Agri-environmental schemes
IBT	Island biogeography theory
CG	Calcareous grassland
GI	Green infrastructure
N	Nitrogen
P	Phosphorus

Chapter 1

Introduction

1.1 Calcareous grasslands

1.1.1 Definition

European calcareous grasslands (CGs) are of high conservation interest since they are one of the richest herbaceous environments of the continent (Maalouf et al., 2012). These plant communities are considered the most species rich in the world at a scale less then ten square meter, containing up to thirty or forty different plant species per square meter. Some form of disturbance is needed to maintain the high plant species diversity, making them dependent on human interference (Jacquemyn et al., 2011; Poschlod and Wallis-DeVries, 2002; Willems, 2001). These habitats are even considered the most species rich habitat for butterflies and grasshoppers and are associated with higher species richness of arthropods (Poschlod and WallisDeVries, 2002; Adriaens, 2008). Generally, the CGs of Central Europe are dry, semi-natural and nutrient poor (Rico et al., 2014). More specifically, the North-Western CGs contain calcareous rock or chalk material. The soil covering the rock is superficial, dry and nutrient poor. Typical is the general absence of woody species and profound abundance of herbaceous species (Adriaens, 2008; Helsen, 2013).

When phytosociologically analyzing CGs, the continental and subcontinental parts of almost all of Europe and a part of Asia consist of xero-mesophytic grassland communities within the class of *Fesuco-Brometea* (Vassilev, 2007; Dutoit et al., 2004). This class includes steppe vegetation and dry grassland of the sub-boreal and temperate region (Terzi et al., 2016). More specifically, the vegetation of North-West Europe belongs to the order of *Brometalia erecti*, comprising mountainous and lowland grasslands. This order is divided in two suborders; *Xerobromenalia erecti* on dry to very dry soils and *Mesobromenalia erecti* on rather buffered soils (Adriaens, 2008).

Different hypotheses have been put forward to explain the coexistence of a high number of plant species in semi-natural habitats. Niche differentiation is one of these theories, the ability of plants to use the environment differently which leads to coexistence (Tilman, 2004). This can be achieved by different trophic and competitive strategies, for instance symbiosis with mycorrhiza (van der Heijden et al., 1998) and phosphorus resource partitioning (Ceulemans et al., 2017). If two plant species were to use the environment exactly the same, it would lead to competitive exclusion with one species driving the other to extinction. Additionally, the intermediate disturbance hypotheses states that species diversity reaches its optimum when disturbance is neither too frequent nor too rare. The number of species coexisting is also positively influenced by a fluctuating environment containing a wide variety of (micro)habitats (Tilman, 2004). Furthermore, species represented by different taxonomic groups can coexist when they perform the same function (e.g. nitrogen (N) fixing), according to the functional equivalence theory (Weiss et al., 2014). All these theories try to clarify why CGs are among the most plant species rich habitats.

1.1.2 Importance

CGs are reservoirs for both fauna and flora and comprise a large variety of available habitats. The numerous ecosystem services that it provides are often classified under four categories (Costanza et al., 2017). First, the supporting services are basic ecosystem processes such as nutrient cycling, soil formation, primary productivity and biogeochemistry. Nutrient cycling is responsible for the plant available phosphorus (P), limiting the primary productivity of plants in calcareous grasslands. Secondly, the provisioning services encompass clean water supply via aquifers located underneath the grassland. Additionally, it includes conservation of wild species and their genetic diversity. Grasslands can also serve for food production when grazing livestock is present, although also non-commercial food sources like berries and nuts serve this purpose.

Thirdly, services such as water storage, control of flooding, carbon sequestration, pollination, air quality maintenance, climate control and pest regulation are regulating ecosystem services (Ford et al., 2012). One of the essential regulating ecosystem services that semi-natural grasslands (SNGs) provide is pollination and a refuge for populations of natural enemies of crop pests (Deák et al., 2016). A service that is both a provisioning and regulating is the supply of clean groundwater due to storage of nutrients and prevent its leakage into the groundwater. This process is greatly influenced by the community composition. A loss of species results in a loss of productivity and even a decreased stability of primary production (Van Rooijen et al., 2015), a changing soil nutrient status, increased N leaching and reduced ecosystem stability (Phoenix et al., 2008). Lastly, the cultural services include cultural heritage, landscape aesthetics, ecotourism and outdoor recreation. These services can be defined as non-material benefits to society (Plieninger et al., 2015; Deák et al., 2016).

1.1.3 Historical origin and land use

CGs are generally man-made, although there are some assumptions based on scarce deposits and pollen that small and isolated patches of CG were already present before humans settled down. There were areas of “Steppenheide” along the Jurassic mountains and gravel banks and steep slopes along mountain rivers in the Alps. Between the Neolithic age and the time of the Roman Empire, the first human mediated SNGs were established. This started with the use of forests for grazing practices which were readily converted into common pastures. For Central Europe, the first increase in CGs only happened during the time of the Roman Empire. A this moment in time, new land-use practices of mowing for hay were introduced. Flocks of sheep grazed the CGs mainly to produce dung that was used on arable fields together with hay produced on these grasslands (Willems, 2001). Two of these new land-use practices were primarily responsible for the origin of CGs; alternate husbandry and the three-field-rotation system. Characteristic is that both methods use an abandoned field that is used for arable farming and followed by grazing for a longer period or every third year. This alternating practice favored the migration of CG specialist plant species.

During the plague of the fourteenth and fifteenth century, there was a crises in agriculture leading to the formation of large flocks of livestock (mainly sheep) and the upcoming of transhumant shepherding. This encompasses a seasonal movement of domestic livestock between winter and summer pastures. These pastures were distant from each other and even more distant from the abattoir. This transhumant shepherding shaped vast and connected regions of CG since sheep very effectively disperse seeds through epizoochory and endozoochory (Poschlod and WallisDeVries, 2002), even regardless of the seeds adaptation to epizoochory by adhesive appendages. Although the number of dispersed seeds was higher for seeds with adhesive appendages than those without specific adaptations (Rico et al., 2014).

The number of sheep as ideal seed dispersal vectors started to decline at the end of the 19th century and aggravated after 1940, along with the general decline in CGs (Jacquemyn et al., 2011). Waldén and Lindborg (2018) stress that both ecological factors and social factors play an important role in the success of CGs. Since the management of SNGs has a low profitability, abandonment of the sites is a persisting issue across Europe. Several major changes were responsible for this upcoming abandonment. First, hay was no longer produced on CGs since the appearance of fibrous hay and mineral fertilizer. Second, transhumant shepherding ceased to be economically beneficial, resulting in smaller sheep flocks which were fixed in paddocks. These higher productive paddocks became increasingly available since the invention of the railways. More agricultural products were imported instead of produced locally, leading to a surplus of unused land. Third, ancient breeds of cattle were replaced by high-performance breeds that could no longer feed on CGs alone to meet their nutritional demands. Later, the number of sheep decreased due to intensification of agriculture, the import of cheaper wool, less winter pastures and the difficult social facet of being a shepherd.

These changes negatively affected the size and number of CGs (Poschlod and WallisDeVries, 2002). Mainly in hilly areas of Western Europe, many CG grasslands were transformed in high productivity grasslands (Willems, 2001). A large proportion of the former CGs of Belgium were afforested with *Pinus nigra* and *Pinus sylvestris*. Abandonment was followed by natural succession which led to oak woodlands (Piqueray et al., 2011). A thorough analysis on all the potential threats and their magnitude is crucial in guiding restoration efforts with the greatest positive consequences for these remnant CGs.

1.1.4 Threats

The strong decline in low-intensive farming gave rise to some major threats to SNGs. These habitats became subject to either abandonment or conversion to other land-uses; intensive agriculture and forest (Lindborg et al., 2014). Afforestation is self-explanatory and therefore will not be discussed further. Habitat fragmentation is one of the most important threats to the biodiversity of CGs (this will be thoroughly discussed in Section 1.2). The patches of CG that remained are dependent on human interference for their continued existence. These areas are isolated and surrounded by a hostile matrix of forest, intensive agriculture and roads. Resulting in a dramatic decline of the species richness during the 20th century and a subsequent reduced aesthetic and cultural value (Poschlod and WallisDeVries, 2002; Deák et al., 2016). The research on both direct and indirect threats on SNGs gained attention in the past decades, providing insight in the processes responsible for the observed biodiversity decline (Lindborg et al., 2014).

Abandonment

When grasslands are abandoned, the changes in plant communities are characteristic for each specific locality. The local site conditions (i.e. the soil and nutrient availability) play a role during the course of changes and influence the eventual outcome. Nonetheless, there are some generally accepted transitions. During early abandonment a rise in species richness can be observed, which is then followed by a strong decline when forest closure increases and grassy species begin to dominate (Poschlod and WallisDeVries, 2002). The grasses start to become dominant due to their high competitive ability. Rosette plants and small herbs disappear quickly due to decreased light penetrating to the soil (Jacquemyn et al., 2011). When shrubs and trees appear, even less light penetrates to the lower levels of the vegetation. This light is crucial for the survival and growth of short lived plant species. Abandonment can cause forest establishment after only eight years, eventually leading to the disappearance of specialized grassland species by the process of succession (Štýbnarová et al., 2017; Öckinger et al., 2006).

According to Hansson and Fogelfors (2000), it is key to remove woody plants frequently in order to preserve species richness and community structure. An early successional stage is generally favoring CG species richness since this prevents competitive species from dominating the community. Meaning that restoration of grasslands can be achieved when the abandonment is recent and the signs of succession are still generally absent (Öckinger et al., 2006). The state of the grassland is of great importance to plant species but also to various animals. Birds, butterflies, grasshoppers, wasps and bumblebees are typically favored by taller vegetation and a more structurally rich habitat. This suggests that their numbers will first increase after the CG is abandoned. Nevertheless, when succession occurs leading to an increase in coverage of shrubs and trees, these species will decline (Hansson and Fogelfors, 2000). Additional creation of (micro)habitats and structural diversity by trampling disappeared with abandonment and cessation of grazing in the grassland. This underlines the detrimental effects of abandonment and subsequent succession on both plant species diversity and animal diversity of CGs (Öckinger et al., 2006).

Nutrient enrichment

Nutrient enrichment on a large scale is the most profound threat to biodiversity. The enrichment can have different origins such as atmospheric deposition, runoff of nutrients and addition of fertilizer and subsequent leaching (Piessens et al., 2006). The additional N and P leads to a severe decline in species richness (Štýbnarová et al., 2017; Phoenix et al., 2008). Different mechanisms cause species loss and competitive exclusion of less productive species. This includes eutrophication, toxic effects, alteration of mutualistic relations, acidification of the soil and a higher susceptibility to environmental stressors and pests (Stevens et al., 2010). Agricultural fertilization by the use of biologically active N is detrimental for the number of plant species, even when the N addition is chronic at a low level (Clark and Tilman, 2008). The N addition mainly leads to severe eutrophication (Van Den Berg et al., 2011) and acidifying N compounds (NH_4^+, $H_xNO_3^{y-}$) which cause soil acidification. Ceulemans et al. (2013) found that acidification is associated with toxic metals (Al^{3+}), accumulation of toxic NH_4^+ and mineral nutrient deficiencies (Ca^{2+}, K^+, Mg^{2+}). Survival, germination and growth of forb species are affected by a combination of the previous effects. On the other hand, the effect of P addition is also disadvantageous. Mostly because small changes in P balances can already create adverse effects on the plant community and consequently hamper biodiversity conservation efforts. Plant species living in symbiosis with arbuscular mycorrhizal fungi are especially sensitive to P, leading to a decline in mycotrophic species. Overall, both N and P availability need to be limited in order to obtain the highest biodiversity levels (Ceulemans et al., 2013).

Climate change

Not only abandonment and land use change are threatening CGs. Climate change is a threat with increasing importance that is rarely taken into account. It is known that climate change has an impact on the species diversity and range. The applied management approaches might intensify the effects of climate change, leading to an even greater loss of biodiversity in these already vulnerable areas. Additionally, increasing drought events due to climate change can diminish competitive exclusion by tall competitive species since stress-tolerant species possess an advantage. The increase in drought could be beneficial in abandoned mesic grasslands, nevertheless it puts xeric communities under high stress for instance in Southern Europe (Maalouf et al., 2012). Subsequently, dispersal is an important process to permit the geographic shifting of species to more suitable habitat and the possibility to rapidly adapt to that new habitat by the spreading of genes. Climate change affects the dispersal of species directly and indirectly by altering the environmental conditions. When a species is unable to disperse and climate change causes a high local stress, this can lead to extinction of the species (Travis et al., 2013).

1.1.5 Management

The economic function of CGs was completely lost after World War II and more sites were converted to nature reserves. A few of the extremely species rich CG areas were already converted to nature reserves in the 1930s and questions about the right management and restoration strategies arose soon. The idea of a non-interference management was an unpopular opinion from the beginning. Mowing was the first management action to be introduced, preventing shrubs and trees to invade the open landscapes. Some other practices were also executed in addition to mowing (i.e. shrub clearance, mulching and burning). Management efforts started to increase from the 1960s onwards and were aimed towards improving sustainable conditions that are crucial to both specific plant and animal species of a CG ecosystem (Willems, 2001; Poschlod and WallisDeVries, 2002).

Nowadays, the management of these species rich grasslands is maintained for historical or biodiversity reasons by non-government organizations, nature conservation authorities and farmers. The management costs should not be overlooked since certain practices demand more resources and manpower and even a higher frequency of intervention, for example mowing and grazing. Furthermore, new management methods are emerging (e.g. prescribed fire), which are likely to induce other selections pressures compared to the historical traditional management, demanding a profound investigation before implementation (Milberg et al., 2018).

The most abundant practices are different regimes of mowing, grazing or shrub clearing. It is common knowledge that an intermediate disturbance encompasses a higher amount of species (Catford et al., 2011). Coexistence of species is encouraged by biomass removal and disturbance which restrains highly dominant species, leading to less competition (Cousins, 2006). Still, different incentives are at the base of the efforts; preserving endangered species, the plant communities or the ecosystem and landscape as a whole (Dutoit et al., 2004). The conservation objectives determine the preferred management although trade-offs between ecosystem services should be taken into account (Ford et al., 2012). Furthermore, the European nature conservation mainly focuses on the needs of the plant community. Although it is important to look beyond this and integrate both fauna and flora. This poses a challenge since specific management practices often support some taxonomic groups and have detrimental effects on others (Bonari et al., 2017).

Grazing

Management by grazing is considered to be the preferred method when maintaining species richness is the primary concern. The consumption of plants by large grazers favors coexistence due to changing competitive interactions between species (Hansson and Fogelfors, 2000). The grazing of livestock also creates gaps which are (micro)habitats providing ideal light conditions and space for seedling recruitment and germination (Jacquemyn et al., 2011). According to Öckinger et al. (2006), grazing by sheep is less beneficial to species richness compared to horses or cattle. Mostly because sheep prefer herbs over grasses, leading to a dominance of the latter. Contrarily, the appetite of sheep for leaves and young saplings of shrubs and trees makes them efficient in suppressing woody encroachment. Additionally, sheep are good seed dispersers since the seeds more easily stick in their woolly coat compared to the coat of horses and cattle. In the study of Rico et al. (2014), ewes were herded to reconnect neighboring abandoned and high-quality patches of CG, separated by a distance of five to five hundred meter. Regardless of the presence of a dispersal adaptation to epizoochory, more then fifty percent of the seeds fell out of the sheep coats within the first few hours after grazing. Although numerous seeds are dispersed by epizoochory, the largest number of dispersed seeds were specifically adapted to this manner of zoochory. The adaptations encompasses longer seeds with specialized appendages that are easily caught in the wool of sheep.

The most common grazing practices are mixed grazing and rotational grazing. Mixed grazing is a management practice where different species of large grazers are used. On the other hand, rotational grazing encompasses large grazers which are displaced at regular time intervals to allow plant species to recover (Ford et al., 2012). Most beneficial for conservation would be the case where all the successional stages of a CG are present in one system, containing the full species diversity. The former dynamics that maintained the grasslands should be taken into account in future management and restoration to ensure the viability of specialist species (Poschlod and WallisDeVries, 2002; Bonari et al., 2017; Piqueray et al., 2011).

Mowing and shrub clearance

According to Poschlod and WallisDeVries (2002), the management practice of mowing and shrub clearing is successful in reducing dominant species such as *Brachypodium pinnatum* and increasing the number of specialist species. Although more specialist species were observed, the species diversity per square meter decreased. They confirm that mowing as conservation effort was not successful in maintaining characteristic species and species richness. Contrarily, Hansson and Fogelfors (2000) state that periodic mowing can result in a significant increase in species diversity. The difference in results might be explained by several factors influencing the success of mowing. First, the timing and frequency of mowing is important. Disturbances during the period where many endangered species flower and bear fruit must be avoided to conserve grassland herb diversity (Nakahama et al., 2016). Second, removing the mown plant material afterwards is important to avoid enrichment of the soil.

1.2 Habitat fragmentation

1.2.1 Definition

Not solely habitat quality but also habitat fragmentation causes a serious reduction in biodiversity. According to Butaye et al. (2005), habitat fragmentation operates on three levels; (1) the loss of pure habitat, (2) a decline in the fragment size and (3) increasing isolation of the habitat fragments. Both island biogeography theory (IBT) and metapopulation theory are important in modelling the dynamics of species colonization and extinction between fragmented habitat patches.

Island biogeography theory

The IBT was proposed by MacArthur and Wilson in 1967 and states that the species diversity is largely influenced by habitat area and isolation at a particular spatial scale (Leibold et al., 2004). The CG patches fit in this model although the theory was originally designed for oceanic islands. Some adjustments were included to create a more suitable model for human-dominated ecosystems. This included the incorporation of the heterogeneity of a habitat surrounding the grassland patches, that this matrix has a certain structure and has the possibility to change. These human-made habitats in the surrounding matrix provide resources for different species in contrary to true islands surrounded by water, strongly influencing both immigration and extinction processes (Mendenhall et al., 2014b). In the IBT, the relations between species richness and spatial patterns is assessed. Each island reaches an equilibrium number of species according to its size and the distance to other islands. The species richness of an island can increase when the habitat area is larger and when the island is in close connection to other islands. When the area of a habitat is larger, it includes a wider variation in microhabitats containing more niches which leads to a higher species richness. The fact that larger fragments include larger populations makes these species less prone to extinction. Subsequently, there is an increased probability of a viable and persisting population of species (Lindgren and Cousins, 2017).

Here, the SNG patches can be seen as islands. An isolated patch has lower immigration rates and higher extinction rates which adds up to an overall lower species richness in comparison to less isolated and larger patches of SNG. The amount of surrounding habitat seems of less importance compared to distance to the nearest patch and patch size. These isolated remnant patches of SNG habitat can function as stepping stones for dispersal and are important for biodiversity since they provide sources for plant dispersal and can serve as refugia (Lindgren and Cousins, 2017; Mendenhall et al., 2014a).

Metapopulation theory

The metapopulation theory became a principal paradigm in conservation biology. This is quite ironic since it was brought forward by Levins in 1969 to eradicate organisms instead of conserving them. This theory was later enhanced by Hanski and Gilpin around 1991. The focus of this theory lies on the spatial structure (connectivity) of populations and how this in turn influences population dynamics (Heard et al., 2012; van Nouhuys, 2016). The models of metapopulation theory handle a fragmented landscape as a combination of habitat patches which are separated by an unsuitable matrix habitat. Although recently this matrix is seen less as hostile but increasingly as heterogeneous, affecting the fitness of dispersers (Shima et al., 2010).

Each population can be seen as almost independent and only connected with other populations by infrequent, distance-limited dispersal (Figure 1.1). Single populations are prone to extinction due to demographic stochasticity, which are random demographic events causing fluctuations in population size. If these small populations go extinct, the empty patches can be re-colonized by immigration from other populations nearby. It can be stated that a single population is not capable of guaranteeing long-term survival of a species. Contrarily, the combination of many populations is able to achieve this by sustaining a balance between colonization and extinction rates. This ensures the survival of the whole metapopulation. These models offer a prediction of how threatened species can respond to habitat degradation, fragmentation and loss (Heard et al., 2012). Additionally, the theory emphasizes the importance of dispersal and connectivity in a landscape for species to persist in fragmented habitat areas (van Nouhuys, 2016).

1.2.2 Consequences

Increased extinction risk

For sparsely placed small remnant populations, a range of processes affect the fitness within a population, for instance genetic drift. This is the random change in allele frequencies during the process of sexual reproduction. The frequency of an allele can increase or decrease in a population, still there is a trend towards homozygotic individuals. This happens more frequently when mating opportunities are scarce leading to self-fertilization

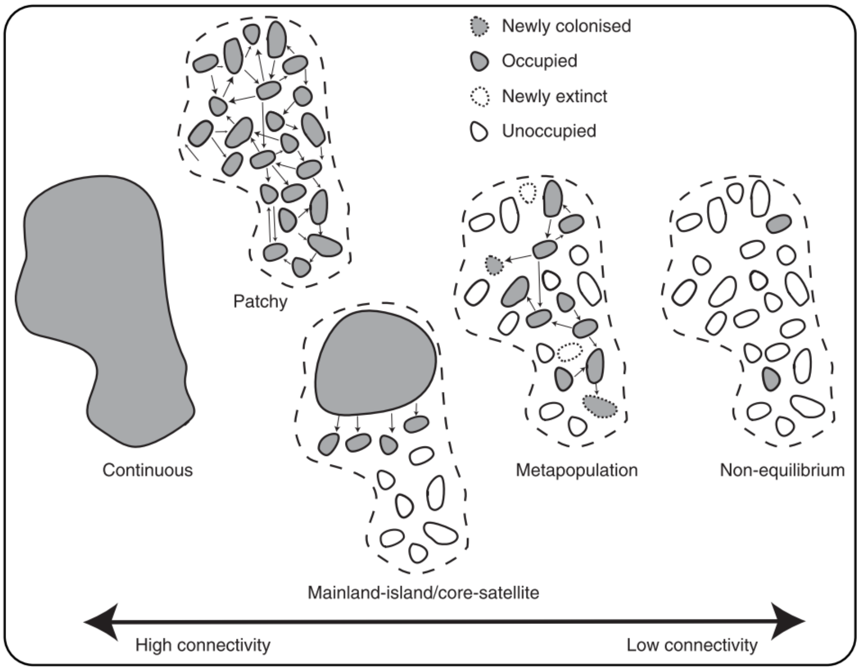

Figure 1.1: Metapopulations lie in between two extremes; one large well-mixed population and populations that are completely independent. In the metapopulation, populations are weakly interacting through dispersal

and non-random mating. This aggravates into the process of inbreeding depression where the frequency of homozygotic individuals increases, containing deleterious gene combinations which can lead to extinction of the population (Adriaens, 2008). Moreover, edge effects increase when patch size decreases because the edge to area ratio is higher. Piessens et al. (2006) found that the soil and flora of heathlands were affected up to eight meter from the edge. Eutrophication effects were more pronounced compared to the effects of forest presence near the edge. The typical forbs of CG vegetation were replaced by grasses and species thriving on nutrient-rich soils.

The fragmentation of habitat can increase to the extent that habitat patches are too isolated to support sufficient gene flow between populations (Adriaens, 2008). The probability of extinction rises when small areas are isolated, preventing a genetic rescue effect (to restore alleles lost to genetic drift) by immigration of individuals from other CG patches. Moreover, empty fragments are less prone to be recolonized when isolation is high, disrupting the metapopulation equilibrium and hampering restoration. Additionally, fragmented habitats are less resistant against environmental disturbances and have a lower ability to recover. The population size of species decreases, making them more vulnerable to extinction (Piessens et al., 2009). This suggests that small populations can be swept away by random environmental disturbance whereas the same amount of disturbance might not harm a larger population (Adriaens, 2008).

Reduced dispersal

The fragmentation and decreased connectivity of the contemporary landscape caused an extremely reduced seed and pollen flow between CG patches, aggravated by the disappearance of sheep as traditional dispersal vectors. Generally, the fragmentation results in an increased reliance on wind dispersal because seeds are not as easily attached and dispersed by current management practices. The dispersal traits of specialist grassland species are important predictors of the dispersal distance, especially the release height of diaspores. Grasses are generally good wind dispersers by releasing seeds above vegetation height, increasing the possibility of encountering higher wind speeds. The opposite is true for forbs which release seeds below vegetation height. This indicates that wind dispersal alone is insufficient for natural regeneration of CGs, since forbs account for the greatest part of the specialist species present in an CG community (Diacon-Bolli et al., 2013). Summarized, habitat fragmentation causes a reduction in population size, lower population growth rates, hampered dispersal and limited colonization (reducing the rescue effect). This leads to an increased risk of extinction and an increased recovery time (Piessens et al., 2009).

Shifting ecosystem services

The abandonment of CGs led to both positive and negative effects on the ecosystem services stated earlier. For the provisioning ecosystem services, the disappearance of grazing decreases floral diversity which hampers the conservation of wild species and their genetic diversity. The meat production from grazing sheep and cattle is reduced, negatively affecting the ecosystem service of food production. Without grazing by large herbivores, primary succession of shrubs can dominate the vegetation and alter water infiltration and storage. The soil compaction by trampling is reduced, the infiltration rates of water into the soil and aquifers increases, leading to an improvement in water storage. In addition to provisioning ecosystem services, the regulating ecosystem services are also impacted. Pollinators are generally more abundant on grazed grasslands since there are more floral resources or sward structures providing nectar. In addition to the service of improved water infiltration, the water experiences less contamination and less run-off which enhances the regulation of flooding (Ford et al., 2012).

When focusing on the cultural ecosystem services, the aesthetic value of the grassland declines with cessation of grazing because less plant diversity remains to be admired. Lindemann-Matthies et al. (2009) found that plant diversity is attractive to humans, not solely some individual species. Their study underlines the economic argument for conservation of the diversity in CGs, since it has a negative impact on tourism. It can be concluded that a change in the grazing regime impacts individual services of SNGs, which in turn leads to a shifting balance between the four groups of ecosystem services. The management determines which ecosystem services are the main conservation goal and which trade-offs between the services are present (Ford et al., 2012).

Unequal severity of the consequences

The plant traits may play a role in the experienced severity of fragmentation. For example, self-fertilizing species are less affected by a reduced gene flow since their genetic diversity can be mainly found within populations. Contrarily, obligate or mainly outcrossing small plant populations or the ones that depend on pollinators are more affected by isolation (Honnay and Jacquemyn, 2007; Rico et al., 2014). Furthermore, Honnay and

Jacquemyn (2007) found that not solely specialist species are affected by habitat fragmentation. Common species and rare species showed the same population genetic response to the detrimental effects of a shrinking population size. Both experience a loss of genetic diversity and suffer from genetic erosion. The loss of genetic diversity in species that are common can have major consequences for the species richness and the community composition of a fragmented habitat. Meaning that even common species are seriously affected and might already have reached the critical threshold in population size.

Habitat fragmentation also has consequences for species of higher trophic levels, which are often more susceptible to extinction due to lower colonization rates. Especially if a species of a higher trophic level depends on one plant species for reproduction and survival, which is often the case in specialist herbivore butterflies (Piessens et al., 2009). It can be concluded that passive protection of CG habitats alone is insufficient in guaranteeing a long-term persistence of plant species due to all previously mentioned effects. Habitat restoration with a focus on defragmentation and enlargement of the remnant habitats is therefore a fundamental priority (Helsen et al., 2013).

1.2.3 Restoration

A striking issue in highly fragmented landscapes is that even if restored patches of grassland are directly bordering a species rich CG, the increase in species diversity through dispersal is limited. This is due to the extremely limited dispersal of typical CG seeds (Jacquemyn et al., 2011). The poor dispersal capacity is linked to a step-wise dispersing mechanism and propagating vegetatively. This leads to a longer establishment time on new sites, aggravated by a lower rate of emergence compared to generalist species. These generalists disperse rather swift and evenly across the area of a field. Dispersal limitation is regarded as the main mechanism restraining the distribution of plant species in the landscape (Fagan et al., 2008; Cousins and Lindborg, 2007). Especially because the dispersal of pollen and seeds between communities is important for local colonization of remnant patches by new species. Additionally for recolonization of formerly present species and an enrichment of the genetic material (Diacon-Bolli et al., 2013).

As previously mentioned, the conservation of grasslands started around the 1970's in Europe. In 1992, orchid-rich *Festuco-Brometalia* was included in the EU Habitat Directive. By that time, remaining CGs were already small and isolated. Furthermore, different restoration projects and funds arose; the European Regional Development Fund (ERDF), Financial Instrument for the Environment (LIFE) and the European Agricultural Fund for Rural Development (EARDF). Furthermore, there is an EU Strategy on Green Infrastructure (GI) with the following aim; *"by 2020, ecosystems and their services are maintained and enhanced by establishing GI and restoring at least 15% of degraded ecosystems"* (European Commission, 2016) [1]. The SNG communities are also included in the EU Natura 2000 network (Helsen, 2013; Butaye et al., 2005). Another European funding instrument are agri-environmental schemes (AES). However, the results of AES are highly variable, which might be associated with the unsuccessful selection of appropriate areas for these measures that often ignore habitat connectivity and the landscape context (Arponen et al., 2013; Fagan et al., 2008).

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[1] http://ec.europa.eu/environment/nature/ecosystems/index_en.htm

Wagner et al. (2019) did research on indicator species to be able to assess the degree of success of local restoration goals, leading to two approaches. First, comparing the floristic similarity between an ancient (reference) plant community and the restored plant community. This is an instant indicator of the degree of progress in a habitat. Second, information on individual species (i.e. presence and abundance) can also provide information on the progress of restoration. These species can be seen as 'positive indicator species'. They found thirty plant species that are positive indicator species for different stages of natural regeneration towards high-quality ancient calcareous grassland. This is especially interesting when no records or maps are available to prove site age. This way, non-statutory sites that underwent long-term management can be uncovered. Using this knowledge, some sites can be prioritized for conservation and linked to AES payments with higher rates for older grasslands.

Up to today, restoration usually involves shrub and tree clearing, followed by a mowing or a grazing regime without actively adding species. A spontaneous colonization by the specialist species is assumed, mostly from adjacent grasslands or the soil seed bank (Helsen, 2013; Waldén et al., 2017). Although most CG species only have a transient seed bank which is insufficient to restore the diversity or buffer extinction of rare species if the aboveground vegetation has already disappeared (Karlík and Poschlod, 2014; Fagan et al., 2008; Piqueray et al., 2011; Bossuyt et al., 2006). Additionally, these semi-natural habitats are man-made and therefore depend on management. This accentuates that solely creating protected areas is insufficient for restoration (Arponen et al., 2013; Fagan et al., 2008).

Other efforts encompass artificial introduction of species by sowing of seed mixtures, hay seeding and transplanting sods or plant material (Jacquemyn et al., 2011; Diacon-Bolli et al., 2013; Bossuyt et al., 2006). Although Fagan et al. (2008) found that only natural regeneration caused restored grasslands to resemble the target site better, suggesting that seeding might suppress grassland development. Additionally, the seed mixtures that are commercially available are expensive (Smart et al., 2002). Nonetheless, a high-diversity seed mix can be applied in small blocks within a large grassland area which creates small species rich sources from where the surrounding area can be colonized (Török et al., 2011). A consensus is that restoration of CGs has the highest potential of succeeding when remnant populations of typical CG species are still present in the landscape, emphasizing the importance of connectivity (Jacquemyn et al., 2011).

Comparison restored and ancient focal patches

To evaluate the efficiency of restoration, it is important to compare restored sites to reference sites. A site can be species rich, still the species might be unrepresentative for the natural community. These concerns are resolved using a reference or control site (Piqueray et al., 2011). Furthermore, the classical climax approach of succession assumes that a community assembly follows a predictable path towards a fixed final endpoint. Apparently, other factors also determine the outcome of a community assembly, for instance historical processes and landscape characteristics. The species assembly in a restored grassland habitat is deterministic on the level of plant traits, in contrary to the species level. Meaning that isolation can be seen as a trait filter for plants with good dispersal traits. More low-mass seeds of small species with an attachment potential are found in extremely isolated remnant grasslands, suggesting that isolation causes a delay in community assembly in restored sites (Helsen et al., 2013). The study of Helsen et al. (2013) was executed in the Viroin valley in southern Belgium where migratory sheep flocks are part of the management, explaining the presence of seeds with attachment potential in isolated patches. Without these migrations, the isolation would have caused animal-dispersed seeds to reach the highly isolated patches much slower or even not at all (Fagan et al., 2008).

Piqueray et al. (2011) compared restored sites that once were pine forests and oak coppices to reference grasslands. The restoration was accomplished by forest clearing followed by management (mainly grazing). They observed that older restored sites are more similar to reference sites. Although floristic surveys show that some rare species were not able to colonize the restoration site despite being in close proximity to ancient grassland. This indicates dispersal limitation and might also be due to the high cover of invasive grasses in these restored plots. The soil characteristics did not diverge much between pre-restoration forest patches and reference grassland ecosystem, encouraging restoration from afforested sites. These abiotic conditions are often the major constraint when restoring arable fields. The species richness in a restored site increases over time and the floristic similarity with reference sites increased. There were some persisting differences between the two, being lower availability of bare soil and a higher number of native invasive grasses cover in restored patches. Especially these invasive grasses must be counteracted if restoration is to be successful.

When restoring CG starting from arable land, it can be observed that older restored sites better resemble the plant community of reference sites compared to younger restored sites. Although the process is slow, exceeding sixty years of research covered by Fagan et al. (2008). According to these authors, it is crucial that a good quality grassland is in close proximity if any degree of success is ought to be achieved when sites are left to restore naturally. Especially stress-tolerant species with large and heavy seeds or animal-dispersed seeds are slow at entering restored sites when there is a large distance to overcome. Additionally, more generalist species an opportunists are found in restored ex-arable patches since there is initially no competition for nutrients or space. Regeneration of restored habitat is most beneficial only if sufficient ancient grasslands sites are nearby to mitigate the dispersal limitation (Fagan et al., 2008). It was already clear that conservation by mowing, grazing or shrub cutting alone would not be sufficient in yielding viable (meta)populations. Crucial is the physical interconnection of remnant grassland patches (Helsen et al., 2013; Maccherini et al., 2014). This could be achieved by adding GI to the landscape.

Impact of green infrastructure

The attention and conservation efforts are mostly directed towards exceedingly important habitats in nature reserves. In highly modified landscapes, most habitats are not conserved or protected although they bear a significant value in the spatial context. They might mitigate dispersal limitation because of their ideal placement in the landscape. The use of poorer sites in the matrix habitat can diminish negative effects of isolation on species richness in isolated patches since they contain a substantial amount of diversity. After all, the species pool of the landscape is not solely present in CGs (Öckinger et al., 2012; Cousins and Lindborg, 2007). Summarized, if few SNGs are present and the connectivity of the landscape is degraded, GI can be a potential source of specialist species and serve as stepping stones in facilitating dispersal (Waldén et al., 2017; Lindborg et al., 2014). Dispersal is not simply one movement, it involves three distinct steps at source and recipient patches; departure, transfer and arrival (Bonte et al., 2012). The species should persist long enough to produce seeds for dispersal between different habitats of high quality (Lindborg et al., 2014). Although persistence and dispersal of remnant populations from refugia or core sites will be most efficient in landscapes where species diverse biota is still present (Smart et al., 2002).

This GI may be seen as refugia and encompass roadside verges, midfield islets, abandoned grasslands, field boundaries and forest borders (Cousins and Lindborg, 2007; Deák et al., 2016; Waldén et al., 2017). Especially grazed ex-arable fields have potential to increase grassland diversity. Still, it will take a very long period of time for ex-arable grazed fields to gain the same species diversity as ancient SNGs (Cousins and Lindborg, 2007). These measures of conservation using GI should predominantly be implemented in areas where they will be most effective. The small habitat elements can become a part of the CG network, buffering against disturbances in the future and mitigate climate change (Lindborg et al., 2014). Additionally, not only the specialist plant species of CG benefit from GI but also forest species (mainly in hedgerows), plants adapted to semi-closed conditions and the regional fauna such as birds and insects benefit as well (Marshall and Moonen, 2002; Wehling and Diekmann, 2009; Cousins, 2006).

Some studies already assessed the effects of GI on the surrounding habitat. Most sources investigate two specific types of GI, which are midfield islets and road verges. Lindborg et al. (2014) examined how a regional plant species pool, normally associated with SNGs, is sustained by these two types of remnant habitats. They found that SNGs had a higher specialist and total richness compared to the midfield islets and road verges. Mainly because of the smaller size and higher extinction probability in these types of GI. The midfield islets contained more species per unit area than the road verges. This difference is due to the fact that midfield islets were former managed small habitats in contrary to road verges, rendering an effect of landscape history. Additionally, and in accordance with Cousins and Lindborg (2007), the number of specialist species decreased with increasing distance from the source habitat. Remarkable is that (Lindborg et al., 2014) found this effect in fragmented landscapes, but not in a more heterogeneous landscape. Furthermore, Cousins and Lindborg (2007) found that a longer period of grazing led to an increased species diversity in both midfield islet (source habitat) and surrounding remnant habitats. The number of specialist species increased over time and the species composition became more similar. Both Lindborg et al. (2014) and Cousins and Lindborg (2007) indicate that the plant communities of midfield islets and road verges of small remnant habitats can serve as sources for grassland specialist species to disperse to the surrounding habitat, accelerated when grazing is introduced.

Broader research on the landscape composition has also been implemented. Cousins (2006) investigated species occurrence in midfield islets and road verges in two types of surrounding habitat. Habitat still encompassing many grasslands and habitat with grassland reduction. Connectivity, area and surrounding vegetation were more important in the landscape with less grasslands left. The number of specialist species was also lower compared to the habitat with many grasslands left. It was also clear that midfield islets, not road verges, contained a considerable share of the CG species pool and several specialist species.

Waldén et al. (2017) performed research on the effect of landscape composition. They found that the specialist richness in restored grasslands was positively influenced by the frequency and specialist richness of the surrounding remnant grassland habitats and SNGs. Additionally, there was a positive relationship between the proportion of remnant grasslands and SNGs in a landscape and the specialist richness present in restored grasslands. This suggests that the specialist species pool of the surrounding landscape can be important for the outcome of restoration efforts. There was no significant difference in specialist richness between ancient and restored grasslands, suggesting that the specialist species can become equal over time. Both spatial and temporal processes have an impact on the eventual success of restoration efforts. In conclusion, there are only limited studies on the effect of landscape composition on recently restored SNGs and most studies are only limited to two types of GI; midfield islets and road verges.

Chapter 2

Objectives

GI is a well-known concept in conservation and management policies at various levels of government. Although there is not much empirical evidence on the actual ability of GI to enhance functional connectivity and biodiversity in the landscape. The risk is allocating time and resources at an ineffective management, leading to detrimental effects on ecosystem services and biodiversity.

The aim of this master thesis is to determine the importance of GI for conservation of plant species in fragmented CGs. To achieve this, an inventory was made of the plant species within a set of CGs and in different types of GI. The main aim can be split up in two smaller goals, firstly assessing if there is a difference in the species composition and species richness of three types of plant species groups (all plant species, specialist species and epizoochorous species) between ancient and restored grassland patches. Secondly, the impact of GI on the CGs is investigated. It is determined how similar the specialist species composition of these CG patches is, compared to the specialist species composition of the surrounding GI. Additionally, investigating if the specialist community composition of the GI surrounding a CG changes significantly when the distance to the focal patch increases. Furthermore, the hypothesis is that the dissimilarity in specialist species composition between an ancient/restored CG and the surrounding GI increases with increasing distance. Lastly, assessing if the species richness of the three plant species communities are correlated to specific types of GI; total GI, road verges, linear feature coverage (excluding road verges) and islets.

Knowledge will be generated about how to manage remnant CGs and GI to uphold biodiversity and ecosystem services in the light of a rapidly changing environment. The results may be formulated into guidelines to create a more effective management for species rich SNGs in order to maintain biodiversity and ecosystem services for the future. Hopefully, communicating relevant information will enforce the connection between science, policy makers and stakeholders.

Chapter 3

Material and methods

3.1 Study area

The study region is the Viroin Valley situated in the Southern part of Belgium in the province of Namur. The SNG fragments are located in the Southwest part of the Calestienne region, which mostly corresponds to the valley of the river Viroin. The sediments are of Devonian and Carboniferous age, an alternating pattern of hard calcareous rock on the hills and soft schist rock in the valleys. Before 1900, vast areas of CG were present which declined after the abandonment of grazing practices. Now, the remaining CGs are present on these hilly domes, which are called 'tiennes' by the local people. These tiennes are surrounded by a matrix of weathering schist. Phytosociologically, the grasslands belong to the *Festuco-Brometea*, which is common in central Europe. Still, affinities are shown to other classes; *Trifolio-Geranietea*, *Sedo-Scleranthetea* and *Molinio-Arrhenatheretea* (Adriaens et al., 2006). There is a mean annual temperature of 8.6°C (47.5°F) and mean annual precipitation of 781 mm (Royal Meteorological Institute Belgium, 2019) [1]. Microclimatic conditions are due to the mostly Southwest-Northeast orientation of the calcareous hills (Adriaens, 2008). The spontaneous succession or transformation in conifer plantations led to decreased CG area and increased isolation (Bossuyt et al., 2006; Adriaens et al., 2006). The current management consists of annual grazing by migrating sheep flocks and goats. This is complemented by chopping and mulching of encroaching woody species. The restoration of CGs was executed in two phases, the first operation in 1995 and a larger one in 2001-2007, yielding a variety in restoration age classes (Addendum; Table A.1). Moreover, there was no evidence of an extinction debt in this area (Helsen et al., 2013; Adriaens et al., 2006).

For this study, twelve circular locations were selected in the Viroin Valley (Figure 3.1). In the center of each circle, a CG is present; recently restored CG (6 sites) or continuously managed CG (6 sites). Continuously managed CG and restored CG are further referred to as 'focal grasslands'. The restorations were around ten years old and the twelve locations were at least three kilometers apart from each other to avoid non-independence. The continuously managed CGs (referred to as 'ancient sites') represent reference communities that are still intact and that are the desirable state at which restoration is aimed. Restoration of the younger sites was achieved by clearing of shrubs, felling trees and also

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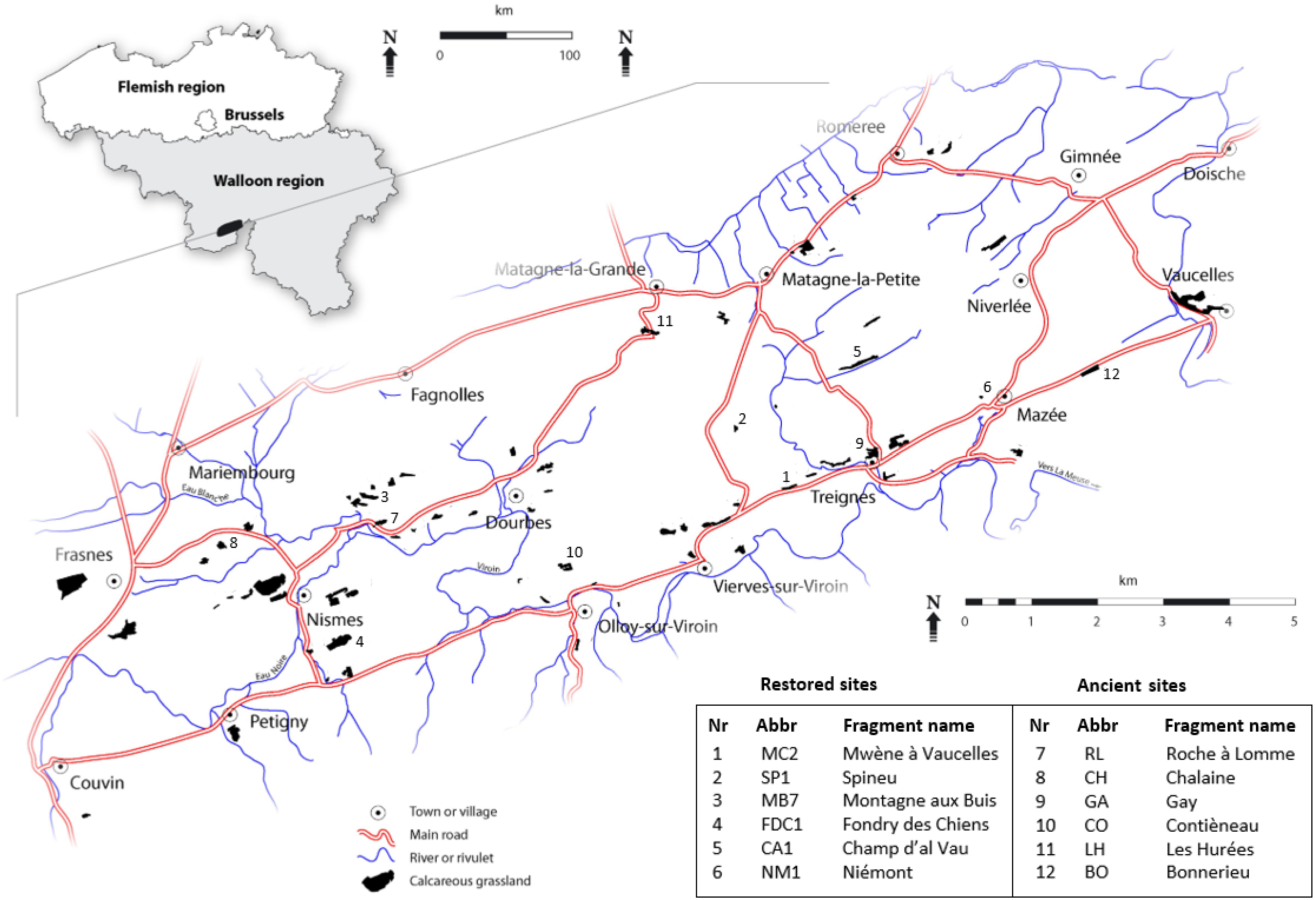
[1] https://www.meteo.be/nl/klimaat/klimaat-in-de-wereld

by the introduction of grazing livestock (mostly sheep) for management. The area of the CGs varied from $0.6\,\mathrm{km}^2$ to $5\,\mathrm{km}^2$ (Addendum; Table A.1).

Surrounding the CG center of each location, ten sites were chosen that consist of linear elements of spatial GI. This is defined as habitats that can potentially contain grassland specialist species, for instance road verges, hedgerows, grass strips, small remnants and formerly grazed forest borders. These sites were located circularly around the CG center at five different distances (300 m, 600 m, 900 m, 1200 m and 1500 m from the center), with two GI sites at each distance. The habitat matrix surrounding the GI mostly consists of agricultural fields with both crops and grazing livestock (Addendum; Figure A.1 and A.2).

3.2 Field inventories

The floristic surveys were executed from July until begin October in 2018. In each of the twelve focal grasslands, all the plant species were surveyed using ten quadrats of 1m x 1m (which equals $10\,\mathrm{m}^2$ per focal patch). The plots were established randomly across the available area. For the plant species present, the abundances of each species (%) and the vegetation height were recorded in each quadrat. After surveying the plots, the total area of the focal patch was also surveyed for plant species that were absent in the ten quadrats through transect walking. Over or under sampling of the vegetation was avoided by standardizing the sampling time based on the area of CG. For each of the ten GI areas surrounding a CG site at the different distances, the present GI was first categorized in the field as hedgerow, road verge, forest border, grass strips or a combination of the latter. All the plant species present in the GI were documented and their abundance was scored; abundant (A; 80 %), common (C; 65 %), frequent (F; 50 %), occasional (O; 30 %) and rare (R; 10 %).



Restored sites			Ancient sites		
Nr	**Abbr**	**Fragment name**	**Nr**	**Abbr**	**Fragment name**
1	MC2	Mwène à Vaucelles	7	RL	Roche à Lomme
2	SP1	Spineu	8	CH	Chalaine
3	MB7	Montagne aux Buis	9	GA	Gay
4	FDC1	Fondry des Chiens	10	CO	Contièneau
5	CA1	Champ d'al Vau	11	LH	Les Hurées
6	NM1	Niémont	12	BO	Bonnerieu

Figure 3.1: Study area located in the Viroin valley. Each sampled fragment consists of a legend number, abbreviation and full fragment name. Six sites are restored and six sites are ancient semi-natural grassland (Adriaens et al., 2006).

3.3 Statistical analyses

3.3.1 Comparison restored and ancient focal patches

Statistical analyses of the data were implemented using R to compare restored and ancient focal patches. The surveyed plant species were divided in three categories of interest; all species, specialist species and species dispersed by epizoochory (Table 3.1). Specialist species are defined as species limited to calcareous grasslands of Belgium. Plant species dispersed by epizoochory are considered plants from which the seeds are transported on the outside of vertebrate animals, mostly sheep in this study site (Adriaens (2008), appendix 4.1 page 90). To evaluate differences in plant species between old and restored focal patches, different analyses were performed; four distinct Non Metric Multidimensional Scaling analyses (NMDS), with Bray-Curtis distance matrix and hellinger transformed. This was implemented to examine differences in species community composition of ancient and restored patches. Additionally, it was evaluated if the species richness differed between ancient and restored focal patches for the three categories of interest.

First, an exploratory NMDS was performed to evaluate differences in plant species composition of *all* the surveyed plants (n=176) among all quadrats of the restored and ancient focal patches (Addendum; Results A.2.1). The NMDS was performed using the *metaMDS* function in the *Vegan* package of R. An *adonis* analysis was performed afterwards to compare ancient and restored patches. This function partitions sums of squares by using the distance matrix.

Second, an exploratory NMDS was executed on the mean of all the quadrats per focal patch, which contained specialist species counts. This was performed to evaluate if there is a difference in specialist species community composition between ancient and restored focal patches. The three other NMDS analyses were always performed on the data encompassing all the separate quadrats surveyed. An *adonis* analysis was implemented to compare ancient and restored focal patches (Addendum; Results A.2.2).

Third, differences in specialist plant species community composition (n=33) between all quadrats of the ancient and restored focal patches were examined by an NMDS. Using the *adonis* function, the specialist species community composition of restored and ancient focal patches were compared.

Fourth, a similar NMDS was performed on the plant species dispersed by epizoochory (n=12) of all quadrats and followed by an *adonis* analysis, to compare ancient and restored focal patches.

Fifth, the species richness of all plant species, specialist plant species and species dispersed by epizoochory was calculated for ancient and restored focal patches using the *specnumber* function. Mann-Whitney U Tests were executed using the function *wilcox.test* to determine if the species richness of the three categories of species differed between the ancient and restored focal patches.

3.3.2 Impact of green infrastructure

Next, four analyses were performed to evaluate the impact of surrounding GI on the species community composition and species richness of the twelve focal patches. First, differences in specialist plant species community composition (n=40) between all quadrats (ancient and restored) and the surrounding GI was tested (Table 3.1). The NMDS was performed on the joined data of the focal patches and the data of the GI with a hellinger transformed Bray-Curtis distance matrix. Using the *adonis* function, the specialist species community composition of all focal patches was compared to the specialist species community composition of all the surrounding GI.

Second, the dataset encompassing joined data on all focal patches and all the GI was separated. This resulted in twelve datasets of each of the twelve focal patches with its ten surrounding locations of GI. The *envfit* function of the R *Vegan* package was used to test if the specialist community composition of the GI surrounding a focal patch changes significantly when the distance to the focal patch increases. This function can fit environmental factors or vectors onto an ordination.

Third, for each focal patch and its ten surrounding GI locations, an analysis tested the dissimilarity in specialist species community composition of the focal patch and its surrounding GI over distance using the *vegdist* function in the R *Vegan* package with Bray-Curtis distance matrix.

Fourth, the species richness of the twelve focal patches (for specialist species, all species and species dispersed by epizoochory) were correlated with four types of environmental data; total GI (ha), road verges (ha), linear feature coverage excluding road verges (ha) and islets (ha). Correlating the species richness with these four types of GI was done for GI surrounding ancient and restored patches together and for ancient an restored patches separately. In R, comparing if the species richness differed between GI surrounding ancient and restored focal patches was done by a spearman rank correlation test using the *cor.test* function. The species richness was obtained by the *specnumber* function.

Table 3.1: Overview of the plant species (n=50) used for three distinct NMDS analyses (NMDS: 1, NMDS on specialist species; 2, NMDS on focal patches and surrounding GI; 3, NMDS on species dispersed by epizoochory) and the group the plant species belong to (Group: E, dispersed by epizoochory; S, specialist species) (Adriaens (2008), Appendix 4.1 page 90). GI; containing the 35 specialist plant species observed during the floristic surveys in the GI.

Species	Group	NMDS	GI
Allium oleraceum	S	1,2	x
Allium sphaerocephalon	S	1,2	x
Anthericum liliago	S	2	
Anthyllis vulneraria	S	1,2	x
Arabis hirsuta	S	1,2	x
Arrhenatherum elatius	E	3	
Aster linosyris	S	1,2	
Bupleurum falcatum	S	1,2	x
Carex flacca	S	1,2	x
Centaurea scabiosa	S	1,2	x
Cirsium acaule	S	1,2	x
Clematis vitalba	S	1,2	x
Clinopodium vulgare	S	1,2	x
Echium vulgare	S	1,2	x
Epipactis atrorubens	S	2	x
Euphorbia cyparissias	S	1,2	x
Galium verum	S	1,2	x
Genista tinctoria	S	1,2	x
Gentianella germanica	S	1,2	
Geranium columbinum	S + E	1,2,3	x
Helianthemum nummularium	E	3	
Helleborus foetidus	S	2	x
Hippocrepis comosa	S	1,2	x
Knautia arvensis	E	3	
Koeleria macrantha	S	1,2	x
Melica ciliata	S	1,2	x
Ophrys insectifera	S	2	
Pimpinella saxifraga	S	1,2	x
Plantago lanceolata	E	3	
Polygala comosa	S	1,2	
Polygala vulgaris	S	1,2	x
Polygonatum odoratum	S	2	x
Primula veris	S	1,2	x
Prunella laciniata	S	1,2	x
Prunella vulgaris	E	3	
Ranunculus acris	E	3	
Ranunculus bulbosus	S + E	1,2,3	x
Scabiosa columbaria	S + E	1,2,3	x
Sedum acre	S	1,2	x
Sedum album	S	1,2	x
Seseli libanotis	S	1,2	x
Sesleria caerulea	S	1,2	x
Teucrium chamaedrys	S	1,2	x
Thlaspi perfoliatum	S	2	x
Trifolium medium	E	3	
Trifolium pratense	E	3	
Urtica dioica	E	3	
Verbascum lychnitis	S	2	x
Vincetoxicum hirundinaria	S	1,2	x

Table 3.2: Stressvalue of the NMDS analyses on the hellinger transformed Bray-Curtis distance matrix and p-value of the adonis analyses.

	Stressvalue	p
Specialist species	0.17	0.001
Species epizoochory	0.14	0.078
Focal patches and GI	0.18	0.001
All species	0.25	0.001
Mean per focal patch	0.11	0.046

Chapter 4

Results

In this chapter, the data analysis results are presented. In section 4.1, a comparison between restored and ancient focal patches is made. Section 4.2 investigates the impact of green infrastructure on the focal patches.

4.1 Comparison restored and ancient focal patches

4.1.1 NMDS specialist species

The dataframe consists of 33 specialist plant species as variables (Table 3.1) and the 1 m^2 quadrat names (i.e. BO_1, BO_10) as rownames. Of the 120 quadrats, 4 quadrats were omitted for analysis since these contained no specialist plant species (MC2_7, GA_9, LH_4, RL_2). Visual examination of the NMDS plot suggested a difference in specialist plant species composition between the quadrats of ancient and restored focal patches (Figure 4.1). The NMDS had an acceptable stressvalue (0.17) (Table 3.2) and was visualized in a Shepard plot (Addendum; Figure A.5a). The *adonis* analysis was highly significant (p = 0.001) (Table 3.2). It can be stated that there is a difference in specialist plant species community composition between ancient and restored focal patches.

4.1.2 NMDS epizoochorous species

The dataframe consists of 12 species dispersed by epizoochory as variables (Table 3.1) and the 1 m^2 quadrat names as rownames. Of the 120 quadrats, 25 quadrats were omitted since these contained no species dispersed by epizoochory. Inspection of the NMDS plot suggested no difference in epizoochorous species community composition in the quadrats of ancient or restored focal patches (Figure 4.2). The NMDS had an acceptable stressvalue (0.14) (Table 3.2) and was visualized in a Shepard plot (Addendum; Figure A.5b). The NMDS was followed by an *adonis* with a marginally significant result (p = 0.078) (Table 3.2). It can be concluded that there is a marginally significant difference in epizoochorous species composition between ancient and restored focal patches.

4.1.3 Species richness

A dataframe was made consisting of *all* the surveyed species during the floristic surveys. This contained 176 plant species as variables and the 120 quadrat names as rownames.

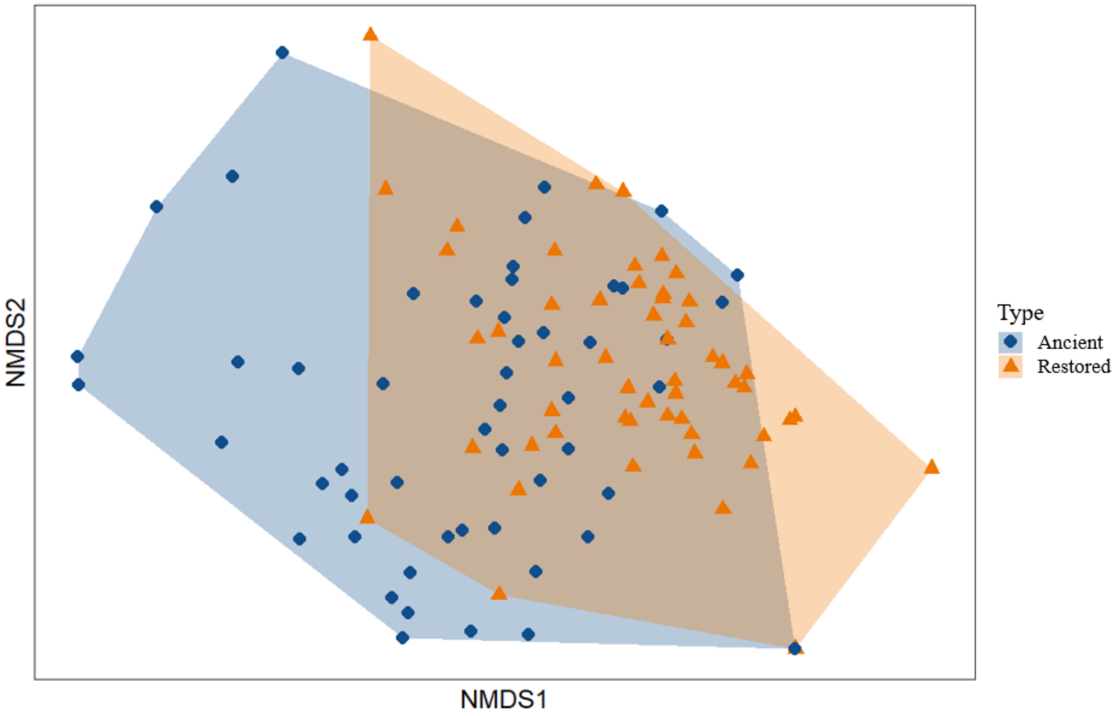


Figure 4.1: Non Metric Multidimensional Scaling (NMDS) on the hellinger transformed Bray-Curtis distance matrix on the quadrats of ancient and restored focal patches. Note that because in four of the quadrats no specialist species were present, these quadrats were omitted from the NMDS analysis.

For each of the 12 focal patches (for example BO), 10 quadrats were surveyed (BO_1 - BO_10). Here, the mean of the 10 quadrats was taken for each of the focal patches, resulting in a dataset with 12 means as variables. Using this dataset, the species richness for the three categories of interest was calculated. Mann-Whitney U Tests were executed to determine if the species richness of the three categories differed between the ancient and restored focal patches. First, the analysis on all the plant species rendered an insignificant result ($p = 0.47$). It can be concluded that the species richness of all plants surveyed did not differ between the ancient and restored focal patches. Second, the analysis on the specialist species resulted in a Mann-Whitney U Test that was not significant ($p = 0.17$). The specialist species richness does not differ between ancient and restored focal patches (Figure 4.3). Third, the analysis on the species dispersed by epizoochory was also not significant ($p = 0.58$), leading to the conclusion that the richness of epizoochorous species did not differ between ancient and restored focal patches. In all three Mann-Whitney U Tests, some ties were present making it more difficult to find an exact p-value since the test is based on ranks.

4.2 Impact of green infrastructure

4.2.1 NMDS focal patches and GI

The dataframe consists of 40 specialist species as variables (Table 3.1) and as rownames the 12 focal patch names and 94 GI locations. Normally each focal patch is surrounded by

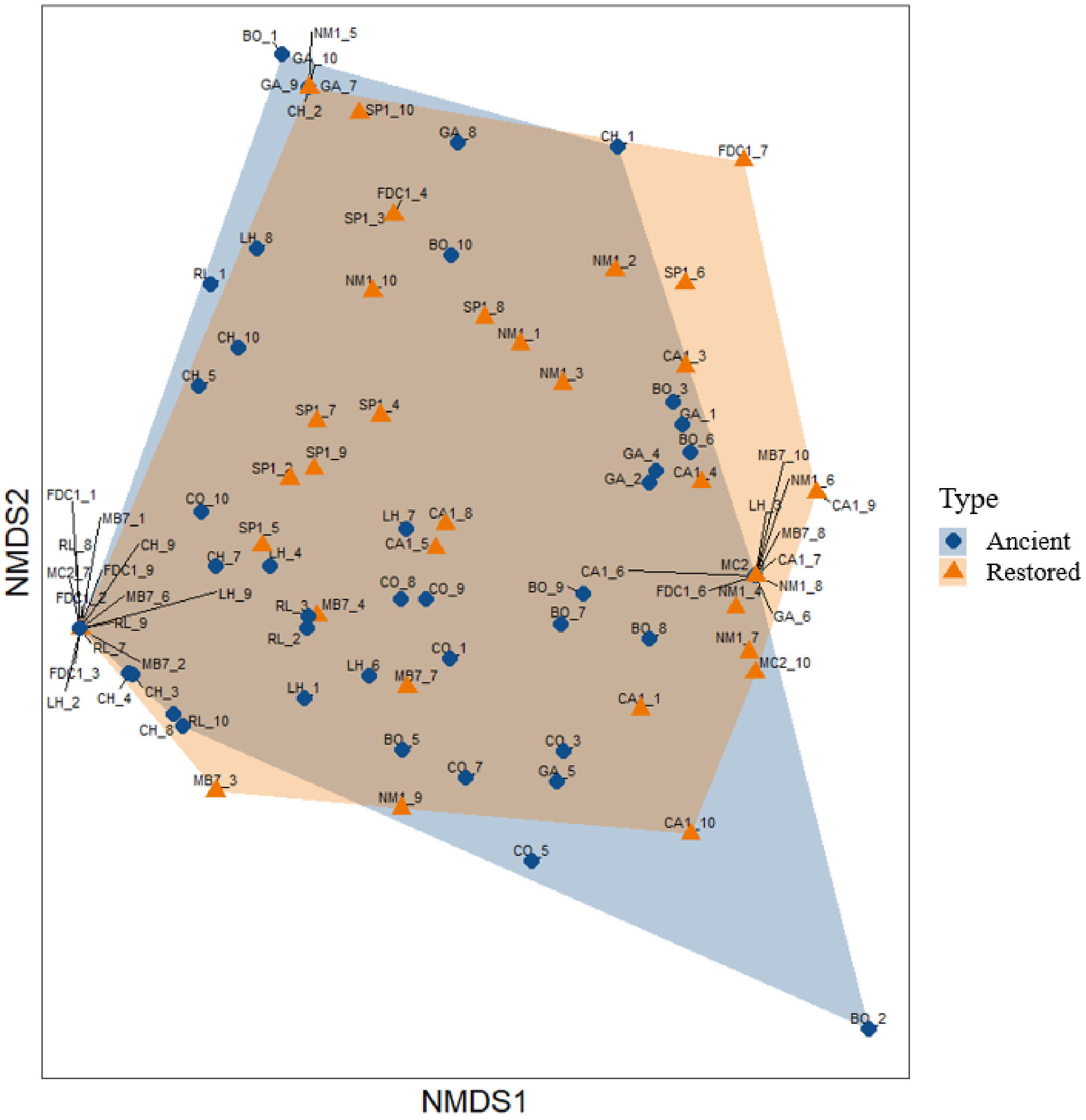


Figure 4.2: Non Metric Multidimensional Scaling (NMDS) on the hellinger transformed Bray-Curtis distance matrix on the quadrats of ancient and restored focal patches. Note that twenty-five quadrats were disregarded from the NMDS analysis because these contained no species dispersed by epizoochory.

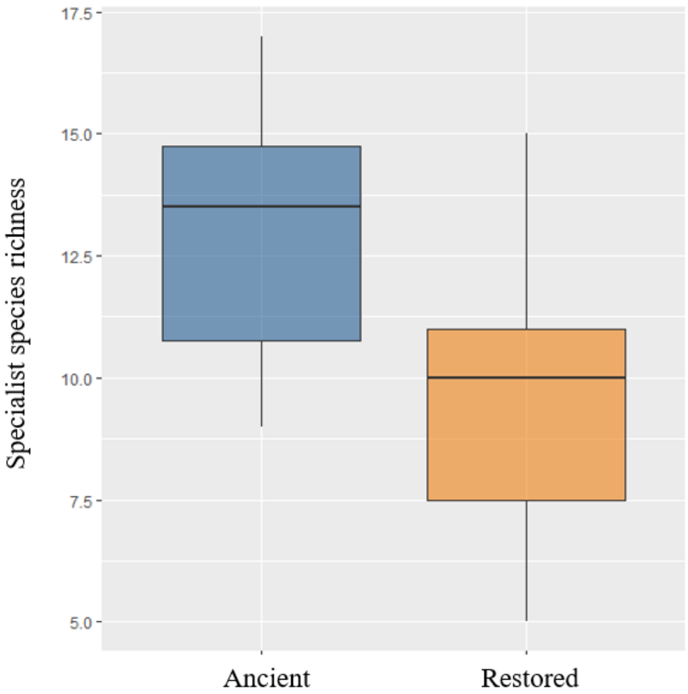


Figure 4.3: Boxplot of the specialist species richness in the six ancient and six restored focal patches. The Mann-Whitney U Test showed a non significant difference (p = 0.17) in the number of specialist species between ancient and restored focal patches.

10 GI locations. Here, 26 GI locations were omitted from the analysis since they did not contain any specialist species. The dataset containing specialist species in focal patches were counts, the dataset containing specialist species in GI (Table 3.1) were estimated counts using the ACFOR scale (Material and Methods 3.2). The data were normalized after joining both datasets. Visual examination of the NMDS plot implied a difference in specialist plant species community composition between the focal patches and the surrounding GI (Figure 4.4). The NMDS showed an acceptable stressvalue (0.18) (Table 3.2) and was visualized in a Shepard plot (Addendum; Figure A.5c). The *adonis* analysis was highly significant (p = 0.001)(Table 3.2). A difference in specialist plant species community composition can be observed between focal patches and the surrounding GI.

4.2.2 Impact of distance on species composition in GI

Visual inspection of the 12 plots suggests a change in GI specialist species community composition in 3 plots (Figure 4.5). The *envfit* analysis revealed 3 significant p-values for GI surrounding restored focal patches (CA1, p = 0.004; MB7, p = 0.005; MC2, p = 0.026). None of the GI surrounding ancient focal patches gave a significant result (Table 4.1). The specialist species community composition of GI surrounding *restored* focal patches changes with increasing distance to the focal patch. Of the 12 datasets, 4 had insufficient data to accurately conduct an analysis since some of the 10 sampled GI locations per focal patch did not contain any specialist plant species (LH, MC2, RL and SP1). Subsequently, the results have to be interpreted with caution.

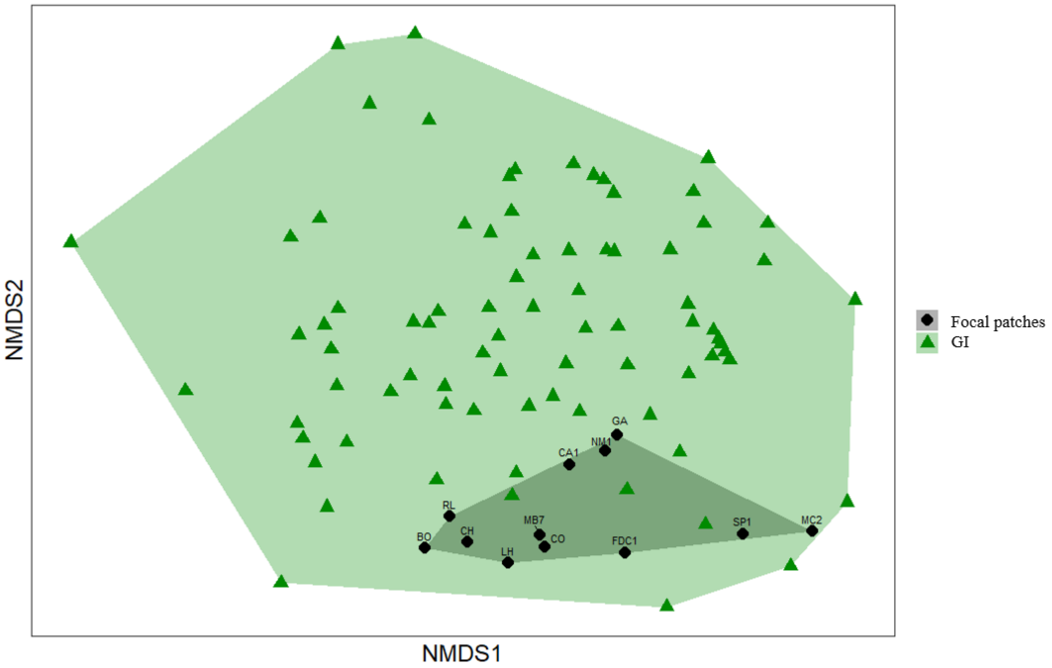


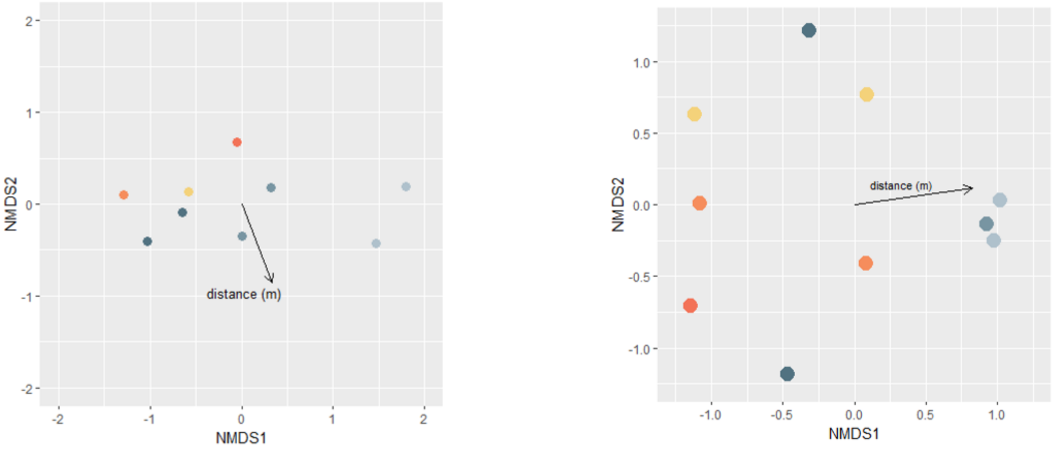
Figure 4.4: Non Metric Multidimensional Scaling (NMDS) on the hellinger transformed Bray-Curtis distance matrix on the twelve focal patches and surrounding GI locations. Note that because in twenty-six of the GI locations no specialist species were present, these locations were omitted from the NMDS analysis.

4.2.3 Dissimilarity focal patches and GI

The 12 separate datasets from section 4.2.2 were used, containing normalized counts of the focal patch and its 10 surrounding GI locations. A *vegdist* analysis tested the dissimilarity in specialist species community composition in the focal patch compared to its surrounding GI over distance (Figure 4.6). When the dissimilarity is 0, the specialist species community composition present in the GI would be equal to the specialist species community composition found in the focal patch. Conversely, when the GI reaches a dissimilarity of 1, it has no specialist species in common with the focal patch. The dissimilarity of the specialist community composition in the GI increases with increasing distance to the focal patch in 4 out of the 12 analyses. Of these results, 3 were in restored focal patches (CA1, FDC1 and MB7) and 1 in an ancient focal patch (GA). Of the 8 remaining analyses, 1 restored focal patch has a reversed trend (SP1) and the other 7 focal patches have a rather unexpected trend (BO, CH, LH, RL, CO, NM1, MC2) (Addendum; Figure A.6).

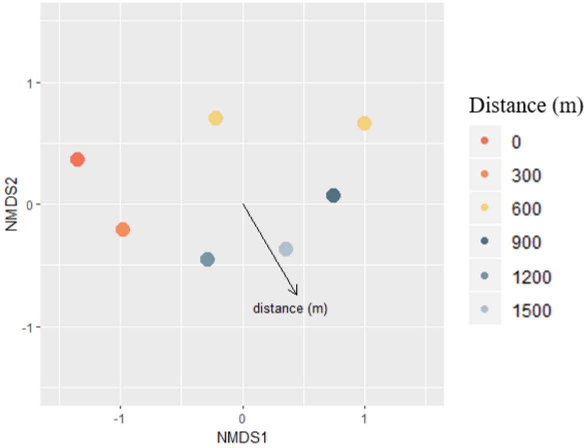
4.2.4 Correlation species richness and environmental data

The species richness was calculated from 3 datasets. The dataset containing *all* surveyed species used in section 4.1.3, the dataset of species dispersed by epizoochory from section 4.1.2 and another dataset was created using the dataset of section 4.1.1. Instead of using all the separate quadrats and their counts, the mean of the 10 quadrats per focal patch was taken, resulting in a dataset with 12 rownames and 33 specialist species as variables. The results revealed that 3 of the spearman rank correlation tests were significant. First, when



(a) CA1

(b) MB7



(c) MC2

Figure 4.5: Visualization of the three significant envfit analyses on focal patches and their surrounding GI; CA1 (p = 0.004), MB7 (p = 0.005), MC2 (p = 0.026). The specialist species composition of GI surrounding restored focal patches changes with increasing distance to the focal patch.

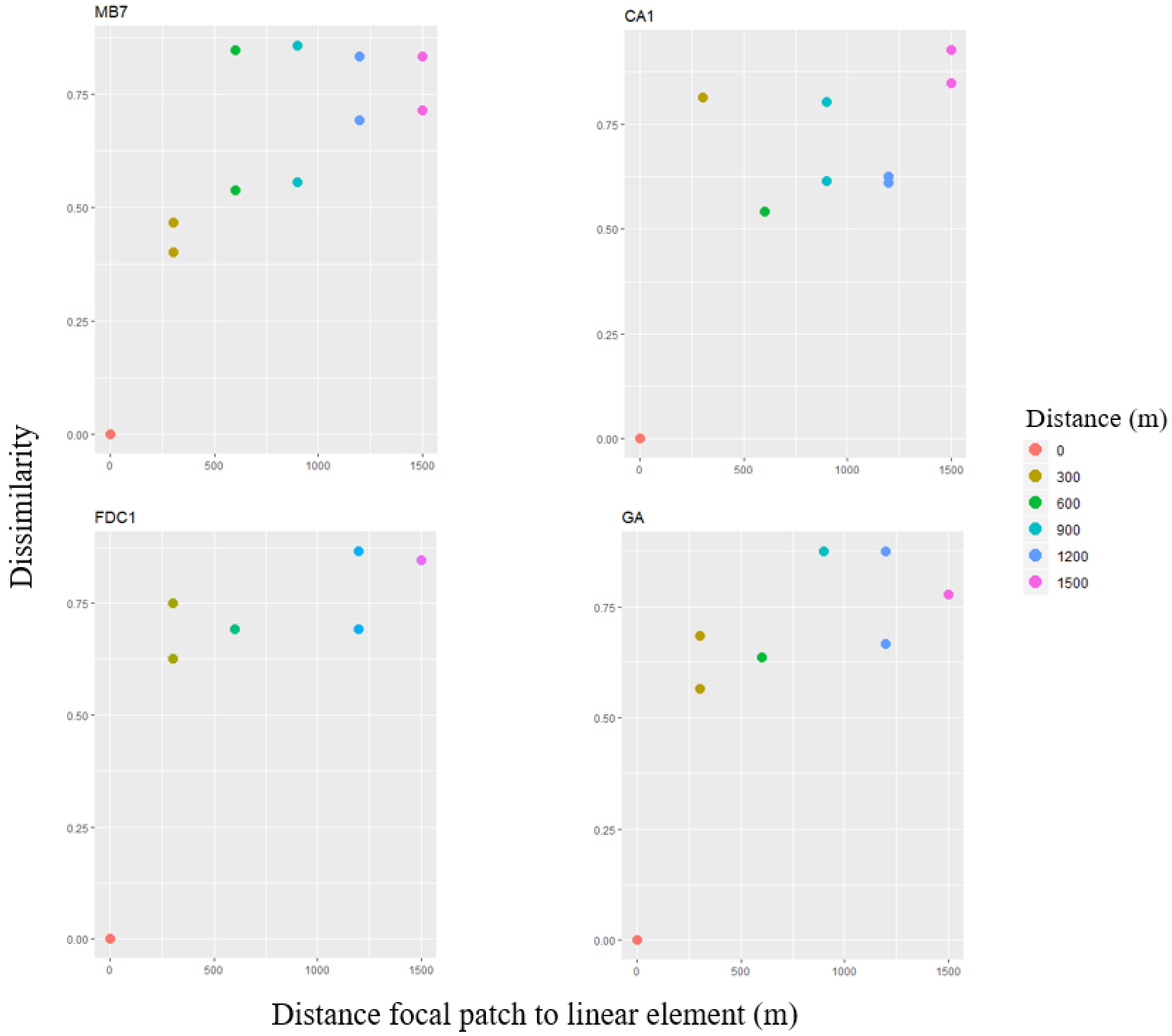
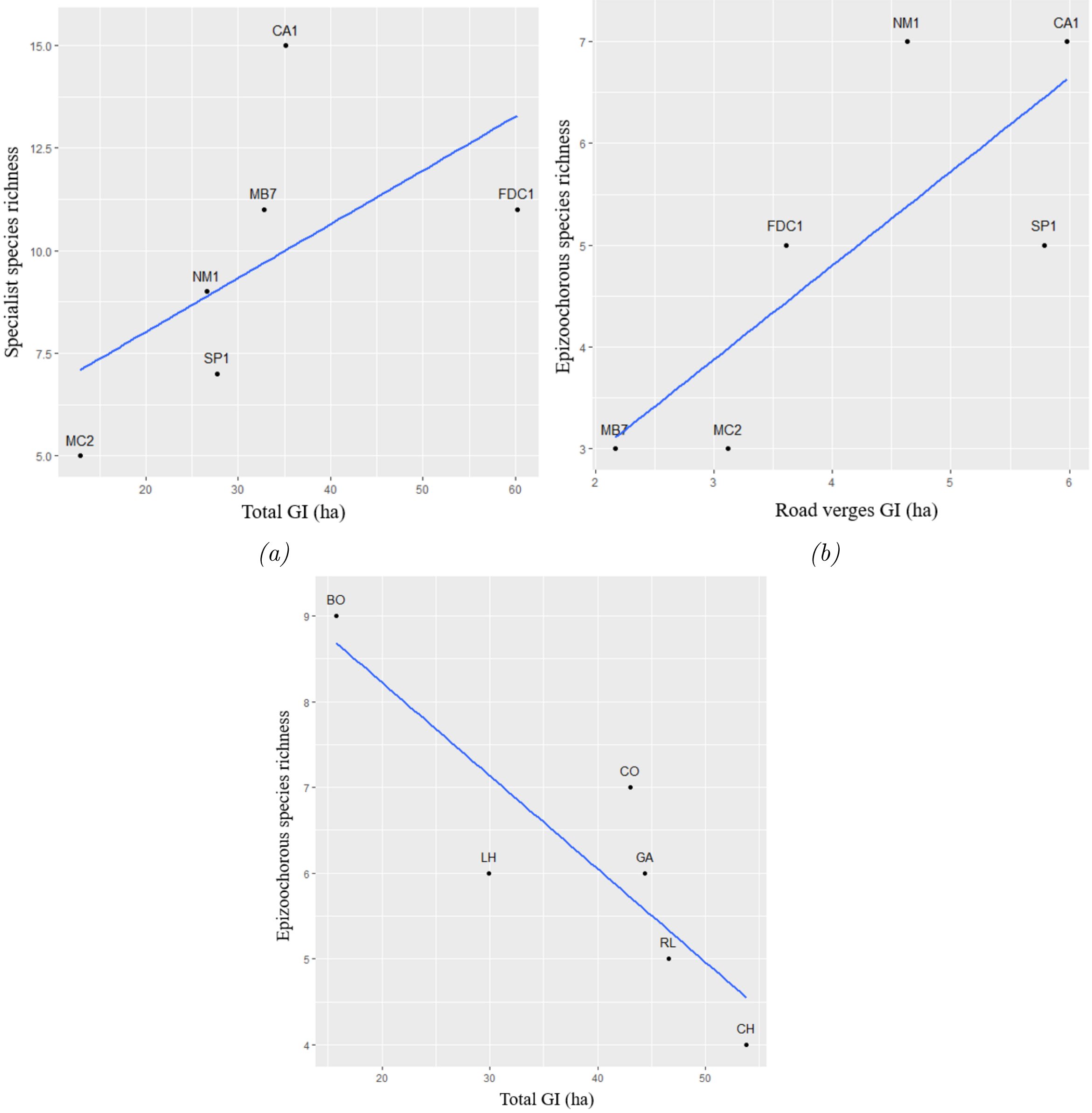


Figure 4.6: The vegdist analysis on the four significant focal patches and their surrounding GI (MB7, CA1, FDC1, GA). The dissimilarity of the specialist species composition in the GI increases with increasing distance to the focal patch. Note that three out of the four significant results were found in restored focal patches.



(a) *(b)*

(c)

Figure 4.7: Visualization of the three significant correlations. Figure 4.7a; when the total GI (ha) surrounding the restored focal patches increases, the specialist species richness of these patches also increases. Figure 4.7b; when the amount of road verges (ha) surrounding the restored focal patches increases, the epizoochorous species richness in these focal patches increases. Figure 4.7c; when the total GI (ha) surrounding an ancient focal patch increases, the epizoochorous species richness decreases.

Table 4.1: Results of the envfit analyses on focal patches and their surrounding GI examining if the species composition of the GI surrounding a focal patch changes significantly with increasing distance to the focal patch. Note that in four of the datasets some GI locations were removed since these contained no specialist species (LH, MC2, RL and SP1), resulting in insufficient data.

	p
BO	0.548
CH	0.817
CO	0.603
RL	0.172
GA	0.368
LH	0.642
CA1	0.004
MB7	0.005
MC2	0.026
NM1	0.284
FDC1	0.404
SP1	0.517

the total GI (ha) surrounding the *restored* focal patches increases, the *specialist* species richness of these patches also increases ($p = 0.036$; $rho = 0.840$) (Figure 4.7a). Second, when the amount of road verges (ha) surrounding the *restored* focal patches increases, the richness of epizoochorous species in these focal patches increases ($p = 0.038$; $rho = 0.837$)(Figure 4.7b). Third, when the total GI (ha) surrounding an ancient focal patch increases, the epizoochorous species richness in these focal patches decreases ($p = 0.015$; $rho = -0.89$) (Figure 4.7c).

Chapter 5

Discussion

5.1 Comparison restored and ancient focal patches

The main aim of this thesis was to determine the importance of GI for the conservation of plant species in fragmented CGs, which was split up in two smaller goals. The first goal was to assess if there is a difference in the species community composition and species richness of three categories of interest (all plant species, specialist species and species dispersed by epizoochory) between ancient and restored CGs. The ancient grassland patches function as reference or control sites (Piqueray et al., 2011; Fagan et al., 2008). The second aim of this thesis was to determine the impact of GI on the species community composition and species richness of the CGs.

For the first aim, it is clear that the species community composition of all three categories differs between ancient and restored CG patches. Although the difference in epizoochorous species community composition was only marginally significantly different between ancient and restored patches. The expectation was that there would be no difference in community composition of epizoochorous species between ancient and restored patches because of grazing regimes with sheep that disperse seeds in their woolly coat, which act as living corridors between grassland patches. Adriaens (2008) also emphasized the major role of long distance seed dispersal by grazing sheep flocks. An explanation for the difference could be the source used in creating the epizoochorous species list; Adriaens (2008) Appendix 4.1 page 90. Their list was constructed using literature and existing databases, supplemented with the knowledge of Poschlod et al. (2005) on attachment potential. Furthermore, notice that only floristic surveys were performed in this study, not detecting plant seeds directly in the coat of the animals. The fact that the composition of species dispersed by epizoochory is different between ancient and restored patches can also be due to both pre-dispersal and post-dispersal processes.

Pre-dispersal processes are becoming and staying attached to the fur, and are influenced by plant traits (e.g. plant height, seed production and species abundance in the vegetation) altering a species chance to disperse across long distances (Adriaens, 2008; Poschlod et al., 2005). Post-dispersal processes (e.g. seed predation, unfavorable abiotic conditions, species establishment and recruitment) may be the cause of a less straightforward relation between the observed species distribution pattern and the potential dispersal by grazing animals. Additionally, 25 % of the observed species dispersed by epizoochory were also specialist species. This may further influence the chance of establishment since specialist species demand more specific abiotic conditions compared to generalist species. Nonetheless, this result is only marginally significant, suggesting that many of these species do get transferred and establish in restored CGs through epizoochory by moving animals.

The difference in specialist species composition between ancient and restored CGs is not so surprising since the poor dispersal capacity of specialist species and the longer establishment time on recently restored sites has been emphasized by previous research (Jacquemyn et al., 2011; Fagan et al., 2008; Cousins and Lindborg, 2007). Although the sheep grazing regime could help bridge the large distances between different CGs, only 9.1 % of the specialist species are also epizoochorous plant species. Additionally, the restored patches are relatively young (restored between 1995 and 2066) causing the abiotic conditions to be less ideal compared to the ancient grassland patches (through N and P enrichment causing acidification, toxic metals and mineral nutrient deficiencies) (Piqueray et al., 2011; Štýbnarová et al., 2017; Phoenix et al., 2008; Ceulemans et al., 2013; Stevens et al., 2010; Clark and Tilman, 2008; Van Den Berg et al., 2011). Older restored sites better resemble the plant community composition of reference sites compared to younger restored sites. The process is slow and can easily exceed sixty years (Fagan et al., 2008). Furthermore, not only temporal factors but also landscape composition (i.e. extreme isolation) can cause the delay in community assembly in restored patches (Helsen, 2013), a phenomenon referred to as a colonization credit.

When assessing the species richness of the three plant categories, no difference could be observed in species richness between ancient and restored patches. Restored CGs did showing a larger variation in specialist species richness compared to the ancient CGs. Even though the specialist community composition is different, the specialist species they do have in common are rather similar in numbers.

Summarized, the specialist species that overcome dispersal limitation and colonize restored CGs are able to establish a population of equal size as in the ancient CGs. This suggests that the abiotic conditions of the ancient and restored CGs are more similar then previously thought, and that the main problem is overcoming dispersal limitation which is aggravated by habitat fragmentation. The fact that ancient CG patches contain more different species of specialist plants can probably be attributed to the older age and continued management. Subsequently, there was more time for the plant species to overcome dispersal limitation and establish a population elsewhere.

5.2 Impact of green infrastructure

The second aim of this thesis was to determine the impact of GI on the species community composition and species richness of the CGs. First, through comparing the specialist community composition between CGs and all the surveyed GI in the landscape. Second, through investigating if the specialist community composition of the GI changes with increasing distance to the CG. Third, examining the change in dissimilarity of specialist community composition over distance, assuming that closer GI will be more similar and GI further away will be less similar in specialist community composition to the CG. Fourth, a correlation was established between environmental data (total GI (ha), road verges (ha), linear feature coverage excluding road verges (ha), islets (ha)) and the species richness of the three categories of interest (all species, specialist species and species dispersed by epizoochory).

First, it was clear that the specialist community composition of the CGs was remarkably different from the specialist community composition of the surrounding GI. Presumably, this can be due to stronger edge effects in the GI leading to a higher extinction probability since the area is considerably smaller compared to the CGs (Lindborg et al., 2014). Especially eutrophication effects are more pronounced compared to the effects of forest presence near the edge of GI (Piessens et al., 2006). Additionally, it is possible that certain specialist species are unable to disperse to the surrounding GI. Furthermore, abiotic factors that differ too much from the CG can withhold specialist species from developing and sustaining a viable population, even though they might have managed to disperse. A combination of these factors may be the cause that specialist species are unable to maintain a population in the GI.

Second, in half of the restored CGs (CA1, MB7, MC2), the specialist species composition of GI surrounding these restored CGs changes with increasing distance to the CG (Cousins and Lindborg, 2007; Lindborg et al., 2014). No change with distance was observed in species composition of GI surrounding the other three restored CG patches (FDC1, SP1, NM1). In addition, there was no change in the species composition of GI surrounding all the ancient CGs. It may be assumed that the GI surrounding restored CGs has an influence on that CG and thus might serve as stepping stones for specialist plant species (Cousins and Lindborg, 2007; Lindborg et al., 2014). This in contrary to GI surrounding ancient CGs were no significant influence was observed.

Why only three of the six restored CGs and their GI had a specialist community composition changing over distance might be attributed to the still unknown requirements of GI to be functional. Factors contributing to the success of GI might be the total area of GI (ha), the type of GI (Lindborg et al., 2014) (see chapter 7; future perspectives), the habitat surrounding the GI (Piessens et al., 2006), age of the GI and placement of the GI in the landscape (Heard et al., 2012; van Nouhuys, 2016; Shima et al., 2010). Even factors that are not related to the GI itself could influence its success. For example, age of the nearest CGs (encompassing a higher amount of different specialist species increases the chance of spreading a specialist plant to the surrounding GI) and seed dispersal mechanisms. It can be concluded that these requirements were fulfilled in three of the restored CGs since a significant effect was discovered.

Data was available to investigate some of the factors that might influence the success of GI; age of the nearest CG and the total area of GI (ha). For age it could be expected that older restored CGs have a specialist composition in the surrounding GI that does not change much over distance since the species had time to overcome potential dispersal limitation and establish a population there. However, comparing the age of restored CGs that had no significant difference in specialist composition over distance (2001, 2002 and 2004 as restoration years) and the restored CGs with a significant difference (1995, 2003 and 2006 as restoration years) do not differ substantially. Age is probably not causing the change in GI specialist composition over distance. Presumably, a stable specialist composition in the GI surrounding a CG might only be noticeable around very ancient CGs.

Another explanation could be that there was less total area of GI (ha) surrounding the restored patches that had GI with a changing specialist composition over time. This could be associated with smaller patches of GI and subsequently stronger edge effects and increased difficulty establishing a population of specialist species. Additionally, less total GI surrounding the restored CGs might lead to less space for new specialist species to establish. The total area of GI (ha) surrounding the patches with a significant change (35.19, 32.80 and 12.93 ha) and without significant change (60.21, 27.68 and 26.56 ha) do not differ substantially. The change in specialist community composition over distance can presumably not be attributed to smaller total area of GI surrounding the CGs.

Although age of the restored patches and the total area of GI (ha) were not substantially different here, it can not be excluded as influencing elements since only six restored patches were analyzed. An analysis on a larger number of restored patches is required to truly exclude these two factors in influencing the specialist species composition observed in the surrounding GI.

Third, the dissimilarity in specialist community composition between the CG and the surrounding GI increases with increasing distance to the CG. This change was observed in half of the restored CG patches (MB7, CA1, FDC1) and only in one ancient CG patch (GA). In these cases, it is clear that the surrounding GI serves as stepping stones for specialist species to disperse from the surrounding landscape into the CGs. Remarkably, GI around restored CG patches seems to have a more substantial effect, emphasizing the importance of GI for restoration of these types of CGs.

In four of the six ancient CG patches (BO, CH, RL and LH), a more concave function was observed. First the similarity in specialist species decreases with distance and starts to increase again after approximately one kilometer. This is rather unexpected since the assumption was that the specialist species composition of the several GI locations surrounding ancient patches would be almost equal. This assumption was made since there are more specialist species in ancient CG patches to disperse and establish in the surrounding GI, resulting in some of them establishing a population. It might be possible that the specialist species did disperse to all the GI locations, but in some locations the abiotic conditions did not match the demands of most specialist species to establish and maintain a viable population. This might be attributed to the still unknown requirements for GI to be functional.

Summarized, the stepping stone effect is observed in the GI of three of the six restored CGs, and only once in the GI of an ancient CG. This suggests that the influence of GI is more important on restored CGs compared to ancient CGs. When combining all data, two of the restored CGs have both a significant change in specialist composition and an increasing dissimilarity over distance (CA1 and MB7). In restored CG patch MC2, only the specialist composition changes over distance. The dissimilarity with the GI increases over distance but ceased to have any specialist species in common after 600 m distance.

In restored CG patch FDC1, there is no significant change in specialist composition over distance, but the dissimilarity with the GI increased over distance. Consequently, it can be stated that in four of the six restored grasslands (CA1, MB7, MC2 and FDC1), GI is important in providing specialist species that disperse towards the restored CG and aid restoration. Presumably, the specialist species flow goes in the other direction for ancient CGs and their GI. The species rich ancient CGs serve as a source population and the surrounding GI as sink (Honnay and Jacquemyn, 2012). In this point of view, an ancient CG is more important for the surrounding GI than vice versa.

Fourth, when the total GI (ha) surrounding the restored CG patches increased, the number of specialist species in these restored patches also increased. This implies that GI has a positive effect on the specialist richness of the restored CGs. On the contrary, this trend was not observed for the ancient CGs and their surrounding GI. Furthermore, when the number of road verges surrounding the restored CG patches increased, also the number of species dispersed by epizoochory increased. Potentially, these additional road verges provide a refuge for smaller animals that carry seeds in their fur and transfer it to the restored CG.

A less expected trend was also observed; when the total GI (ha) surrounding an ancient CG increased, the number of species dispersed by epizoochory *decreased*. Since more GI should be linked to more plant species dispersed by epizoochory, the lower numbers might be due to trouble establishing in the ancient CG patches by different post-dispersal processes mentioned earlier. Potentially, the species pool of non-epizoochorous species is larger in these ancient CGs, which might lead to competition that hampers colonization of species dispersed by epizoochory. Otherwise this result is rather illogical, leading to no additional explanations.

Summarized, it is clear that the amount of GI (total GI and road verges) has a greater influence on the plant community composition in restored CGs and less effect on the plant community composition in ancient CGs. This could be due to the fact that restored CGs contain less specialist species and less species dispersed by epizoochory and still have potential to be colonized by new species from the surrounding GI. It is clear that GI positively assists the restoration process in recently restored CGs by a specialist species flow towards the restored CGs. In contrary, ancient CGs are almost not affected by the surrounding GI. Potentially, they rather serve as sources of specialist species to disperse from the ancient CG towards the GI of the surrounding landscape.

Chapter 6

Conclusion

Calcareous grasslands (CGs) are of high conservation interest since they are one of the richest herbaceous environments of the European continent. They comprise a large variety of available habitats and are reservoirs for both fauna and flora, providing numerous ecosystem services. Since the decline in low-intensive farming, these habitats have been subject to various threats of which habitat fragmentation is the most important one. Especially since specialist species of CGs have extremely limited dispersal capacity. A consensus is that restoration of CGs has the highest potential of succeeding when remnant populations of typical CG species are still present in the landscape, emphasizing the importance of connectivity. Remnant patches and road verges in the landscape matrix might serve as refugia and stepping stones for dispersal of these specialist species. Such refugia are referred to as green infrastructure (GI). The concept of GI is well-known in management and conservation policies. Although there is not much empirical evidence on the actual ability of GI to enhance functional connectivity and biodiversity in the landscape. Additionally, most studies focused on two types of GI; road verges and midfield islets. This study took a broader view on GI, encompassing road verges and midfield islets, hedgerows, field boundaries, small remnant and formerly grazed forest borders.

For this study, twelve circular locations were selected in the Viroin Valley in the Southern part of Belgium. In the center of each circle, a CG was present (six restored CGs and six ancient CGs). Floristic surveys were performed in the CG using ten quadrats of $1m^2$ and scoring the abundance of each species. Surrounding the CG center of each circular location, ten sites with GI were randomly selected at five different distances. The plant species present in the GI were documented and their abundances were scored.

When comparing the ancient and restored CGs, there was a difference in species community composition of specialist species and epizoochorous species. The community composition of epizoochorous species was only marginally significantly different between ancient and restored CGs, meaning that many species do get transferred and establish through moving sheep or other animals. The slight difference might be due to pre and post-dispersal processes. The difference in community composition of specialist species could be due to the older age of ancient patches and their continuous management, giving the species more time to overcome dispersal limitation and establish a population. Additionally, it can be expected that restored patches encompass less ideal abiotic conditions compared to ancient patches. Resulting in the fact that both temporal factors and the landscape composition play a role in the observed difference. We also found that there was no difference in species richness of specialist plants between ancient and restored CGs. Meaning that the ancient and restored CGs have a different community composition of specialist species, yet the species they do have in common are similar in numbers. This leads to the assumption that the abiotic conditions between ancient and restored patches are not that different, indicating that dispersal limitation aggravated by habitat fragmentation is the main cause of a colonization credit in the restored CGs.

When evaluating the impact of GI on the CGs, there is a remarkable difference in specialist species community composition between the GI and the CGs. This can be attributed to a combination of factors. The smaller area of the GI results in stronger edge effects (e.g. eutrophication) that leads to a higher extinction probability. Furthermore, the abiotic conditions might differ too much from these of the CGs. This potentially causes difficulties for specialist species to establish and sustain a viable population in the GI, even though they might have managed to disperse.

Furthermore, two of the six restored CG patches have surrounding GI with both a significant change in specialist composition over distance and an increasing dissimilarity between the specialist composition of the CG and GI over distance. In one of the restored CGs, only the specialist composition changes over distance. The dissimilarity with the GI also increases over distance but ceased to have any specialist species in common after a shorter distance. In one other restored CG patch, there is no significant change in specialist composition over distance, but the dissimilarity with the GI does increase over increasing distance from the CG.

Consequently, it can be stated that in four of the six restored grasslands, GI is important in providing specialist species that disperse from the surrounding GI towards the restored CG and aid restoring biodiversity. The fact that not all the restored patches showed benefits from the surrounding GI, might be due to the yet unknown requirements for GI to be functional. Ancient CGs are remarkably less affected by the presence of GI compared to restored CGs. When looking at different types of GI, the number of road verges and the total amount of GI have a significant influence on the community composition present in restored CGs and less effect on community composition in ancient CGs. Restored patches surrounded by more GI lead to a community composition encompassing more specialist species and more epizoochorous species.

To conclude, the specialist community composition of ancient and restored CGs is different, but the species they do have in common are similar in numbers. This points towards dispersal limitation as the main cause. Furthermore, restored CGs contain less specialist species and less species dispersed by epizoochory and consequently still have potential to be colonized by new species from the surrounding GI. It seems that total GI in the landscape and GI of road verges positively affects the restoration process of restored CGs by a specialist species flow from the GI towards the restored CGs. The GI is more important for the restored CG than vice versa. Presumably, the specialist species flow goes in the other direction for ancient CGs and their GI. The species rich ancient CGs might serve as a source population and the surrounding GI might be a sink. In this point of view, an ancient CG is more important for the surrounding GI than vice versa.

Consequently, GI surrounding recently restored CGs seems a promising measure for specialist species of calcareous grasslands that use it as stepping stones in the landscape. It enables them to overcome dispersal limitation to some extent, caused by the current habitat fragmentation.

Chapter 7

Future perspectives

The design of the experiment revolved around twelve calcareous grasslands (six old and six restored locations), each surrounded by ten locations of GI. In the study area of the Virion Valley in Southern Belgium, a total of seventy-one CGs are present in the landscape (Adriaens, 2008). Due to time constrains, only twelve CGs were selected for floristic surveys. This implies that there is unexploited potential to obtain more data on the species present in the other CGs. Especially since the epizoochorous species community composition was marginally significantly different between ancient and restored CGs, more data on these species might provide a more conclusive result. Additionally, and also related to time constrains, only ten locations of GI were surveyed surrounding each CG. A consequence is that not all the surveyed locations of GI contained specialist species or epizoochorous species and were subsequently omitted from certain analyses, resulting in less data or even insufficient data. This could be avoided by sampling more locations encompassing GI.

Furthermore, the surveyed GI were always categorized in the field as hedgerow, road verge, forest border or grass strips. Although sometimes a combination of GI types occurred in one location. The type of GI was then noted according to the predominant type in that location. There should have been a subdivision between different types of GI in one location. This way, different types of GI and their effects could be compared. The research of Lindborg et al. (2014) was able to conclude that there is a difference in effects related to the type of GI; midfield islets are more beneficial compared to road verges. A subdivision could have explained why only half of the GI surrounding restored patches showed a significant change in specialist composition over distance, in contrary to the other restored patches. Maybe the GI surrounding restored patches with a significant change in specialist composition over distance were surrounded by a higher quality type of GI.

Additionally, it is remarkable that the requirements for GI to be functional are still largely unknown. It could be the total area of GI, the type of GI, the habitat surrounding the GI, age of the GI and its location in the landscape (Piessens et al., 2006; Heard et al., 2012; van Nouhuys, 2016; Shima et al., 2010). More research on these requirements is highly important to guide restoration efforts and it can positively influence the success of the intervention.

Lastly, it was assumed that specialist species dispersed from ancient CGs towards the surrounding GI in the landscape. It would be interesting to investigate if the plant populations in the GI are viable or if there are source-sink dynamics. Research on the specific life cycle transitions that influence the population growth rates of some key specialist species can determine if a population is viable and increases in size. If the population growth rate is insufficient in GI, it can be assumed that populations will disappear if the seed inflow from ancient CGs terminates (Honnay and Jacquemyn, 2012).

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Appendix A

Addendum

A.1 Material and methods

Table A.1: Details of the surveyed CGs in the Viroin Valley

Abbreviation	Name	Type	Surface area (ha)	Restoration year
RL	Roche à Lomme	Ancient	0.94	-
CH	Chalaine	Ancient	1.52	-
GA	Gay	Ancient	1.54	-
CO	Contièneau	Ancient	1.14	-
LH	Les Hurées	Ancient	0.87	-
BO	Bonnerieu	Ancient	1.05	-
MC2	Mwène à Vaucelles	Restored	5.85	2006
SP1	Spineu	Restored	5.25	2002
MB7	Montagne aux Buis	Restored	1.83	1995
FDC1	Fondry des Chiens	Restored	2.66	2004
CA1	Champ d'al Vau	Restored	1.12	2003
NM1	Niémont	Restored	1.77	2001

Figure A.1: Example of one out of the twelve surveyed areas, site Les Hurées (LH). The CG is situated in the center and surrounded by the ten sites containing GI.

Figure A.2: Close-up example of one of the LH sites containing GI (LH_540 in Figure A.1). Here, the the GI is characterized as 'road verge' and its floristic diversity was surveyed.

A.2 Results

A.2.1 exploratory analysis: NMDS all species

The dataset containing *all* the species observed during the floristic surveys from section 4.1.3 was used to perform an exploratory NMDS to evaluate differences in plant species community composition among all quadrats of the restored and ancient focal patches. This was done by an NMDS analysis on the hellinger transformed Bray-Curtis distance matrix. Visual observation of the NMDS plot (Figure A.3) suggests a difference in plant community composition between ancient and restored focal patches. The stressvalue (0.25) related to the visualization in reduced dimensions is relatively high (Table 3.2), which can also be observed in the subsequent Shepard plot by scatter around the line (Figure A.5d). This suggests that the original dissimilarities are not optimally preserved in this figure. Next, the community composition of restored and ancient focal patches were compared using an *adonis* analysis. This showed that there is a significant difference in total plant community composition between ancient and restored focal patches (Table 3.2).

A.2.2 exploratory analysis: NMDS mean focal patches

The dataset from section 4.2.4 was used with the 33 specialist species as variables and mean of the 12 focal patches as rows. This exploratory NMDS was performed on the hellinger transformed Bray-Curtis distance matrix. Visual observation of the NMDS plot (Figure A.4) suggests a difference in specialist community composition between ancient and restored focal patches. The NMDS had an acceptable stressvalue of 0.11 (Table 3.2), visualized in a Shepard plot (Figure A.5e). Eventually, the mean specialist species composition was compared between ancient and restored focal patches using an *adonis* analysis (Table 3.2). There seems to be a marginally significant difference in specialist species composition between ancient and restored focal patches (Table 3.2).

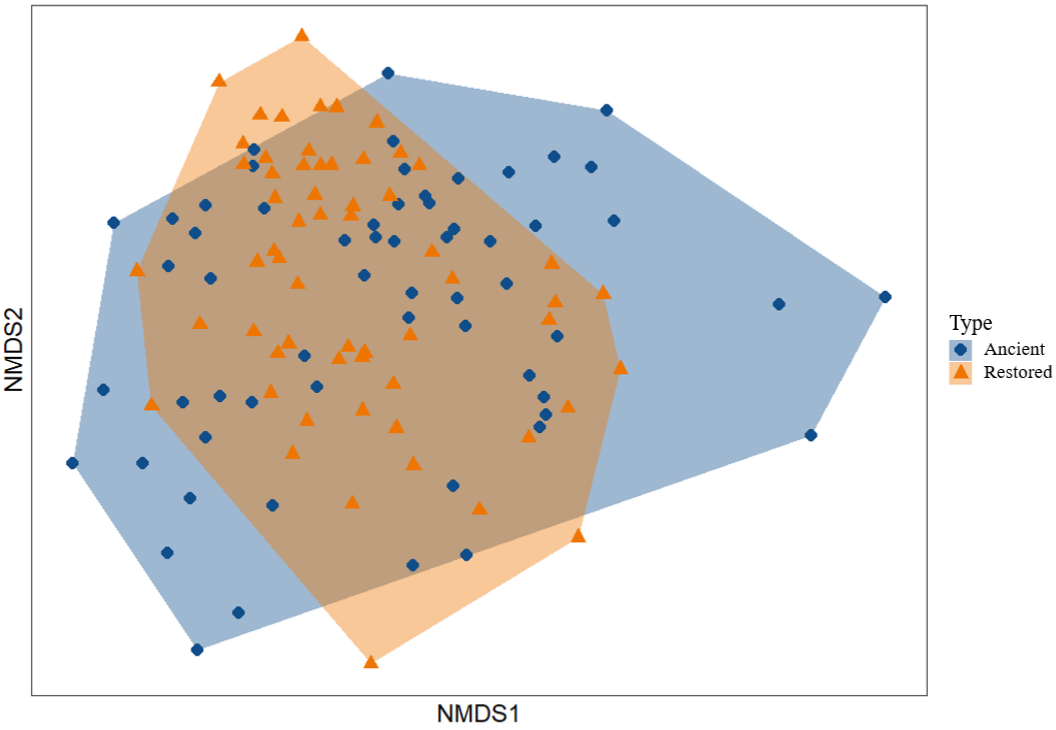


Figure A.3: Non Metric Multidimensional Scaling (NMDS) on the hellinger transformed Bray-Curtis distance matrix on all the species observed in all the quadrats of ancient and restored focal patches.

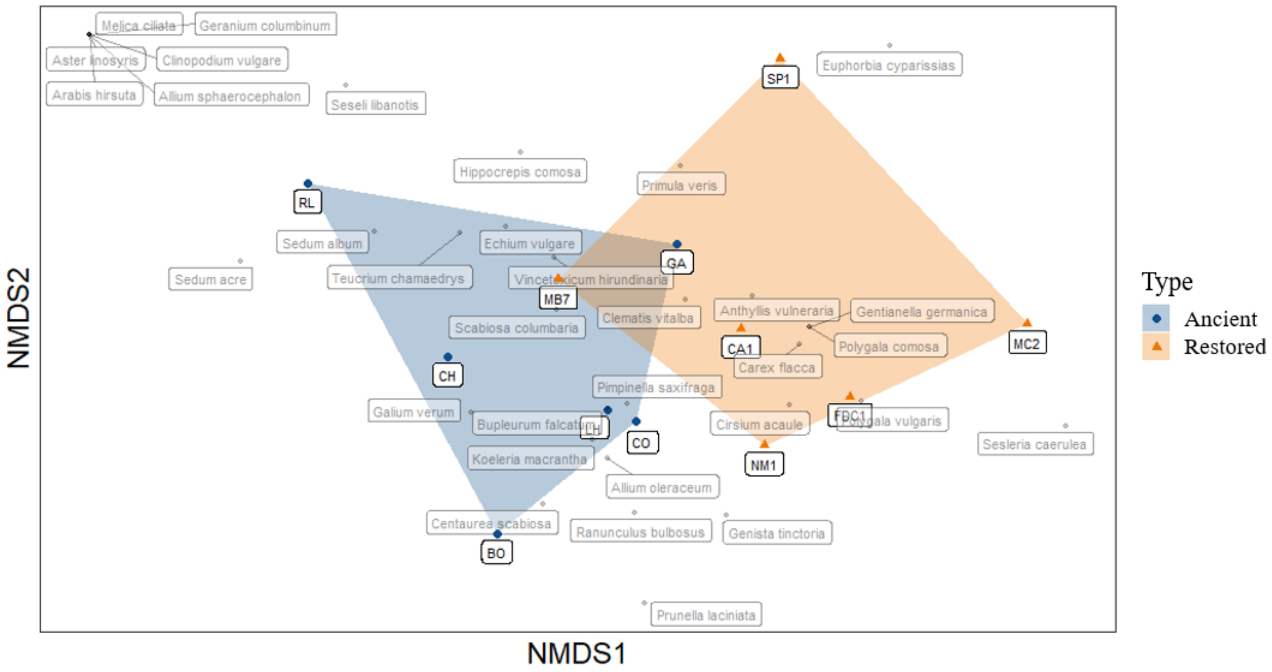
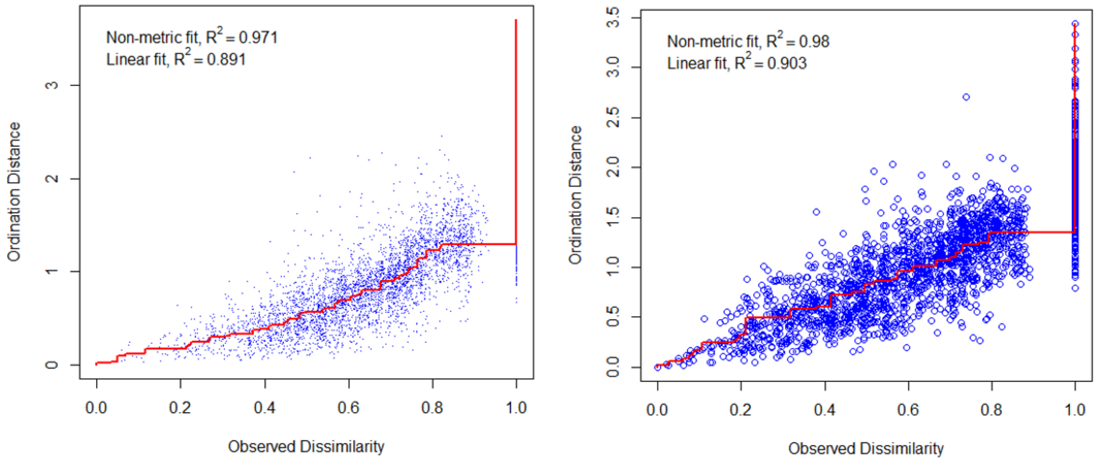
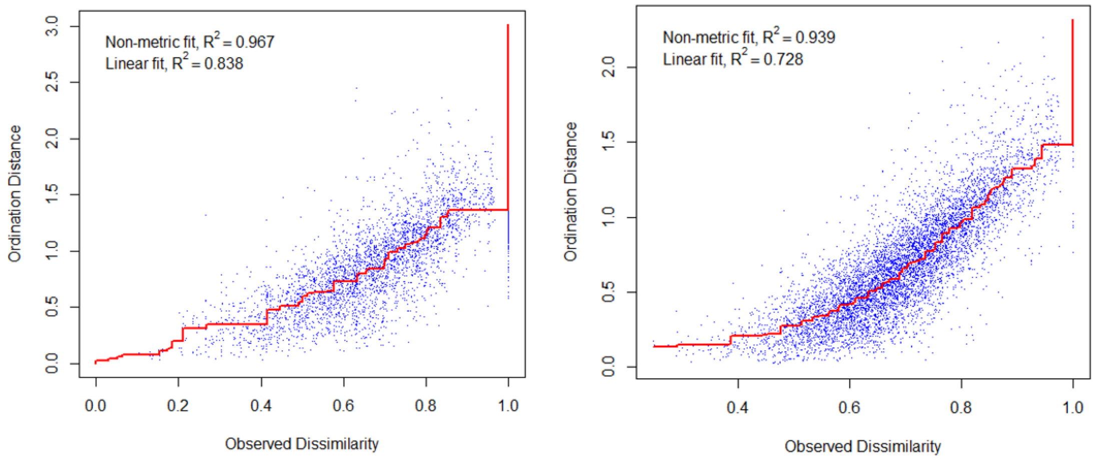


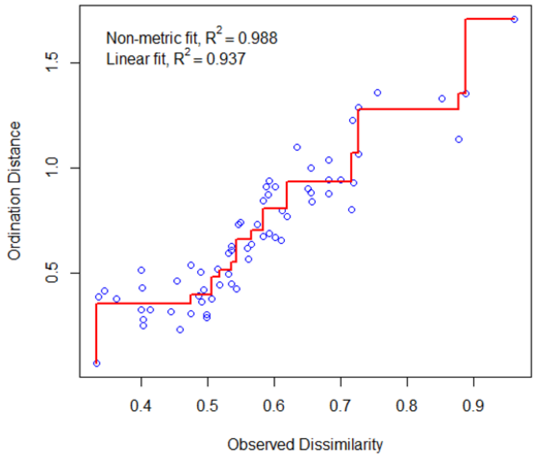
Figure A.4: Non Metric Multidimensional Scaling (NMDS) on the hellinger transformed Bray-Curtis distance matrix on mean counts of the specialist species observed in ancient and restored focal patches.



(a) Specialist species dataset *(b) Epizoochorous species*



(c) Dataset containing counts of focal patches and surrounding GI *(d) Explorative analysis on the dataset containing all the plant species surveyed*



(e) Mean counts of the specialist species per focal patch

Figure A.5: Assessment of each NMDS by a Shepard plot

Dissimilarity focal patch and GI

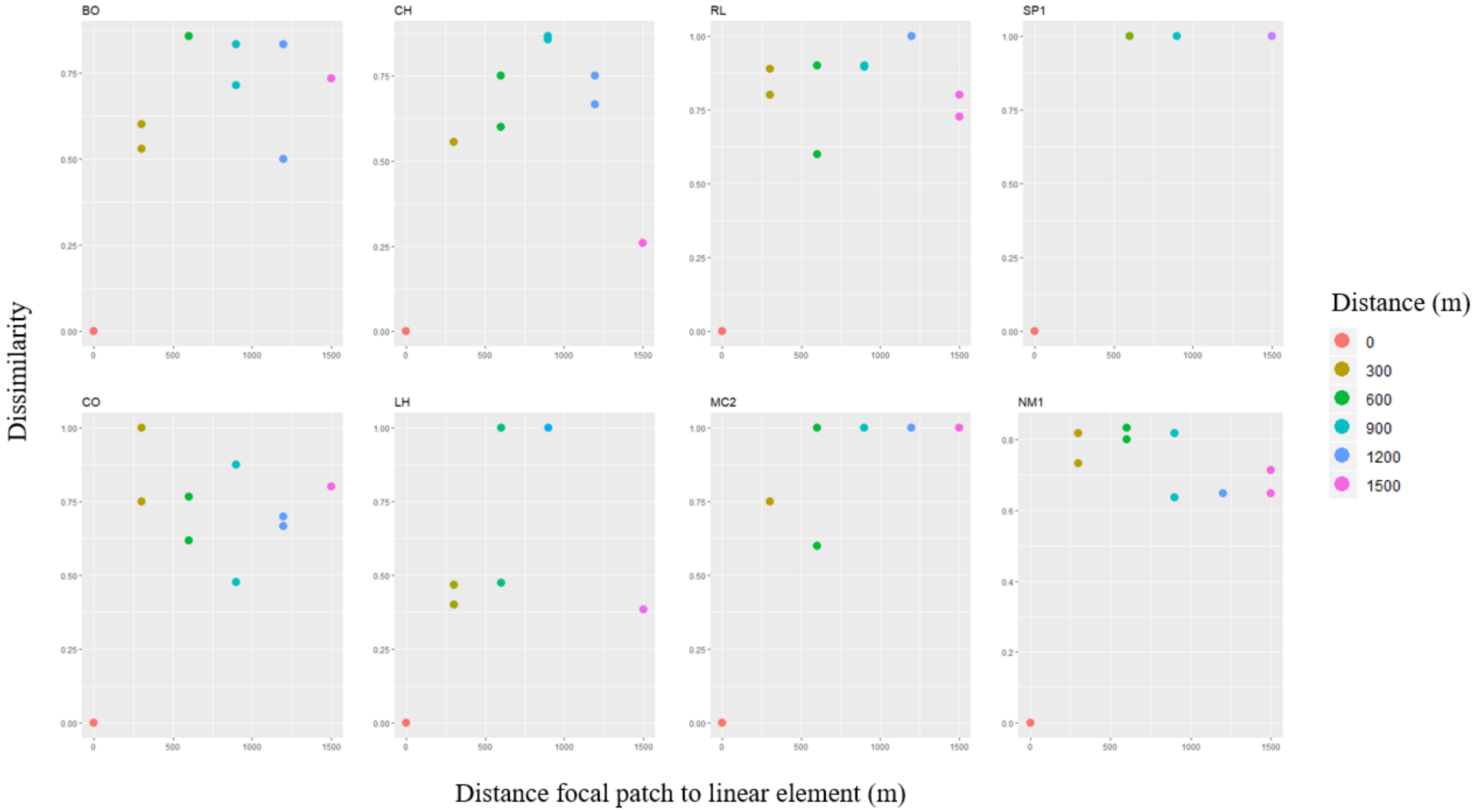


Figure A.6: The eight remaining dissimilarity analyses with a rather unexpected trend. Assessment of the dissimilarity in presence of specialist species in the focal patch compared to its surrounding GI over distance. When the dissimilarity is zero, the specialist species community composition present in the GI would be equal to the specialist community composition found in the focal patch. Conversely, when the GI reaches a dissimilarity of one, it has no specialist species in common with the focal patch.

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