COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE
GEMBLOUX AGRO-BIO TECH

IDENTIFICATION DES MENACES SUR LA DIVERSITÉ FLORISTIQUE DES PELOUSES CALCICOLES DE BELGIQUE ET OPPORTUNITÉS POUR LA RESTAURATION : DE L'ESPÈCE AU PAYSAGE

JULIEN PIQUERAY

Document présenté en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur: Prof. Grégory MAHY **Année 2010**

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Julien PIQUERAY (2010). Identification des menaces sur la diversité floristique des pelouses calcicoles de Belgique et opportunités pour la restauration : de l'espèce au paysage (Thèse de doctorat). Université de Liège, Gembloux Agro-Bio Tech, 170 p., 24 fig., 24 tabl.

RÉSUMÉ: Les activités anthropiques modernes peuvent être responsables de la perte ou du maintien de la biodiversité à l'échelle régionale. L'étude de l'influence de ces activités sur les patrons de diversité biologique est donc d'une importance majeure dans le cadre de l'établissement de stratégies de conservation. Nous avons étudié l'influence des activités humaines sur la diversité floristique des pelouses calcicoles de Belgique. Durant le siècle dernier, ces milieux ont subi une fragmentation importante. Cependant, nous avons montré qu'un délai existait entre le processus de fragmentation et la réponse des espèces, ce qui crée une dette d'extinction dans les taches d'habitat relictuelles. Bien que la fragmentation ait été la tendance principale, nous avons pu montrer que des habitats ont aussi été créés du fait des activités humaines durant la période considérée. Les nouvelles taches d'habitat présentaient des richesses et des compositions en espèces similaires à celles des anciennes pelouses. En réponse à la fragmentation des pelouses calcicoles, des programmes de restauration ont été lancés dans la zone d'étude. Les habitats restaurés présentaient des conditions édaphiques similaires à celles des pelouses témoins. Les communautés végétales des restaurations les plus anciennes tendaient à se rapprocher de celles de pelouses témoins. Cependant, après 15 ans, des différences persistaient. Entre autres choses, certaines espèces n'ont pas colonisé les sites restaurés. Il a été montré que les disponibilités en graines et en microsites pour la germination pouvaient être des facteurs limitant la colonisation des sites restaurés. Cependant, l'absence ou la plus faible émergence de plantules dans les sites restaurés en comparaison avec les sites témoins suite à l'addition de graines et à la création de microsites de germination suggère que la qualité de l'habitat pourrait être moindre dans les sites restaurés. Au vu de ces résultats, il apparaît que l'effort de restauration devrait être poursuivi. Cet effort n'est pas vain, étant donné que de nombreuses espèces typiques sont capables de coloniser les sites nouvellement créés. Enfin, nous avons étudié une autre menace potentielle pour les pelouses calcicoles : la naturalisation d'une espèce ornementale, *Cotoneaster horizontalis*. L'analyse a révélé que l'espèce était au début de sa phase de naturalisation. La présence de cette espèce avait un impact sur la structure et la composition de la végétation ainsi que sur la diversité des pelouses. La gestion de cette espèce devrait s'accompagner d'une limitation de son commerce.

Julien PIQUERAY (2010). Identification of the threats on calcareous grasslands floristic diversity in Belgium and opportunities for restoration : from species to landscape. University of Liege, Gembloux Agro-Bio Tech, 170 p., 24 fig., 24 tabl.

SUMMARY : Current human activities can be responsible either of the loss or promotion of biodiversity at the regional scale. The study of the influence of human activities on patterns of biological diversity is therefore of primary importance in order to set up conservation strategies. We studied the influence of human activities on calcareous grassland floristic diversity in Belgium. During the past century, a high fragmentation of this habitat occurred within the study region. There was a delay between the process of fragmentation and the subsequent response in terms of species richness, which created an "extinction debt" in remnant calcareous grasslands. Although fragmentation was the main process involved, we also showed that some new habitat patches were created thanks to human activities during this period. These new habitat patches exhibited a species richness and composition that was found similar to old habitat patches. In the face of fragmentation, restoration programs were launched in the study region. Restored areas exhibited similar soil conditions as compared to reference grasslands. Plant species communities found in the oldest restorations tend to resemble reference grassland communities. However, some differences in species composition persisted after 15 years. It was also shown that some species did not colonize restored sites at all. With this respect, seed and germination microsite availability could be limiting factors for the colonization of restored sites. However, the absence or the lower colonization in restored areas compared to reference grasslands following seed addition and disturbance suggested that habitat quality could be considered as lower in restored habitats. Our results suggest therefore that restoration effort should be maintain in the study site. This effort is not vain as many species are able to colonize newly created sites. Finally, another threat to calcareous grasslands was studied i.e. the naturalization of the ornamental species *Cotoneaster horizontalis*. The analysis revealed that, although being at an early stage of naturalization, the species had an impact on vegetation structure and composition and on grassland diversity. Trade limitation for this species should be promoted in addition to management.

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INTRODUCTION GENERALE

Contexte général

Les activités humaines, l'occupation du sol et l'exploitation des ressources sont les principaux moteurs de la dynamique des paysages et de ce fait du maintien ou de la perte de la diversité biologique (Baudry & Tatoni 1993). En Europe occidentale, l'homme a commencé à façonner les paysages de manière significative à partir du Néolithique. A cette période, les premiers défrichements destinés à l'agriculture ont contribué à favoriser les habitats ouverts par rapport aux habitats forestiers. Cette dynamique d'ouverture du milieu s'est ensuite poursuivie à l'époque romaine et au Moyen-âge. Ce processus s'est accompagné de l'arrivée d'espèces végétales provenant d'autres régions d'Europe, qui ont trouvé dans ces milieux de nouvelles opportunités de développement (Poschlod & WallisDeVries 2002). Progressivement, ces espèces se sont mêlées aux espèces indigènes pour former de nouvelles communautés et de nouveaux habitats, les habitats semi-naturels. Parmi eux, les pelouses calcicoles constituent un habitat parmi les plus riches en espèces d'Europe occidentale (Prendergast et al. 1993; Willems 2001). Durant le siècle dernier, les changements sociétaux ont conduit à une toute autre évolution du paysage qui s'est faite en défaveur des milieux semi-naturels dans de nombreux cas (Adriaens et al. 2006; Helm et al. 2006; Piessens & Hermy 2006). Les principaux changements ont été l'intensification de l'agriculture et l'urbanisation, qui ont contribué au remplacement des milieux semi-naturels par des milieux artificiels ; les zones les moins intéressantes en terme économique ont été boisées artificiellement ou laissées à leur dynamique naturelle (Poschlod & WallisDeVries 2002). Ces différentes causes ont mené en tout ou en partie à une fragmentation importante des habitats semi-naturels.

L'érosion globale de la biodiversité est une crise dont l'importance est maintenant bien comprise et largement acceptée (Wood et al. 2000). Mais l'impact des activités humaines modernes sur la biodiversité n'est pas à sens unique. D'une part, les causes principales de l'érosion de la biodiversité, telles que la fragmentation et la destruction des habitats (Ehrlich 1988; Sih et al. 2000) ou l'introduction, volontaire ou non, d'espèces exotiques dans les écosystèmes indigènes (D'Antonio & Kark 2002), sont d'origine anthropique. D'autre part, il arrive que des activités anthropiques (construction de route, activité minière, exploitation forestière) conduisent à la création de nouveaux habitats pour des espèces menacées (Collins et al. 1985; Krüger et al. 2002; Quintana-Ascencio et al. 2007). De plus, la prise de conscience de l'érosion de la biodiversité dans les années 1990 a eu pour conséquence la mise en place d'actions anthropiques directement destinées à conserver la diversité biologique, telle que la restauration d'habitats en réponse à leur fragmentation (Perrow & Davy 2002; Jongman & Pungetti 2004). Ces phénomènes de destruction et de création d'habitat créent une double dynamique à l'échelle du paysage. Les conséquences des activités anthropiques sur la biodiversité sont donc diverses.

Selon les cas, leur rôle peut s'avérer positif ou négatif pour la conservation de la diversité biologique. Dès lors, il apparaît que la compréhension du rôle des activités anthropiques sur la biodiversité à l'échelle régionale est fondamentale pour la mise en place de stratégies de conservation.

Structure spatiale des habitats et richesse spécifique

Introduction

Selon la théorie de la biogéographie insulaire (MacArthur & Wilson 1967), on s'attend à ce que la richesse en espèces d'un habitat soit en relation avec ses caractéristiques spatiales. Cette théorie, dans sa version originale, stipule qu'une île est d'autant plus riche en espèces que sa taille est importante et que son isolement est faible. Appliquée aux habitats terrestres, cette théorie considère que les taches d'habitats sont des îles au sein d'une matrice hostile aux espèces de l'habitat cible. Son application dans ce contexte est donc critiquable, puisque certains effets ne sont pas pris en compte par cette théorie (effet lisière, persistance des espèces dans les habitats dégradés, …) et que l'hypothèse de l'hostilité de la matrice est rarement tout à fait observée. Il n'en demeure pas moins que la théorie elle-même se vérifie souvent dans les habitats fragmentés terrestres, sous forme d'effet de la surface et de l'isolement des taches d'habitat sur la richesse en espèces (Laurance 2008). En réponse à la dynamique de fragmentation/création d'habitats, on s'attend à ce que les communautés végétales, et en particulier leur richesse en espèces, évoluent afin de se remettre en équilibre avec les nouvelles caractéristiques spatiales (surface et isolement).

Fragmentation et dette d'extinction

La fragmentation de l'habitat se définit comme la réduction d'un habitat continu de grande taille en taches d'habitat plus petites et plus isolées au sein d'une matrice paysagère de nature différente (Fahrig 2003). La fragmentation peut entraîner une dégradation de la qualité de l'habitat, notamment du fait de l'augmentation de l'effet lisière, c'est-à-dire de la modification des conditions abiotiques (microclimat, niveau trophique, …) et biotiques (prédation, compétition, …) du fait de la présence à proximité de l'habitat-cible, d'un habitat de nature différente. Par ailleurs, la fragmentation de l'habitat a pour conséquence de réduire la taille et la connectivité des populations d'espèces qui lui sont inféodées (Wilcox & Murphy 1985). Les petites populations d'espèces présentent un risque d'extinction plus élevé que les grandes populations du fait de leur plus grande sensibilité aux aléas environnementaux et démographiques (Lande 1993). De plus, ces petites populations présentent souvent une réduction de fitness (succès reproducteur) qui réduit leur probabilité de maintien à long terme (Leimu et al. 2006). De ces considérations, il ressort qu'on s'attend à ce que la fragmentation d'un habitat entraîne la disparition locale ou totale de certaines espèces, et qu'en conséquence la richesse en espèces des taches d'habitat diminue.

L'extinction de ces espèces peut être retardée du fait de leurs caractéristiques intrinsèques telles que la capacité à se reproduire de manière clonale (Fischer & Stöcklin 1997; Poschlod et al. 2000) ou la longue durée de vie des graines dans le sol (Stöcklin & Fischer 1999; Fréville et al. 2007). Par ailleurs, les phénomènes d'ordre génétique rendant les petites populations plus sensibles à l'extinction (dépression de consanguinité, dérive génétique) ont besoin de plusieurs générations pour que leurs conséquences puissent s'exprimer. Par conséquent, suite à la fragmentation, il peut apparaître un état transitoire où des espèces persistent dans des taches d'habitat alors qu'elles sont vouées à s'éteindre. L'ensemble de ces espèces crée une dette d'extinction dans les communautés auxquelles elles appartiennent (Tilman et al. 1994). La dette d'extinction est plus susceptible de se former dans le cas d'organismes à longue durée de vie, tels que les plantes pérennes (Krauss et al. 2010). Dans les milieux dominés par ces organismes, la présence d'une dette d'extinction a pu être montrée: dans les forêts (Vellend et al. 2006), les landes (Piessens & Hermy 2006) et les pelouses calcicoles (Lindborg & Eriksson 2004a; Helm et al. 2006; Krauss et al. 2010). Toutefois, dans les pelouses calcicoles, la présence d'une dette d'extinction n'est pas une généralité, spécialement dans les paysages fortement fragmentés (Adriaens et al. 2006; Cousins et al. 2007).

Création d'habitats et crédit de colonisation

Lors de la création d'une tache d'habitat, on ne s'attend pas à ce que toutes les espèces colonisent le site immédiatement. Aux différents stades de la colonisation, des filtres sont susceptibles d'empêcher ou de ralentir le processus (Tonn et al. 1990). Les trois filtres généralement considérés sont : le filtre à la dispersion, le filtre lié aux conditions abiotiques et le filtre lié aux conditions biotiques. Du fait de l'action conjointe ou successive de ces filtres, le pool d'espèces observé à un endroit donné, à un moment donné, sera un sous-ensemble du pool d'espèces local capable de passer outre ces filtres (Figure 1).

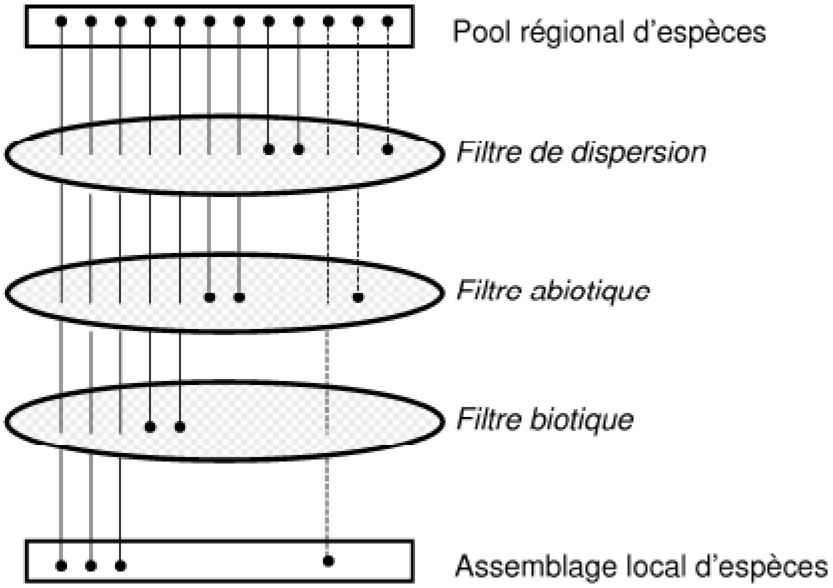


Figure 1 : Règle d'assemblage des communautés végétales selon la théorie des filtres (D'après Lake et al. 2007, adapté.).

FILTRE À LA DISPERSION

Pour coloniser un site nouvellement (re-)créé, soit de manière intentionnelle via la restauration d'habitat, soit de manière non-intentionnelle, les espèces disposent de trois stratégies non-exclusives. Premièrement, elles peuvent atteindre le site par la dispersion spatiale de propagules (graines, fruits, rhizomes, ...). Cette dispersion peut s'effectuer par l'intermédiaire de différents vecteurs (vents, animaux, eau, ...) ou grâce à des adaptations particulières de l'espèce, telle que la capacité à éjecter ses graines (ballochorie), à se fixer sur la toison des animaux… . Deuxièmement, les espèces végétales peuvent émerger dans un site à partir des propagules contenues dans le sol (banque de graines). Cela implique toutefois que l'espèce ait été présente précédemment sur le site et que les graines contenues dans le sol aient pu survivre jusqu'à la réapparition des conditions favorables à leur développement (Harper 1977; Thompson & Grime 1979; Cavers & Benoit 1989). Enfin, il arrive que certaines espèces puissent survivre un certain temps dans des habitats dégradés (Poschlod et al. 1998).

Le filtre à la dispersion spatiale opère lorsque la distance qui sépare les populations sources de propagules du site nouvellement créé excède la capacité de dispersion spatiale d'une espèce. Dès lors, la réduction de l'effet du filtre à la dispersion peut s'effectuer soit en augmentant la connectivité entre les taches d'habitat, soit en favorisant la dispersion des espèces.

FILTRE ABIOTIQUE

A l'échelle de la tache d'habitat, la restauration consiste dans un premier temps à recréer les conditions abiotiques nécessaires. Selon l'état initial de la parcelle à restaurer et l'objectif de restauration, il peut être nécessaire de restaurer la luminosité, le niveau trophique, le niveau hydrique, ou des facteurs abiotiques plus spécifiques tels que la salinité (Perrow & Davy 2002). La levée du filtre abiotique ne peut être atteinte que par le rétablissement de l'ensemble des conditions abiotiques nécessaires au développement des espèces cibles.

FILTRE BIOTIQUE

L'installation et la survie dans une tache d'habitat dont les conditions abiotiques sont adéquates ne peuvent se faire que si l'espèce est capable de se développer en relation avec les autres espèces végétales et animales. Dans les deux cas, les relations peuvent être bénéfiques ou non. Dans le rang des relations positives, on peut citer la pollinisation ou la mycorhyzation. Dans le cas des relations plantes-plantes, le cas le plus classique est la compétition interspécifique. Toutefois, de manière plus récente, il a été souligné que les espèces végétales pouvaient tirer un bénéfice de la présence d'une autre espèce, qui joue un rôle de facilitation (Brooker et al. 2008). Lorsque les filtres biotiques sont trop importants, il peut être nécessaire de prendre des mesures de gestion destinées à réduire leur effet (réduction de la compétition, installation d'espèces facilitatrices).

Dans le chapitre 1, l'importance de ces différents filtres et les possibilités de les manipuler sont décrites dans le cas de la restauration des pelouses calcicoles. Il est intéressant de noter que la logique de restauration des habitats est inverse à celle de la construction des communautés. En effet, dans le cadre de la restauration, il s'agira en premier lieu de lever les filtres abiotique et biotique, le filtre à la dispersion étant levé dans un second temps.

La présence de ces filtres lors de la création (volontaire ou non) d'un habitat va avoir pour conséquence que la constitution du pool d'espèces nécessite un délai. Ce délai peut être dû au fait que les conditions biotiques et abiotiques nécessaires présentent elles-mêmes un délai avant de se reconstituer. Le filtre à la dispersion peut en outre rendre rare l'arrivée de propagules sur un site. L'action des filtres peut agir à différents stades : la dispersion des propagules, l'établissement d'individus, la survie des individus. Tout retard ou tout échec à l'un de ces stades aura pour conséquence d'augmenter le délai de colonisation (Jackson & Sax 2009). Il résulte de ce délai que lors de la création d'un nouvel habitat, un état transitoire apparaît durant lequel un pool d'espèces est encore susceptible de coloniser le site. Ce pool d'espèces constitue le « crédit d'espèces » (Hanski 2000), aussi appelé « crédit de colonisation » (Cristofoli et al. 2009) ou « crédit d'immigration » (Jackson & Sax 2009). Peu d'études ont testé de manière explicite ce crédit de colonisation (mais voir Cristofoli et al. 2009; Cristofoli & Mahy 2010), et, à notre connaissance, aucune dans le cas des pelouses calcicoles. Toutefois, plusieurs études sur la restauration de ces milieux ont montré que la richesse en espèces mettait un certain temps à atteindre la richesse des pelouses témoins (Zobel et al. 1996; Dzwonko & Loster 1998), notamment pour ce qui concerne certaines espèces typiques (Willems 2001; Lindborg & Eriksson 2004b).

Dynamique des espèces et traits d'histoire de vie

La réponse des espèces aux changements de structure du paysage peut différer pour chaque espèce (Lindborg 2007; Mildén et al. 2007). Cette réponse peut être appréhandée au travers des traits fonctionnels des espèces. Un trait fonctionnel est une caractéristique mesurable d'un organisme, habituellement mesurée au niveau de l'individu, qui influence fortement les performances de cet organisme (McGill et al. 2006). En ce qui concerne les plantes vasculaires, les performances considérées habituellement sont liées à trois challenges que l'espèce doit relever: (i) la dispersion, (ii) l'établissement, (iii) la persistance (Weiher et al. 1999; Poschlod et al. 2000). En relation à ces challenges, différents traits, plus ou moins facilement mesurables, peuvent être utilisés (voir exemples table 1).

Table 1 : Exemple de traits fonctionnels facilement et difficilement mesurables en relation avec les trois challenges à relever par les espèces végétales (D'après Weiher et al. 1999).

Challenge	Traits difficilement mesurables	Traits facilement mesurables
1. Dispersion		
Dispersion dans l'espace	Distance de dispersion	Masse des graines, mode de dispersion
Dispersion dans le temps	Longévité des propagules	Masse des graines, forme des graines
2. Etablissement		
Croissance des plantules	RGR (taux de croissance relatif)	Masse des graines, SLA (surface foliaire spécifique), LWC (teneur en eau des feuilles)
3. Persistance		
Production de graines	Fécondité	Masse des graines, biomasse aérienne
Aptitude compétitive	Compétitivité	Hauteur, biomasse aérienne
Plasticité	Norme de réaction	SLA, LWC
Longévité	Durée de vie	Histoire de vie, densité en tiges
Acquisition d'espace	Capacité d'extension végétative	Clonalité
Réponse aux perturbations, aux stress	Phénologie, Palatabilité	Aptitude à rejeter de souche, période de floraison, SLA, LWC

Tant dans le cadre de la fragmentation que dans le cadre de la création d'habitats, les traits liés à ses trois challenges sont fondamentaux. En effet, on peut s'attendre, dans le contexte de la colonisation d'habitats, à ce que les espèces soient capables d'atteindre le nouvel habitat, de s'y établir et d'y persister. Ces hypothèses ont toutefois rarement été étudiées dans le cadre de la restauration des pelouses calcicoles (mais voir Pywell et al. 2003; Dzwonko & Loster 2007). Dans le cadre de la fragmentation, on peut notamment s'attendre à ce que les espèces possédant les meilleures capacités de dispersion soient les plus aptes à occuper les taches d'habitats isolées et à compenser les extinctions locales par des évènements de colonisation. Il a d'ailleurs été montré à plusieurs reprises que ces espèces étaient plus largement distribuées dans les habitats fragmentés (e.g. Kolb & Diekmann 2005; Tremlová & Münzbergová 2007), mais ce patron n'a pas été trouvé dans toutes les études (Eriksson & Jakobsson 1998; Maurer et al. 2003). Par ailleurs, les espèces persistantes, notamment les espèces clonales, présentent des risques d'extinction moindres dans les habitats fragmentés (Fischer & Stöcklin 1997), mais sont plus sensibles à l'isolement de leur habitat (Dupré & Ehrlén 2002). La balance entre ces différents éléments est donc susceptible de déterminer le comportement d'une espèce face à la fragmentation. A l'heure actuelle, il est difficile de tirer des conclusions générales, d'autant plus que les résultats peuvent varier en fonction des analyses effectuées (Tremlová & Münzbergová 2007).

Les espèces exotiques invasives

Les invasions biologiques sont reconnues comme une des causes majeures de perte de biodiversité à l'échelle mondiale (D'Antonio & Kark 2002; D'Antonio & Meyerson 2002). A ce titre, il est important de détecter leur présence dans les habitats d'intérêt ainsi que les impacts potentiels qu'elles peuvent y occasionner. Ces impacts peuvent par exemple être observés au niveau de la composition et de la structure de la végétation (Levine et al. 2003) ou au niveau du fonctionnement de l'écosystème (Ehrenfeld et al. 2001). Les impacts peuvent être nombreux (compétition pour les ressources/les pollinisateurs, effets allélopathiques, …) et constituent donc une menace pour les habitats ou ces espèces sont présentes. L'absence de littérature sur le sujet pourrait laisser croire que les pelouses calcicoles ne sont pas concernées par cette menace. Pourtant, depuis quelques années, *Cotoneaster horizontalis*, une espèce ornementale originaire de Chine, tend à se naturaliser dans ces habitats, comme cela a été observé en Grande-Bretagne (Crofts & Jefferson 1999). Il apparaissait donc important, dans le cadre de l'étude des menaces pesant sur cet habitat, de déterminer son état de naturalisation et les impacts potentiels qu'elle peut occasionner.

Objectifs

Le premier objectif de cette thèse était d'analyser la réponse des espèces végétales des pelouses calcicoles aux changements de structure spatiale de leur habitat : la fragmentation et la création d'habitats volontaire ou non. Les questions abordées sont : (i) Quelle a été l'ampleur des changements de structure du paysage ?, (ii) Existe-t-il un délai entre les changements de structure du paysage et la réponse des espèces/des communautés, induisant un état de dette d'extinction, de crédit de colonisation ou de restauration incomplète des habitats ? (iii) Quelles sont les caractéristiques des espèces qui peuvent les rendre plus sensibles à la fragmentation de leur habitat ? (iv) Quels sont les facteurs pouvant limiter la colonisation des habitats restaurés ? Un second objectif, visant à compléter l'étude sur la menace liée à la fragmentation de l'habitat, était de déterminer si les espèces exotiques constituaient une menace supplémentaire pour les espèces végétales des pelouses calcicoles. La question était de déterminer l'impact d'une d'entre elles, *Cotoneaster horizontalis*, sur les communautés et les espèces des pelouses calcicoles.

Cas d'étude et méthodologie générale

Cas d'étude : les pelouses calcicoles

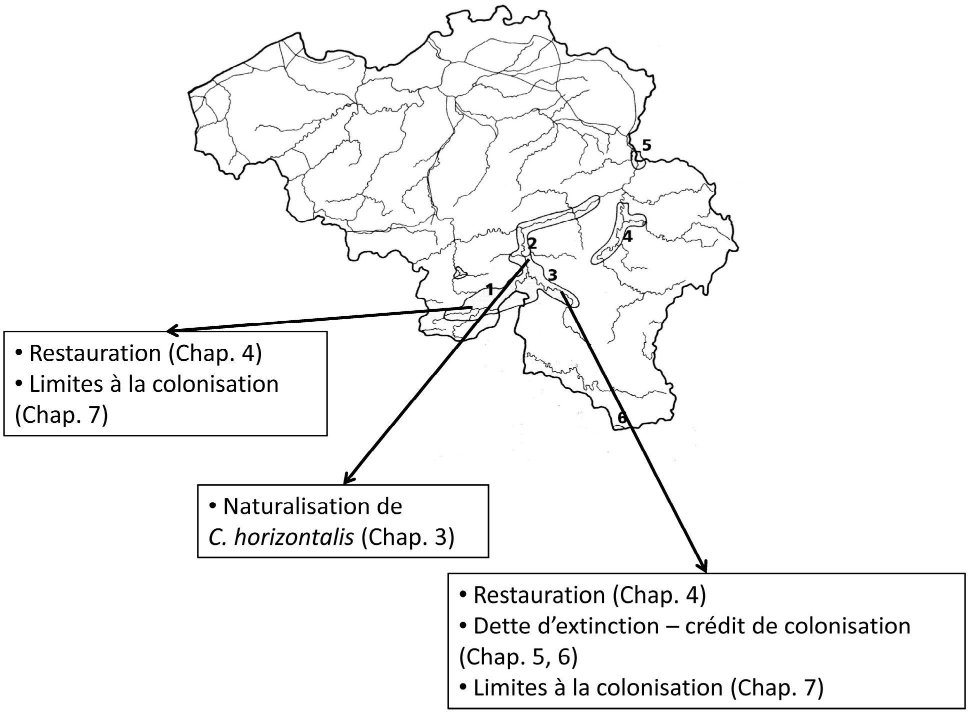
Les pelouses calcicoles sont des formations végétales héliophiles semi-naturelles, principalement composées de plantes herbacées vivaces formant un tapis plus ou moins continu, sur des sols calcaires, crayeux ou schisto-calcaires, superficiels, secs et pauvres en éléments nutritifs (Decocq et al. 1996; Decocq et al. 2004). Ces pelouses sont des écosystèmes qui sont parmi les plus riches d'Europe occidentale pour de nombreux groupes biologiques (Prendergast et al. 1993; Poschlod & WallisDeVries 2002). On y retrouve une grande richesse en espèces végétales et en insectes notamment (Delescaille et al. 1991). Il n'est pas rare de retrouver sur ces pelouses de 30 à 40 espèces de plantes supérieures par m², parfois même plus de 50, ainsi qu'une quinzaine de bryophytes (Bobbink et al. 1987; Dutoit & Alard 1996b). Leur flore est constituée de nombreux éléments originaires des régions subméditerranéennes et des régions steppiques de l'Europe centrale (Delescaille 2004). La flore peut en outre varier selon les conditions hydriques et trophiques, ainsi que selon les régions géographiques (Royer 1982).

En Europe, on retrouve des pelouses calcicoles subméditerranéennes du Nord de l'Espagne au Sud de la Scandinavie et dans les îles britanniques (Royer 1982; Decocq et al. 2004) (Figure 2).

Figure 2 : Répartition des pelouses calcicoles en Europe centro-occidentale (D'après Wolkinger & Plank 1981)

Méthodologie générale

Les différentes questions posées ont été étudiées dans différentes régions de Belgique selon les opportunités en termes de données disponibles et d'occurrence de sites convenant pour répondre aux questions posées. La localisation des sites d'études des différents chapitres est présentée à la figure 3.



- Restauration (Chap. 4)
- Limites à la colonisation (Chap. 7)

- Naturalisation de *C. horizontalis* (Chap. 3)

- Restauration (Chap. 4)
- Dette d'extinction – crédit de colonisation (Chap. 5, 6)
- Limites à la colonisation (Chap. 7)

Figure 3 : Localisation des sites d'études selon les chapitres. Les chiffres représentent les zones de Belgique riches en pelouses calcicoles : (1) vallée du Viroin, (2) haute Meuse, (3) vallée de la moyenne et basse Lesse, (4) basses vallées de l'Ourthe et de l'Amblève, (5) Montagne-Saint-Pierre, (6) sud de la Gaume (Torgny).

Les études à l'échelle du paysage, études de dette d'extinction et de crédit de colonisation (chapitres 5 & 6), ont été réalisées dans la région de Han-sur-Lesse – Rochefort. Durant les saisons de végétation 2002 et 2003, l'inventaire floristique de 190 taches d'habitats a été réalisé dans le cadre de la convention « Développement et test d'une méthodologie pour l'élaboration des arrêtés de désignation des sites Natura 2000 », financée par le Service Public de Wallonie (DGARNE – DNF). Ce jeu de données, combiné à un travail de digitalisation de documents cartographiques anciens (cartes topographiques de 1920 (Institut Royal Militaire de Belgique, échelle 1:20000) et 1965 (Institut Géographique National de Belgique, échelle 1:10000) ; photographies aériennes de 1965 (Institut Géographique National de Belgique, échelle 1:20000)) offrait une opportunité unique d'étudier la dette d'extinction et le crédit de colonisation dans les pelouses calcicoles de la région. Les méthodologies utilisées, qui sont décrites en détail dans les chapitres ad hoc, sont issues d'études similaires afin de permettre la comparaison (voir par expl. Lindborg & Eriksson 2004a; Helm et al. 2006; Cristofoli et al. 2009).

Les études sur la restauration des habitats (chapitres 4 & 7) ont été réalisées dans les régions de Han-sur-Lesse – Rochefort et de Viroinval. Dans ces deux régions, des projets LIFE-Nature, visant à restaurer des pelouses calcicoles ont eu lieu entre 2001 et 2006 (André & Vandendorpel 2004; Graux 2004). Suite à ces projets et aux restaurations réalisées antérieurement par les cantonnements forestiers, de nombreux sites étaient disponibles pour étudier la colonisation des zones restaurées par les plantes vasculaires.

Enfin, l'étude sur la naturalisation de *Cotoneaster horizontalis* dans les pelouses calcicoles a été réalisée dans la région de Dinant du fait de la présence importante de l'espèce dans les pelouses de cette région.

Organisation de la thèse

Chapitre 1: *Revue bibliographique sur la restauration des pelouses calcicoles en Europe : contraintes rencontrées et solutions proposées*. Julien Piqueray, Grégory Mahy.

Dans ce chapitre, les différentes contraintes liées à la restauration des pelouses calcicoles ont été relevées dans la littérature. Les différentes techniques qui ont été proposées pour outrepasser ces contraintes y sont abordées.

Article accepté pour publication dans BASE.

Chapitre 2: *Plant communities and species richness of the calcareous grasslands in southeast* Belgium. Julien Piqueray, Emmanuelle Bisteau, Gaëtan Bottin, Grégory Mahy.

Cet article décrit la variation des communautés végétales de pelouses calcicoles sur une large gamme de conditions environnementales. Elle décrit en outre les patrons de richesse spécifique entre et au sein de ces communautés.

Article publié : Belgian journal of Botany 140 : 157-173.

Chapitre 3: *Naturalization and impact of a horticultural species,* Cotoneaster horizontalis *(Rosaceae) in biodiversity hotspots in Belgium.* Julien Piqueray, Grégory Mahy, Sonia Vanderhoeven. Belgian journal of Botany 141 : 113-124.

Cet article montre l'état de naturalisation de *Cotoneaster horizontalis*, une espèce exotique ornementale, à l'échelle de la Belgique et de celle des pelouses calcicoles de Haute-Meuse. Elle décrit ensuite les impacts que la présence de l'espèce a sur les communautés de pelouses en place.

Article publié: Belgian journal of Botany 141 : 113-124.

Chapitre 4: Rapid restoration of a species-rich ecosystem assessed from soil and vegetation indicators: the case of calcareous grasslands restored from forest stands. Julien Piqueray, Gaëtan Bottin, Louis-Marie Delescaille, Emmanuelle Bisteau, Gilles Colinet, Grégory Mahy.

Cet article est une évaluation du succès des restaurations de pelouses calcicoles qui ont été réalisées en Belgique. Des parcelles restaurées à des moments différents ont été comparées. La comparaison a été faite sur base d'indicateurs de structure de végétation, de composition floristique et de conditions physico-chimiques du sol.

Article en révision dans Ecological Indicators.

Chapitre 5: Plant species extinction debt in a temperate biodiversity hotspot: community, species and functional traits approaches. Julien Piqueray, Emmanuelle Bisteau, Sara Cristofoli, Rodolphe Palm, Peter Poschlod, Grégory Mahy.

Cette étude cherchait à identifier la présence d'une dette d'extinction dans les pelouses calcicoles du bassin de la Lesse. Différentes méthodes ont été testées, ce qui a permis une étude comparative de celles-ci. La dette d'extinction a été décrite des points de vue des communautés, des espèces et des traits fonctionnels.

Article finalisé. A soumettre dans Biological Conservation

Chapitre 6: *Extinction debt and colonization credit? When both phenomena are mingled.* Julien Piqueray, Sara Cristofoli, Emmanuelle Bisteau, Grégory Mahy.

Cette étude visait à identifier la présence d'un crédit de colonisation dans les pelouses nouvellement créées dans le paysage du bassin de la Lesse du fait des activités humaines. L'étude du crédit de colonisation se faisant par comparaison à des pelouses anciennes, nous avons pu déterminer l'influence que la dette d'extinction présente dans ces anciennes pelouses pouvait avoir sur l'estimation du crédit de colonisation.

Article finalisé. A soumettre dans Landscape Ecology

Chapitre 7: Why don't some species colonize restored habitats? The effects of seed and microsite availability. Julien Piqueray, Layla Saad, Grégory Mahy.

Suite à l'évaluation des restaurations de pelouses calcicoles, il a été montré que certaines espèces ne colonisaient pas les sites restaurés. Cette étude visait à déterminer les facteurs pouvant limiter la colonisation de trois de ces espèces. L'étude combinait des expériences ex-situ et in-situ.

Article en préparation pour soumission dans Plant Ecology.

CHAPITRE 1 : REVUE BIBLIOGRAPHIQUE SUR LA RESTAURATION DES PELOUSES CALCICOLES EN EUROPE : CONTRAINTES RENCONTREES ET SOLUTIONS PROPOSEES

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Article accepté dans BASE.

Résumé

Les pelouses calcicoles, qui sont considérées comme des hotspots de biodiversité en Europe, ont subi un fort déclin durant le siècle dernier. Dès lors, il est apparu que la restauration de ces habitats s'imposait en tant que stratégie de conservation. La restauration consiste à recréer les conditions biotiques et abiotiques permettant aux espèces typiques de s'installer. En fonction du type de dégradation du milieu, plusieurs contraintes peuvent survenir lors de la restauration, comme l'enrichissement du sol, l'envahissement par des espèces compétitives, ou des conditions de croissances difficiles pour les plantes. Afin de surmonter ces contraintes, la communauté scientifique a mis au point des techniques qui se sont révélées plus ou moins efficaces. Cet article a pour but de décrire les contraintes qui peuvent survenir lors de la restauration de pelouses calcicoles ainsi que d'exposer les techniques proposées dans la littérature scientifique afin de les surmonter. Il décrit aussi les objectifs spécifiques à la restauration des pelouses calcicoles en tenant compte de l'histoire de ces milieux et dans le contexte des changements globaux. Différents paramètres peuvent être pris en compte pour estimer le succès des campagnes de restauration. Là aussi, une analyse de la littérature scientifique était nécessaire afin d'aborder au mieux le monitoring des pelouses restaurées.

Summary

Calcareous grasslands, that are considered as biodiversity hotspots in Europe, have suffered an important decline during the last century. In this context, restoration of suitable habitats has been promoted as a conservation strategy. Restoration consists in the recreation of favourable abiotic and biotic conditions that permit the recolonization by typical species. Depending on the degradations, several constraints can be encountered during restoration, such as soil enrichment, the presence of competitive species, or harsh conditions for plant growth. In order to overcome these constraints, scientists have proposed techniques that were demonstrated to be more or less efficient. In this article, the different constraints that can occur while restoring calcareous grasslands are described as well as the techniques proposed in the scientific literature. We also describe restoration objectives specific to calcareous grasslands, taking the history of these habitats into account and considering global change issue. Different parameters can be considered to value restoration success. Here again, an analysis of the scientific literature is needed in order to best assess restoration monitoring.

Introduction

Les pelouses calcicoles sont des écosystèmes qui sont parmi les plus riches d'Europe occidentale pour de nombreux groupes biologiques (Prendergast et al. 1993 ; Willems 2001; Poschlod & WallisDeVries 2002; WallisDeVries et al. 2002; Piqueray et al. 2007). On y retrouve une grande richesse en espèces végétales et en insectes notamment (Delescaille et al. 1991). Il n'est pas rare de retrouver sur ces pelouses de 30 à 40 espèces de plantes supérieures par m^2, et jusqu'à 80 espèces si l'on inclut les bryophytes (Bobbink et al. 1987; Dutoit & Alard 1996b; Hutchings & Stewart 2002). Toutefois, cette richesse exceptionnelle est actuellement fortement menacée. Le reboisement artificiel ou spontané, ainsi que l'amendement des sols, l'urbanisation et l'exploitation de carrières, ont mené à une régression intense des surfaces de pelouses calcicoles dans toute l'Europe (Lee et al. 2001; Dutoit et al. 2004; Adriaens et al. 2006). Il en résulte que ces pelouses sont à présent réduites à de petits îlots isolés les uns des autres et qui ne garantissent plus la survie à long terme des espèces qui leur sont inféodées (Saunders et al. 1991). Dans ce contexte, il est apparu clairement que la restauration d'un réseau écologique, c'est-à-dire d'un agencement d'éléments écologiques favorables à la survie à long terme des populations d'espèces, s'imposait comme une stratégie importante pour la conservation de ces espèces typiques (Jongman & Pungetti 2004). Cela demande de restaurer des surfaces d'habitat dégradées ou détruites, c'est-à-dire de diriger leur développement vers un écosystème cible en accélérant ou en outrepassant les stades intermédiaires de la succession (Jordan et al. 1987; Bakker & Berendse 1999). Deux conditions sont nécessaires au succès d'une restauration. Premièrement, il faut recréer et maintenir les conditions environnementales nécessaires au développement d'une pelouse calcicole. Deuxièmement, il faut que les espèces typiques de l'habitat s'installent sur les surfaces restaurées. Ces deux objectifs se heurtent tous les deux à un certain nombre de contraintes et de difficultés.

L'objectif de cette synthèse est d'exposer ces contraintes et d'identifier les solutions que la recherche scientifique nous propose actuellement pour les surmonter. Bien que ce sujet ait déjà fait l'objet de plusieurs revues bibliographiques (Muller et al. 1998; Hutchings & Stewart 2002; Walker et al. 2004), les nombreuses publications récentes sur le sujet justifient de faire de nouveau le point sur nos connaissances en restauration de ces milieux. Par ailleurs, certains concepts récents comme la dette d'extinction ou les mécanismes de facilitation ont permis l'émergence de nouvelles techniques ou ont remis en question notamment les objectifs et les priorités liés à la restauration des pelouses calcicoles. C'est donc dans le but d'intégrer ces données récentes en écologie de la restauration que nous synthétisons dans cet article les connaissances actuelles sur la manière de restaurer les pelouses calcicoles.

Objectifs et planification de la restauration

Avant de se lancer dans les actions de restauration à proprement parler, le gestionnaire doit se poser la question de l'objectif à atteindre. Idéalement, la restauration devrait aboutir à l'écosystème qui était présent avant la dégradation. Toutefois, il est rare que les données existent sur ce qui était présent avant cette dégradation, c'est donc un site ou un ensemble de sites voisins jugés en bon état qui servira d'écosystème de référence (Perrow & Davy 2002). Cela ne pose aucun problème si l'on considère que cet écosystème est le reflet de la gestion actuelle et qu'il est en équilibre avec celle-ci. Cependant, il a été montré que la composition en espèce d'un site dépend largement de l'histoire de ce site. Premièrement, la présence de certaines espèces peut être le reflet de l'utilisation ancienne du site, cet effet pouvant perdurer durant plus d'un siècle (Dutoit et al. 2004; Alard et al. 2005). Par ailleurs, suite à la fragmentation, la composition en espèces des pelouses et leur richesse se trouvera modifiée selon la théorie de la biogéographie insulaire (MacArthur & Wilson 1967). Toutefois, ces changements n'interviennent pas du jour au lendemain et un délai existe entre le moment où la structure spatiale de l'habitat est modifiée et celui où les espèces qui ne rencontrent plus les conditions propices à leur survie à long terme vont disparaître, ce qui crée une dette d'extinction dans la communauté (Tilman et al. 1994). Une telle dette a déjà été mise en évidence au sein de pelouses semi-naturelles (Lindborg & Eriksson 2004a; Helm et al. 2006), mais pas dans tous les cas (Adriaens et al. 2006; Lindborg 2007). La considération d'un habitat qui est un reflet du passé comme écosystème de référence est évidemment problématique, puisque l'on sait alors dès le départ que l'objectif qu'on se fixe n'est pas atteignable. En plus de considérer les effets de l'histoire du site, les gestionnaires du futur auront à prendre en compte les changements globaux dans la définition des objectifs de la restauration. Ils devront tout d'abord considérer les potentiels changements climatiques, au risque de restaurer un habitat qui n'est plus en équilibre avec son environnement climatique. Cet aspect est particulièrement délicat puisqu'il est difficile de prédire les changements climatiques à l'échelle locale (Lavendel 2003). Ensuite, les dépôts d'azote atmosphérique, importants dans certaines régions d'Europe, sont eux aussi susceptibles d'entraîner des modifications dans les écosystèmes de références et en voie de restauration (Stevens et al. 2004; Horswill et al. 2008). Il convient donc de ne pas les négliger tant au niveau de la définition des objectifs que de la gestion elle-même.

Par ailleurs, la restauration ne doit pas s'appréhender uniquement au niveau de la tache d'habitat, mais aussi au niveau du paysage écologique. Bien que le choix des zones à restaurer soit souvent dépendant de contraintes socio-économiques, certains critères d'ordre écologique doivent entrer en compte quand cela est possible. Le but étant d'une part d'optimiser les résultats au niveau des taches d'habitat restaurées et d'autre part que la restauration permette de réduire au mieux les effets de la fragmentation de l'habitat (Hobbs 2002). En raison des capacités de dispersion limitées de nombreuses espèces des pelouses calcicoles, il convient de considérer dans le choix des parcelles à restaurer la présence de ces espèces soit sur la parcelle

elle-même, soit dans le voisinage proche. Plusieurs auteurs ont souligné l'importance de la présence de sources de graines proche pour le succès de la restauration (Bakker et al. 1996; Kiefer & Poschlod 1996; Pärtel et al. 1998; Willems & Bik 1998). Dans le cas de taches d'habitat isolées, la connexion avec d'autres taches peut être améliorée par la création ou l'entretien de corridors écologiques (Hobbs 2002). Ces corridors peuvent prendre la forme de différents éléments linéaires du paysage (bords de routes, lisières, …) où des populations peuvent se développer au moins transitoirement (Kiviniemi & Eriksson 1999). Finalement, l'histoire des taches d'habitat et l'éventuelle dette d'extinction qui pèse sur ceux-ci est à prendre en considération, notamment pour la définition des priorités en terme de restauration.

Habitats restaurables

La restauration des pelouses calcicoles peut être réalisée à partir des habitats issus de la dégradation de celles-ci, c'est-à-dire : les forêts secondaires ou les plantations de résineux (voir Kiefer & Poschlod 1996; Baba 2003; Bisteau & Mahy 2005b) ; les habitats issus de l'agriculture intensive (voir Pywell et al. 2002; Walker et al. 2004; Kiehl et al. 2006) ; les anciennes carrières (voir Wheater & Cullen 1997; Cullen et al. 1998). Dans tous les cas, l'environnement de départ ne permet pas la réinstallation directe des espèces typiques des pelouses calcicoles. Il convient donc dans un premier temps de recréer les conditions abiotiques (principalement le niveau de fertilité) et biotiques (structure de végétation) nécessaires à l'établissement d'une pelouse riche en espèces.

Restauration des conditions abiotiques (niveau de fertilité)

La relation qui existe entre la productivité d'un milieu — ou les facteurs qui l'influencent comme la teneur en éléments minéraux du sol — et sa richesse en espèces est un concept d'intérêt majeur pour la conservation de la biodiversité, qui a déjà été largement étudié (Al-Mufti et al. 1977; Wheeler & Shaw 1991; Janssens et al. 1998; Loreau 2000; Mittelbach et al. 2001; Jacquemyn et al. 2003a). Bien que cette relation puisse varier selon le milieu considéré (voir Mittelbach et al. 2001), la teneur en éléments minéraux du sol est, dans de nombreux cas, un facteur limitant la richesse en espèces (Figure 1).

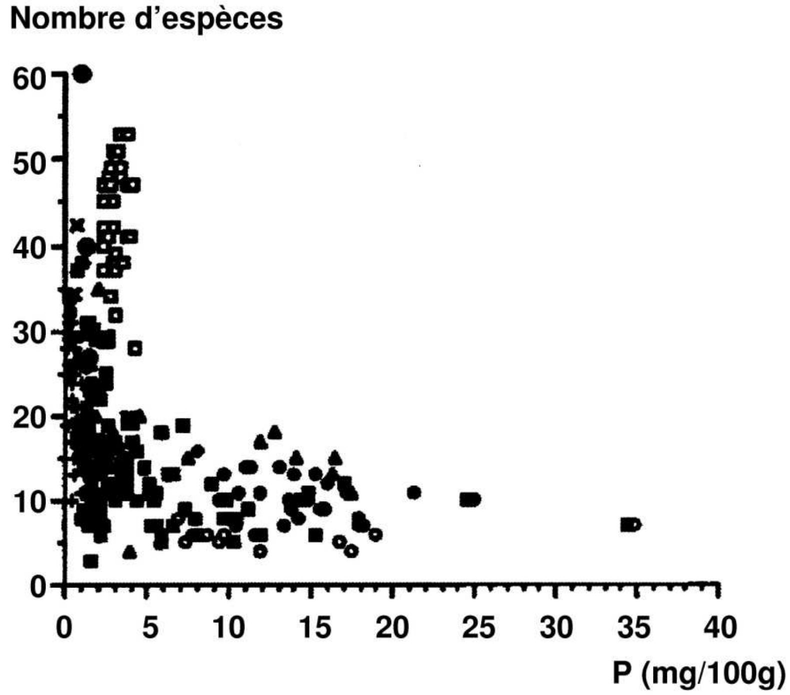

Figure 1 : Relation entre le nombre d'espèces par 100 m² et la teneur en phosphore du sol dans différents types de prairies en Europe. Les symboles différents représentent différents sites. D'après Janssens et al. (1998), modifié.

Par ailleurs, il est généralement admis que l'enrichissement en azote et autres éléments minéraux est l'une des principales causes de perte de diversité pour de nombreux écosystèmes (Marrs 1993; Janssens et al. 1998; Maron & Jefferies 2001). Cet enrichissement entraîne un changement dans les relations de compétition entre espèces, qui mène le plus souvent à la dominance de quelques espèces compétitives au détriment d'un grand nombre d'espèces qui le sont moins (Bakker 1987; Marrs 1993; Bobbink et al. 1998; Pywell et al. 2002), et participe ainsi à un phénomène plus global d'homogénéisation de la flore causé par les pratiques humaines (McKinney & Lockwood 1999). Les pelouses calcicoles n'échappent pas à la règle. Historiquement, ces écosystèmes n'ont jamais été amendés et de grandes quantités de matière en étaient exportées par le pâturage itinérant. Il en résulte que ces milieux sont, par nature, pauvres en éléments nutritifs et de faible productivité (Grime 1979; Critchley et al. 2002b), et leur enrichissement entraîne une perte importante de richesse spécifique (Willems et al. 1993; Jacquemyn et al. 2003a). La restauration d'un niveau de fertilité adéquat est donc une condition importante pour l'établissement d'une pelouse riche en espèces.

Il est évident que c'est lors de la restauration d'anciennes terres cultivées ou de prairies amendées que cette contrainte est la plus forte. Toutefois, Hurst & John (1999a) ont montré qu'un enrichissement en azote du sol pouvait être enregistré durant les premiers stades d'enfrichement des pelouses par les graminées sociales. Il n'est par ailleurs pas exclu que la conversion d'une pelouse en un écosystème forestier provoque, elle aussi, un enrichissement en éléments minéraux, comme cela a été montré pour d'autres milieux semi-naturels (Green 1972; Davis 1994). L'enrichissement en azote peut aussi se présenter comme un phénomène récurrent, sous la forme de dépôts atmosphériques d'oxydes d'azote ou de composés ammoniacaux (Bobbink et al. 1998).

Au cours du temps, les sols ont tendance à s'appauvrir naturellement par lessivage des éléments nutritifs. Toutefois, ce phénomène est très lent (Marrs et al. 1991). Dès lors, différentes techniques permettant d'accélérer la réduction de la fertilité du sol ont été mises au point (Marrs 2002; Walker et al. 2004).

Une première technique consiste à faucher le site à restaurer et à en exporter la biomasse produite. Le principe sous-jacent étant d'enlever, par l'exportation, une quantité d'éléments plus grande que celle qui entre dans la parcelle (Marrs et al. 1998). Bien que l'idée semble cohérente du point de vue théorique, ses résultats sont le plus souvent modestes, que ce soit pour les pelouses calcicoles (Rizand et al. 1989; Kiehl et al. 2003), ou pour d'autres types de pelouses (Marrs et al. 1998; McCrea et al. 2001).

Différents traitements du sol permettent de réduire les teneurs en éléments minéraux, ou leur disponibilité. Parmi celles-ci, le traitement du sol par des composés adsorbants, tels que les oxydes et hydroxydes de fer et d'aluminium, a été proposé comme technique pour réduire la disponibilité du phosphore notamment. Les résultats liés à ces techniques sont relativement intéressants en termes de réduction de la concentration en phosphore dans les plantes (voir Walker et al. 2004) ou de phosphore soluble dans le sol (Janssens 1998). Toutefois, leur utilisation peut poser un certain risque du fait de la toxicité des composés utilisés. Ces techniques sont donc à proscrire à proximité des zones sensibles. D'autres traitements moins risqués ont été proposés. L'ajout de matériel inerte (gravier, boues de carrières) permet de diluer les concentrations en éléments minéraux (Marrs 1993). L'ajout de matériel riche en carbone (écorce, paille, sciure) permet, lui, d'augmenter l'activité microbienne et d'immobiliser ainsi une partie de l'azote contenu dans le sol (Blumenthal et al. 2003; Spiegelberger 2006). Ces techniques, bien que potentiellement intéressantes montrent toutefois des résultats contrastés (Blumenthal et al. 2003; Walker et al. 2004).

Une autre technique, qui est aussi probablement la plus efficace à ce jour, consiste à enlever la couche superficielle du sol, où la concentration en éléments minéraux est la plus forte. Dans de nombreux cas, cette technique a permis de réduire fortement la concentration d'azote et de phosphore dans le foin récolté sur les parcelles restaurées, ainsi que de diminuer la productivité du site (Hopkins et al. 1999; Walker et al. 2004). En conséquence, on peut observer, suite à ce traitement, une meilleure installation des espèces typiques des pelouses, bien que la richesse spécifique n'augmente pas forcément à court terme (Hopkins et al. 1999; Kiehl &

Pfadenhauer 2007). A défaut d'enlever cette couche superficielle, un labour profond permet de la diluer dans les couches moins riches situées en profondeur (Pywell et al. 2002).

Restauration des conditions biotiques (structure de végétation)

Les pelouses calcicoles ne constituent pas, en Europe occidentale, un habitat climacique. Elles font partie d'une série dynamique de la végétation qui va de la roche calcaire mise à nu jusqu'à la forêt climacique, qui est le plus souvent la hêtraie calcicole dans nos régions. Les pelouses calcicoles formant une voie détournée de la succession normale par le pâturage ou la fauche, autrement dit un plagioclimax (Green 1972; Maubert & Dutoit 1995; Dutoit & Alard 1996a) (Figure 2).

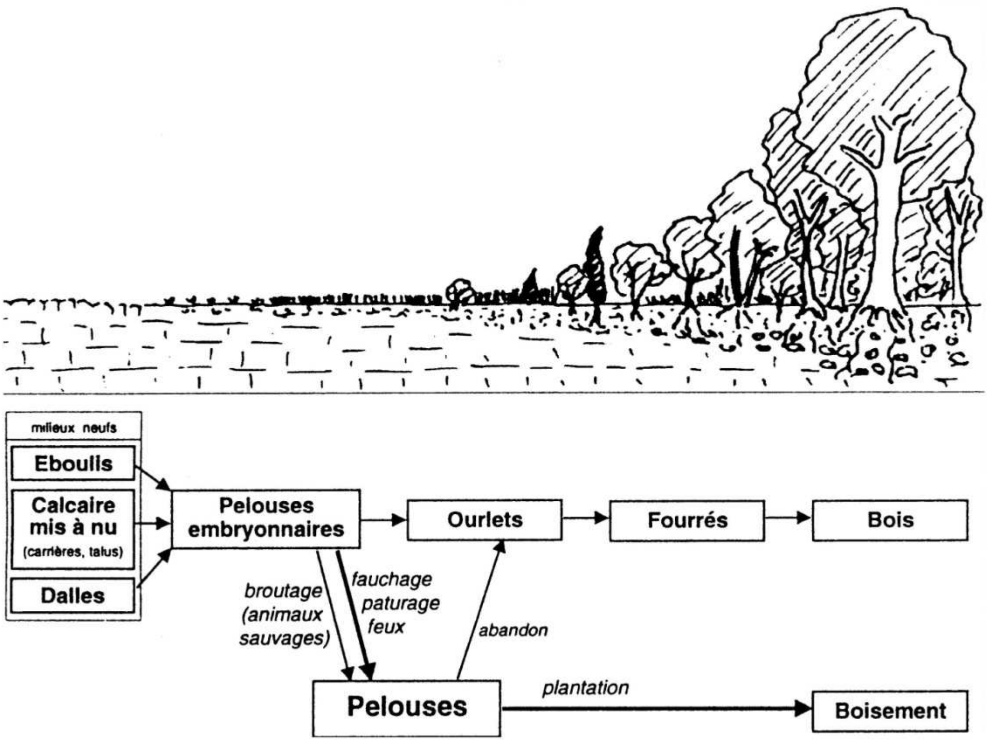

Figure 2 : Dynamique des végétations calcicoles d'Europe occidentale. D'après Maubert et al. (1995), modifié.

Il résulte de cela que les pelouses calcicoles peuvent être restaurées à partir des différents stades de cette série évolutive. Selon que l'état de départ correspond à un stade antérieur (dalle rocheuse, pelouse embryonnaire ; par exemple dans les anciennes carrières) ou postérieur (fourré, forêt), il s'agira d'accélérer ou de faire régresser la succession pour obtenir l'habitat désiré (Prach et al. 2007). Ces manipulations de la succession font appel d'une part aux relations entre les plantes et le sol, qui ont déjà été abordées dans la section précédente, mais surtout aux relations plantes-plantes qu'elles soient intra- ou interspécifiques. Pendant de nombreuses années, l'étude de ces interactions s'est limitée à celle de la compétition pour les ressources. Ce n'est que récemment que la facilitation a été considérée comme une composante essentielle dans le fonctionnement des écosystèmes et de leur développement (Brooker et al. 2008). Ces deux pendants des interactions plante-plante ont des applications directes dans le cas de la restauration des pelouses calcicoles. La limitation de la compétition est primordiale pour restaurer des milieux envahis par les espèces ligneuses ou les graminées sociales. Par ailleurs, le renforcement des effets de facilitation peut être un outil très intéressant lors de la restauration de pelouses sur des sols nus.

En effet, certaines activités humaines, telles que l'exploitation de carrière ou la construction de voies de communication, laissent des zones rocheuses totalement dépourvues de végétation. La recolonisation naturelle de ces zones dénudées mène souvent, dans un premier temps à la formation de milieux semblables aux milieux semi-naturels issus des pratiques agropastorales anciennes, servant ainsi de refuge à des espèces et des communautés devenues rares (Cullen et al. 1998). Toutefois, ces milieux sont fortement hostiles (absence de sol, sécheresse, pauvreté en éléments nutritifs) pour l'installation et pour le développement des espèces végétales, ce qui rend le processus extrêmement lent, et donc souvent incompatible avec les délais imposés pour la réhabilitation de ces sites (Wheater & Cullen 1997; Cullen et al. 1998). Dès lors, l'objectif principal de nombreux projets de réhabilitation écologique de carrières a été d'obtenir rapidement un couvert végétal indigène, mais pas forcément de recréer des communautés végétales riches en espèces (voir par expl. Dixon & Hambler 1984; Davis et al. 1985; Clemente et al. 2004). Pourtant, certaines techniques montrent un intérêt pour la restauration de pelouses calcicoles, ou de communautés affines, à partir d'anciennes carrières, et ce dans un délai raisonnable. Le remodelage du profil topographique à l'aide d'explosifs suivi d'un ensemencement permettent d'accélérer la restauration de communautés végétales des dalles rocheuses calcaires (Cullen et al. 1998). Davis et al. (1993) ont montré que l'application de faibles quantités d'engrais pouvait favoriser le développement des espèces cibles. Néanmoins, il s'agit de rester prudent, car même dans des conditions extrêmement difficiles, l'application trop importante d'engrais peut empêcher le développement de pelouses riches en espèces (Mitchley et al. 1996). Enfin, les avancées récentes dans la connaissance des interactions plantes-plantes a permis la mise en place d'essais de nouvelles techniques de restauration (Brooker et al. 2008). L'utilisation d'espèces-nurses destinées à stabiliser le sol et/ou à créer des conditions micro-climatiques favorables pourrait se révéler un outil intéressant pour la création de pelouses à partir de la roche nue. Cette technique a déjà été utilisée avec plus ou moins de succès pour la restauration d'autres types de milieux (Castro et al. 2002; Gómez-Aparicio et al. 2004; Huber-Sannwald & Pyke 2005), mais rarement pour la restauration de pelouses calcicoles (mais voir Mitchley et al. 1996). Pourtant, il a été montré dans plusieurs études que la régénération d'un certain nombre d'espèces typiques des pelouses calcicoles est favorisée par la présence d'un couvert végétal, et ce d'autant plus que le milieu est xérique et peu productif (Hillier 1990; Ryser 1993). Dès lors, cette technique pourrait s'avérer intéressante dans le cadre de la restauration de pelouses calcicoles à partir de roches nues. Toutefois, leur utilisation n'est pas toujours simple et leurs effets demandent souvent plusieurs années avant de s'exprimer pleinement (Brooker et al. 2008).

D'un autre côté, l'abandon progressif des pratiques agro-pastorales depuis la fin du XIX^{ème} siècle a entraîné l'évolution de grandes surfaces de pelouses calcicoles vers des forêts secondaires. Trois phases peuvent être reconnues au cours de cette évolution : phase de colonisation par les graminées sociales (*Brachypodium pinnatum, Bromus erectus*) ; phase pré-forestière (colonisation par les arbustes *Prunus spinosa, Crataegus monogyna, Corylus avellana*, …) ; phase forestière dominée par des espèces arborées (Wells 1969; Dutoit & Alard 1996a; Delescaille et al. 2004). La plantation artificielle de conifères (principalement *Pinus nigra* et *P. sylvestris*) dès le milieu du XIX^{ème} siècle a contribué à amplifier ce phénomène (Vandermotten & Decroly 1995). Ces forêts naturelles et artificielles issues d'anciennes pelouses calcicoles présentent un certain potentiel pour la restauration de cet habitat. Dans ce cas, les techniques de restauration et de gestion post-restauration visent principalement à maîtriser la compétition par les espèces ligneuses et les graminées sociales.

La première phase de restauration de milieux forestiers consiste à déboiser ou débroussailler la parcelle à restaurer. Cette pratique n'a lieu qu'une fois, mais elle ne suffit pas à assurer la restauration d'une pelouse calcicole (Delescaille et al. 1990; Zobel et al. 1996; Dzwonko & Loster 1998). En effet, les espèces ligneuses qui composent les formations à restaurer ont pour la plupart la capacité de rejeter vigoureusement de souche. Afin de réduire cette vigueur, une technique de brûlage des souches de buis (*Buxus sempervirens*) au chalumeau a été utilisée avec succès dans la vallée du Viroin (Belgique), mais le travail est assez contraignant, l'utilisation de ce matériel étant malaisée sur les terrains fort accidentés. Une autre possibilité consiste à réaliser un gyrobroyage des souches après la coupe, qui va réduire leur capacité de rejet. Toutefois, cette technique occasionne des dégâts à la végétation en place et ne peut donc être utilisée que si celle-ci ne présente pas d'intérêt conservatoire particulier (Delescaille, comm. pers.).

En plus des rejets de ligneux, il importe de limiter ou de réduire l'envahissement par les graminées sociales. Différentes techniques existent, qui permettent de gérer les rejets ligneux et les graminées sociales conjointement. La fauche a été testée comme technique de gestion dans de nombreuses études (e.a. Wells 1969; Green 1980; Burger 1984; Bobbink 1991; Bobbink & Willems 1993; Maron & Jefferies 2001). Elle a principalement été utilisée pour le contrôle des graminées sociales, mais peut aussi servir pour éliminer les rejets de souches ligneux (Delescaille et al. 1991). Il ressort de ces études que les meilleurs résultats sont obtenus en fauchant plusieurs fois par an (Wells 1969; Bobbink & Willems 1993). Si cela n'est pas possible, il est préférable de réaliser la fauche au début de l'été (juin-juillet), au moment où les parties aériennes des plantes contiennent leur maximum de ressources, plutôt qu'en fin de saison (Green 1980; Krüsi 1981; Bobbink & Willems 1987; Bobbink & Willems 1991; Köhler et al. 2005). Dans la plupart des cas, le foin issu de la fauche est exporté (Bobbink & Willems 1987; Bobbink & Willems 1991; Köhler et al. 2005), afin de contribuer à l'appauvrissement du sol. Toutefois, Kahmen et *al.* (2002) n'ont pas pu montrer de différences notables entre des parcelles gérées avec ou sans exportation du foin, et ce sur une période de 25 ans. Lorsque la situation le permet (terrain peu accidenté et facile d'accès), ces actions de débroussaillage et de fauche peuvent être réalisées mécaniquement à l'aide d'engins tractés. Cela permet de réduire fortement les coûts de main d'œuvre liés à la gestion (Delescaille et al. 1995).

Bien que les pelouses calcicoles de certaines régions soient issues de la fauche traditionnelle (voir Köhler et al. 2005), la plupart d'entre elles se sont formées et maintenues grâce au pâturage itinérant (Wells 1969; Delescaille 2002). C'est donc assez naturellement que des techniques de restauration et de gestion par pâturage ont été testées à travers toute l'Europe. La mise en place d'un système de gestion par pâturage est un exercice complexe.

Premièrement, se pose la question du choix de l'espèce animale et de la race à utiliser pour le pâturage. Il est généralement admis que les moutons de race rustique ainsi que les chèvres sont les animaux les mieux adaptés pour la gestion des pelouses calcicoles (Dutoit & Alard 1996a; Delescaille 2002). Les deux espèces sont très sélectives quant aux végétaux consommés. Si les moutons sont très efficaces pour le contrôle des graminées sociales, les chèvres montrent quant à elles une nette préférence pour les éléments ligneux. Il existe donc une certaine complémentarité entre les deux espèces en ce qui concerne la restauration des pelouses (Haumann 1998; Colas et al. 2002; Delescaille 2002; Tischew et al. 2008). Deuxièmement, il faut s'assurer que la charge de pâturage imposée permette de contrôler les ligneux et les graminées sociales sans pour autant en arriver à un surpâturage qui serait préjudiciable pour les espèces cibles. Cela ne laisse parfois qu'une marge de manœuvre très étroite (Dutoit & Alard 1996b). Il est généralement préconisé d'appliquer une charge de l'ordre de cinq moutons par hectare et par an sur les pelouses nouvellement restaurées. Cette charge devra ensuite être ramenée progressivement à un ou deux moutons par hectare et par an, qui est la charge conseillée pour les pelouses à l'entretien (Dutoit & Alard 1996b; Verbeke & Lejeune 1996; Delescaille 2000; Colas et al. 2002). Toutefois, ce ne sont que des valeurs moyennes, la charge étant fonction de la productivité du milieu qui peut fortement varier d'une station à l'autre et d'une année à l'autre dans la même station (Colas et al. 2002). Troisièmement, il existe plusieurs modalités de gestion par pâturage. Idéalement, la gestion devrait se faire par pâturage itinérant, tel qu'il était pratiqué anciennement (Dutoit & Alard 1996b). Cependant, les conditions socio-économiques actuelles ne permettent pas le plus souvent de réinstaurer le système de pâturage traditionnel. C'est donc la plupart du temps un système de pâturage en enclos qui est mis en place. Dans le cas des pelouses sèches, la meilleure alternative est le pâturage tournant, où une forte charge en bétail pâture la pelouse durant une durée assez courte. Cette technique permet en outre d'avoir une bonne maîtrise de la pression de pâturage imposée (Verbeke & Lejeune 1996).

Bien que la fauche et le pâturage soient les techniques les plus couramment utilisées pour contrôler les rejets ligneux et les graminées sociales, d'autres techniques ont été envisagées telles que le feu (voir Dutoit & Alard 1996a; Verbeke & Lejeune 1996; Köhler et al. 2005) ou l'utilisation d'herbicides (Hurst & John 1999b). Globalement, ces techniques donnent des résultats au mieux médiocres, et posent des problèmes éthiques, pratiques et de sécurité. Dès lors, elles sont peu recommandables, bien que le feu puisse s'avérer intéressant pour réouvrir les milieux fortement embroussaillés (Dutoit & Alard 1996a).

Comme cela a déjà été sous-entendu, il faut noter que, quel que soit le milieu de départ, les techniques de fauche ou de pâturage doivent être appliquées de manière récurrente sur les pelouses nouvellement créées afin d'assurer le maintien à long terme de celles-ci. Toutefois, dans le cas de restaurations à partir de sols rocheux, tel est le cas des anciennes carrières, il est connu que le pâturage peut freiner l'installation d'un couvert végétal (Davis et al. 1985; Davis et al. 1993). Dès lors, et bien que l'effet de la fauche sur ces milieux ne soit pas connu, il est préférable d'attendre que le couvert végétal soit comparable à celui des pelouses de référence avant d'y appliquer une gestion régulière.

Restauration de la communauté végétale

Introduction

Une fois les conditions biotiques et abiotiques rétablies, il importe que le cortège d'espèces typiques puisse se reformer. Il arrive qu'un certain nombre d'espèces ait pu subsister dans l'habitat dégradé, parfois de manière peu visible. Pour les autres espèces, trois mécanismes peuvent intervenir dans ce processus de colonisation : l'expression de la banque de graines ; la dispersion spatiale de propagules par voies naturelles ; la dispersion spatiale, plus ou moins dirigée, de propagules par des actions anthropiques.

Persistance des espèces végétales dans les habitats dégradés

Un certain nombre d'espèces végétales sont capables de survivre dans les habitats issus de la dégradation des pelouses calcicoles, en particulier dans les formations forestières ou pré-forestières qui se développent suite à l'abandon de celles-ci. Si une grande partie des espèces typiques des pelouses est capable de survivre dans des pelouses récemment abandonnées, très peu d'entre elles sont par contre capables de survivre sous un couvert forestier dense ; citons toutefois *Brachypodium pinnatum*, *Carex flacca* et *Viola hirta* (Poschlod et al. 1998; Bisteau & Mahy 2005b). Dans les premières années suivant la restauration, le développement végétatif de ces espèces persistantes va être un des mécanismes les plus actifs de recolonisation de l'espace restauré (Hofmans & Delescaille 1990). Il résulte dès lors que l'état initial de la parcelle restaurée va avoir un impact important sur le succès de la restauration (Dzwonko & Loster 1998).

Expression de la banque de graines

La banque de graine est l'ensemble des graines et des fruits présents sur ou dans le sol (Leck et al. 1989). Sa composition en un endroit donné est globalement fonction de la composition historique de la végétation sus-jacente, de la longévité des graines, des stratégies de régénération de chaque espèce et des facteurs environnementaux influençant la persistance des graines (Harper 1977; Thompson & Grime 1979; Cavers & Benoit 1989). Pour que cette banque de graines soit utile à la reconstitution du cortège d'espèces typiques, il est donc nécessaire qu'une pelouse calcicole ait été présente historiquement à l'endroit ou à proximité du site restauré, et que les graines des espèces cibles aient pu persister dans les conditions environnementales rencontrées avant la restauration de conditions favorables à leur germination. Certaines espèces caractéristiques des pelouses calcicoles sont connues pour répondre à ces conditions. C'est le cas par exemple de *Carex flacca*, *Origanum vulgare, Euphorbia cyparissias* et quelques *Fabaceae*. Toutefois, il a été montré à plusieurs reprises que la banque de graines des pelouses calcicoles était majoritairement transitoire (i.e. les graines ne survivent qu'un an dans le sol) et ne jouait donc qu'un rôle limité dans la reconstitution du cortège floristique typique (Dutoit & Alard 1995; Hendry et al. 1995; Kiefer & Poschlod 1996; Pärtel et al. 1998; Bakker & Berendse 1999; Bisteau & Mahy 2005b; Bossuyt et al. 2006).

Dispersion spontanée des espèces cibles

La pluie de graines, c'est-à-dire l'ensemble des graines atteignant un endroit donné suite à une dispersion spatiale, est considérée comme une contrainte importante pour la restauration des pelouses calcicoles et des milieux semi-naturels en général (Bakker et al. 1996; Pärtel et al. 1998; Bakker & Berendse 1999). En effet, plusieurs études ont montré que la plupart des espèces typiques de ces milieux possédaient de faibles capacités de dispersion spontanée. Une étude menée par Verkaar et al. (1983) sur quatre espèces typiques des pelouses calcicoles a donné des capacités de dispersion spontanée de 0,3 à 3,5 m pour ces espèces. D'après Stampfli & Zeiter (1999), peu d'espèces de ces pelouses sont capables de se disséminer à plus de 25 m, et les échanges à longue distance ont parfois été considérés comme insignifiants (Maurer et al. 2003). Toutefois, ces études ne mettent en évidence que le caractère improbable des phénomènes de dispersion à longue distance ; si l'on considère un temps suffisamment long, de tels phénomènes peuvent se produire. Par exemple, Willems (2001) a pu observer l'apparition de *Gentianella germanica* sur une pelouse, 25 ans après sa restauration. Cette espèce connue pour ses faibles capacités de dispersion (Verkaar et al. 1983), provenait probablement d'un site situé à 300 m de là et aurait été dispersée par des blaireaux. Récemment, des modèles ont été mis au point afin de permettre une approche plus quantitative et multi-espèces de la dispersion. De telles modèles existent pour la dispersion par le vent (Tackenberg et al. 2003) et par epi-zoochorie (Römermann et al. 2005). Une application récente de ce dernier modèle a permis de montrer que la dispersion par les mammifères joue un rôle tout à fait significatif dans la distribution des espèces des pelouses calcaires (Adriaens et al. 2007). Une telle application manque encore concernant la dispersion par le vent, mais il est certain que ces modèles pourraient remettre en cause une partie des conclusions sur le potentiel de dispersion de certaines espèces des pelouses calcicoles. Quoiqu'il en soit, il est un fait que beaucoup d'espèces typiques des pelouses calcicoles présentent des capacités de dispersion relativement faibles. Dès lors, la présence de sources de graines dans les environs immédiats du site restauré sera d'une importance considérable pour la reconstitution d'une communauté végétale typique à partir des pools d'espèces et de gènes locaux (Bakker et al. 1996; Kiefer & Poschlod 1996; Pärtel et al. 1998; Willems & Bik 1998).

Techniques permettant d'améliorer la disponibilité en graines

Dans beaucoup de cas, les capacités de dispersion temporelle (banque de graines) et spatiale (pluie de graines) ne suffisent pas à restaurer complètement le cortège floristique après plusieurs années. Ce problème est d'autant plus important que les sites sont isolés et que les espèces typiques ne sont plus présentes dans leur voisinage immédiat (Pärtel et al. 1998; Lindborg & Eriksson 2004b). En outre, il apparaît souvent qu'un certain nombre d'espèces, notamment les espèces rares typiques des vieilles pelouses, ne parviennent pas à recoloniser les milieux réouverts rapidement (Kiefer & Poschlod 1996; Zobel et al. 1996; Dzwonko & Loster 1998; Lindborg & Eriksson 2004b). Il arrive d'ailleurs que certaines espèces ne soient pas présentes dans la pluie de graines, malgré la présence de populations-sources à proximité (voir Bakker et al. 1996). Il en résulte que, dans le cas particulier de ces espèces ou dans le cas de sites isolés, il convient de mettre en place des mesures visant à améliorer la disponibilité en graines voire à introduire volontairement des espèces.

Une première manière d'améliorer la disponibilité en graines sur un site restauré est d'adopter un système de gestion par pâturage itinérant ou tournant. Les animaux, et les moutons en particulier, sont capables de transporter un nombre très important de graines dans leur pelage (exozoochorie) ou leur tube digestif endozoochorie (Fischer et al. 1996; Mitlacher et al. 2002; Couvreur et al. 2004a; Cosyns et al. 2005). Dès lors, dans un système de pâturage où les animaux passent alternativement sur des anciennes pelouses puis des pelouses restaurées, un grand nombre de graines d'espèces caractéristiques peut être transporté en provenance de populations-sources relativement éloignées (Mouissie et al. 2005). Les moutons sont particulièrement efficaces en ce qui concerne l'exozoochorie, puisque leur laine dense, bouclée et grasse permet même le transport de diaspores dépourvues de dispositif d'adhésion (Shmida & Ellner 1983; Fischer et al. 1996). Pour ces raisons, le pâturage tournant a souvent été considéré comme un outil important pour la restauration du cortège floristique des pelouses calcicoles

(Gibson et al. 1987; Barbaro et al. 2001; Hellström et al. 2003). Dans un ordre d'idée similaire, quoique bien moins étudié, les activités humaines de gestion ou de loisirs sur les pelouses calcicoles peuvent être responsables de la dispersion d'espèces typiques de ces milieux, par fixation sur les vêtements ou les machines servant à la gestion (Clifford 1956; Bakker et al. 1996; Strykstra et al. 1997).

Un second type de méthode, plus ciblé que le précédent, consiste à transporter un pool de graines provenant d'une pelouse en bon état (site donneur) vers un site restauré. Différentes méthodes ont déjà été proposées. Premièrement, la transplantation peut se faire directement sous forme de graines. Ces graines peuvent soit provenir du commerce, soit être récoltées sur site à la main, ou mécaniquement (machine à brosses, pompe à vide) afin de réduire les coûts de main d'œuvre, puis ressemées sur le site restauré (Stevenson et al. 1997; Riley et al. 2004; Edwards et al. 2007). Une seconde méthode, consistant à transplanter des mottes d'un site vers un autre, a été proposée par Pärtel *et al.* (1998). Toutefois, le succès de cette technique était assez limité. Enfin, une technique qui a été utilisée avec des résultats prometteurs est le transfert de foin. Elle consiste à faucher un site donneur le matin quand les végétaux sont mouillés et que les graines y restent collées, puis à répandre le foin fraîchement coupé sur le site restauré (Jones et al. 1995; Kiehl & Wagner 2006; Edwards et al. 2007; Kiehl & Pfadenhauer 2007). Quelle que soit la technique utilisée, il est largement conseillé de transplanter du matériel végétal provenant de sites proches, afin de préserver l'intégrité du pool génétique régional des espèces et de profiter des adaptations locales (Stevenson et al. 1997; Jones & Hayes 1999; Smith et al. 2005). Cette remarque est surtout valable dans le cas de l'utilisation de graines provenant du commerce et dont l'origine est souvent incertaine.

Dans le cas d'espèces particulièrement rares, ou présentant des difficultés à se réinstaller dans les milieux restaurés, des mesures particulières pourraient être prises afin d'introduire volontairement l'espèce dans un site donné (semis, transplantation de plantes, plantules, rhizomes, …). De telles méthodes nécessitent évidemment une connaissance approfondie de l'écologie de l'espèce cible. Ces méthodes ont déjà été testées sur plusieurs espèces (voir Gilfedder et al. 1997; Morgan 1999). Une expérience de ce type a été tentée en Suisse sur *Pulsatilla vulgaris*, une espèce typique des pelouses calcicoles, par Pfeifer *et al.* (2002), dans le but de renforcer les populations de l'espèce. Plusieurs espèces ont pu être ainsi réintroduites sur des pelouses restaurées au Royaume-Uni (Hutchings & Stewart 2002).

Evaluation du succès et de la pérennité de la restauration

L'évaluation est une étape délicate du processus de restauration, mais néanmoins tout à fait indispensable. La question se pose quant aux paramètres à prendre en compte lors de cette évaluation. Dans son abcédaire sur l'écologie de la restauration, la Society for Ecological Restoration International Science & Policy Working Group (2004) donne toutefois les grands principes à intégrer pour une évaluation complète du succès de la restauration. D'après ce guide, neuf attributs de l'écosystème restauré doivent être pris en compte : (1) même diversité et même structure de communauté que les sites de référence, (2) présence d'espèces indigènes, (3) présence des groupes fonctionnels nécessaires pour une stabilité à long terme, (4) capacité de l'environnement physique de maintenir des populations reproductrices des espèces à sa stabilité, (5) fonctionnement normal de l'écosystème, (6) intégration dans le paysage écologique, (7) menaces potentielles éliminées ou réduites, (8) résilience aux perturbations naturelles et (9) l'écosystème se maintient lui-même au même degré que son écosystème de référence. La plupart du temps, les limitations de temps et de budget ne permettent pas un monitoring aussi complet du succès de la restauration. Dans leur méta-analyse, Ruiz-Jean & Aide (2005) ont mis en évidence que le succès de la restauration était actuellement basé sur des mesures (1) de diversité (principalement plantes et arthropodes), (2) de structure de végétation et (3) de processus écologiques. Ils concèdent toutefois que la prise en compte de deux mesures relevant de chacun de ces trois paramètres constitue déjà une évaluation valable du succès de la restauration.

Dans le cas de la restauration des milieux semi-naturels sans apport de graines dirigé, il serait cependant intéressant d'évaluer le fonctionnement des populations d'espèces nouvellement apparues sur les sites restaurés. En effet, en raison du caractère exceptionnel des phénomènes de dispersion à longue distance, il est probable que ces populations se sont formées à partir d'un nombre de graines très limité au départ. Dès lors, il résulte que ces populations peuvent présenter des effets dits « de fondation » diminuant leur résilience aux perturbations naturelles et aux changements globaux, ainsi qu'un risque accru de dépression de consanguinité pouvant entraîner une diminution du fitness de la population (Ridley 1997; Society for Ecological Restoration International Science & Policy Working Group 2004).

Enfin, les pelouses calcicoles nécessitant un entretien récurrent, le succès de la restauration ne sera total que si un système d'entretien permanent est assuré suite à la campagne de restauration.

Conclusions et perspectives

La restauration des milieux semi-naturels en général, et des pelouses calcicoles en particulier, est un problème complexe, mettant en œuvre les compétences issues de nombreuses disciplines scientifiques (écologie, pédologie, ingénieries biologique et civile, …). Les processus nécessaires à sa complète réussite sont encore méconnus, bien que ce ne soit peut-être qu'une question de temps (Zobel et al. 1996; Pärtel et al. 1998).

Actuellement, la restauration des pelouses calcicoles a pour but principal de recréer, en partie, les paysages écologiques qui existaient par le passé. Cela implique de restaurer des pelouses dans les sites même où elles existaient jadis. Cependant, certains auteurs ont insisté récemment sur l'opportunité que représentaient les milieux fortement anthropisés (bord de route, parcs des villes, …) pour y installer des espèces indigènes et sur l'intérêt biologique et sociologique de cette pratique (Simmons et al. 2007). La réflexion pourrait être poussée plus loin en proposant que, non seulement les espèces utilisées soit indigènes, mais qu'elles forment en plus des communautés végétales indigènes d'intérêt biologique. Les travaux réalisés jusqu'à présent en restauration de milieux semi-naturels nous ont donné les bases théoriques et pratiques qui permettraient de mener à bien un tel projet.

CHAPITRE 2 : PLANT COMMUNITIES AND SPECIES RICHNESS OF THE CALCAREOUS GRASSLANDS IN SOUTH-EAST BELGIUM

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Abstract

Calcareous grasslands are biodiversity hotspots in Western Europe. In Belgium, a number of phytosociological surveys has been realized in these habitats, but none covers the whole range of calcareous grasslands at the regional scale. The aim of this study was (i) to provide a synthesis of the floristic variation of calcareous grasslands of the Calestienne natural region using a uniform methodology; (ii) to relate floristic variation to environmental variables and (iii) to characterize the specific diversity of the different grassland communities that occur in the study region. Seven different communities were identified with the TWINSPAN method. The originality of the grasslands on calcareous and calcaro-siliceous rocks was statistically confirmed. Significant differences for environmental variables were identified among the seven communities by a MANOVA. Main differences between communities were related to xericity and pH although also a North-South gradient was identified. More xeric grasslands were located in the southern part of the study region while Northern part was occupied by more mesophilous grasslands. Multiple regressions were used to describe the influence of the environmental conditions on plant species richness. The most mesophilous grasslands appeared to be the most species rich while soil acidity negatively affected species richness.

Introduction

Calcareous grasslands of the *Festuco-Brometea* extend from southern Scandinavia to northern Spain, with their main distribution in the Atlantic, central-European and sub-Mediterranean regions (Royer 1991). Calcareous grasslands are among the most species-rich habitats in Western Europe, both at local and regional scales (Willems 2001; Poschlod & WallisDeVries 2002). Belgium coincides with the north-western boundary of the distribution of many grassland plant species, especially thermophilous ones such as *Teucrium montanum* and *Aster linosyris*, and plant communities, such as *Xerobromion* grasslands (WOLKINGER & PLANK 1981; MAUBERT & DUTOIT 1995). Belgian calcareous grasslands are located at the border between the Atlantic and the central European floristic regions (Butaye et al. 2005b).

Since World War II, the extent of calcareous grasslands has dramatically decreased in Western Europe (Dzwonko & Loster 1998). Many surveys have been made during the last decades in order to determine the most suitable management and restoration measures (e.g. Bobbink et al. 1987; Delescaille et al. 1995; Köhler et al. 2005). In Belgium, ca. 100 hectares of calcareous grasslands have been restored since 1990. Many are still occupied by clear cuttings managed with the aim to restore typical grassland communities. The remaining grasslands are frequently dominated by *Brachypodium pinnatum* or *Bromus erectus* due to the absence of grazing or mowing. The total currently managed area is c. 300 hectares (André & Vandendorpel 2004; Graux 2004). However, managing and restoring plant communities at a regional scale require a good knowledge of the floristic composition and diversity variation in order to adapt management objectives to the local situation.

Despite numerous local descriptive studies of calcareous grasslands in Belgium (i.e Noirfalise & Dethioux 1982; Duvigneaud 1989; Brackelaire 1993), no large scale general phytosociological synthesis, based on a large number of relevés and consistent statistical analyses, has been realised yet. Van Speybrouck et al. (1989) performed a classification of calcareous grasslands in Belgium, only using a limited number of relevés and focusing on the *Xerobrometum* types in its northern location. Recently, Butaye et al. (2005b) proposed a detailed study of the floristic variation of calcareous grasslands in Belgium, based on a large number of relevés. The geographic scale of this study, however, was limited to the Viroin valley (western part of the Calestienne natural region).

This paper presents a regional scale study of the floristic variation and diversity of calcareous grasslands across the Calestienne region, with the exception of the Viroin valley already studied by Butaye et al. (2005b). It differs from previous studies because: (i) we covered the whole geological range of calcareous grasslands; (ii) the large scale considered allowed to take into account biogeographical variability; (iii) we based our analysis on a large number of relevés with an undirected relevé selection.

Our aims were (i) to provide a synthesis of the floristic variation of calcareous grasslands of the Calestienne using a uniform methodology in order to classify grasslands into different communities; (ii) to relate this synthesis to environmental variables and (iii) to characterize the species diversity of the different grassland communities identified.

Methods

Study region

The studied grasslands were mainly located in the Calestienne region, Belgium which is characterized by Devonian geological formations consisting of limestone or calcaro-siliceous rocks, the latter supporting soils with higher acidity. The study region also included locally occurring Cretaceous chalk formations near Visé (50°45'N – 5°40'E), and one small Carboniferous calcareous area near Theux (50°32'N – 5°49'E). These two localities were included because of the expected specific floristic composition of their grasslands. The study region is characterised by a hilly landscape with isolated calcareous grasslands in a matrix of forest and arable land.

Data collection

All existing calcareous grassland patches were first localized through literature information and recent ortho-rectified vertical aerial photographs. In the region near Rochefort, grassland locations were obtained from a previous survey (Bottin et al. 2005). A representative subset of patches was selected by stratified sampling, using geographical sub-regions as strata. This subset was representative of the different geological formations present and of the different patch sizes. Within the selected patches, transects were established on a slope gradient. Along these transects, one-meter square plots were located every 20 meters, with a minimum of three plots and a maximum of ten plots per patch. When patch configuration (shape or size) did not allow to establish a transect, plots were randomly located. Plots falling in non-grassland vegetation were moved to the nearest grassland zone nearby their initial location. A total of 477 plots was established (see Appendix 1).

During the 2004 vegetation period, the cover of all higher plant species in the one-meter square plots was recorded using the Braun-Blanquet (1932) scale. A total of 245 plant species were found. Nomenclature followed Lambinon et al. (2004). Species were classified following their preference for phytosociological alliances (see Bisteau & Mahy 2005b for details). Environmental variables were derived directly from field observations for each plot. They included (i) biogeographical variables: Belgian Lambert coordinates and altitude; (ii) topographic and soil variables: slope (degrees), mean soil depth (three measurements) and mean soil pH estimated with a Hellige pH-indicator (two measurements); (iii) community structure variables:

maximum height of the vegetation, bare soil percentage, moss cover, herbaceous vascular species cover and shrub cover (%). Because nutrient status is an important factor explaining grassland floristic composition (Al-Mufti et al. 1977; Critchley et al. 2002b), we derived it indirectly from Ellenberg (1992) as the mean indicator value for soil nitrogen richness (mN), computed over all inventoried species in the plot. Soil moisture status was derived in the same way, using the mean Ellenberg value for humidity (mF).

Data analysis

Prior to the analysis, Braun-Blanquet coefficients were transformed into ordinal coefficients, using the van der Maarel scale (Jongman et al. 1995). To fulfil our first objective, vegetation data was analysed using TWINSPAN (Hill & Smilauer 2005). This method was chosen to make our results comparable with those obtained by Butaye et al. (2005b) in the Viroin valley. Pseudospecies were defined using van der Maarel coefficients 3 and 5 as cut off levels (corresponding to 5% and 25% cover, respectively). A synoptic table was created depicting the constancy of the different species in the groups resulting from the classification. These groups were compared with the communities described in the EUNIS typology (European Environment Agency 2005) based on their indicator species. The distribution of the different communities derived from TWINSPAN was analysed based on the frequency of plots belonging to each community in 10 x 10 kilometres squares.

In relation to our second objective, a Canonical Correspondence Analysis (CCA) was run with CANOCO 4.5 and CanoDraw for Windows (ter Braak & Smilauer 2002). Environmental variables were selected, using 999 Monte Carlo permutations, at a significance level of 0.05. Their values were standardized to give the same weight to each one. We tested whether environmental variables differed significantly between the vegetation types derived from TWINSPAN, through a multivariate analysis of variance (MANOVA) and subsequent ANOVAs, using the SAS CANDISC procedure (SAS Institute Inc. 1999). The considered variables were soil depth, soil pH, slope, height of vegetation, bare soil percentage, shrub cover, herbaceous cover and moss cover. The four latter variables (proportions) were arcsin transformed.

The third objective was first approached through Kruskal-Wallis comparisons of mean species richness per plot among grassland communities (derived from TWINSPAN). Second, the influence of the patch environmental conditions and geographical location on the species richness (one-meter square scale) was examined using best subset multiple regressions. The analysis was carried out both on the whole data set and on the different grassland communities identified. The models were built with the subset of predictor variables for which all *p*-values were significant at a 0.05 minimum level. When necessary, the dependent variables were transformed in order to meet normality and homoscedasticity requirements. Normality of the residuals was tested by Levene's test and their homoscedasticity by Breuch-Pagan's test. Box-Cox transformations on the dependent variable were applied using a Minitab macro (Palm 2002) in order to improve the results for those tests.

Results

Twinspan classification

GENERAL CLASSIFICATION

Calcareous grasslands in the study area belong to the *Festuco-Brometea* class, characterized by, among others, *Festuca lemanii*, *Bromus erectus*, *Helianthemum nummularium*, *Sanguisorba minor* (Table 1). TWINSPAN classification of relevés revealed seven different groups (Fig. 1, Table 1, Table 2). The number of relevés in each group varied considerably (from 7 to 155 relevés, Table 2). Two groups were represented by a very low number of relevés (groups VI and VII), mainly limited to two sites : "Heid des Gattes" (eight relevés out of ten for group VI) and "Heid de Stinval" (five relevés out of seven for group VII).

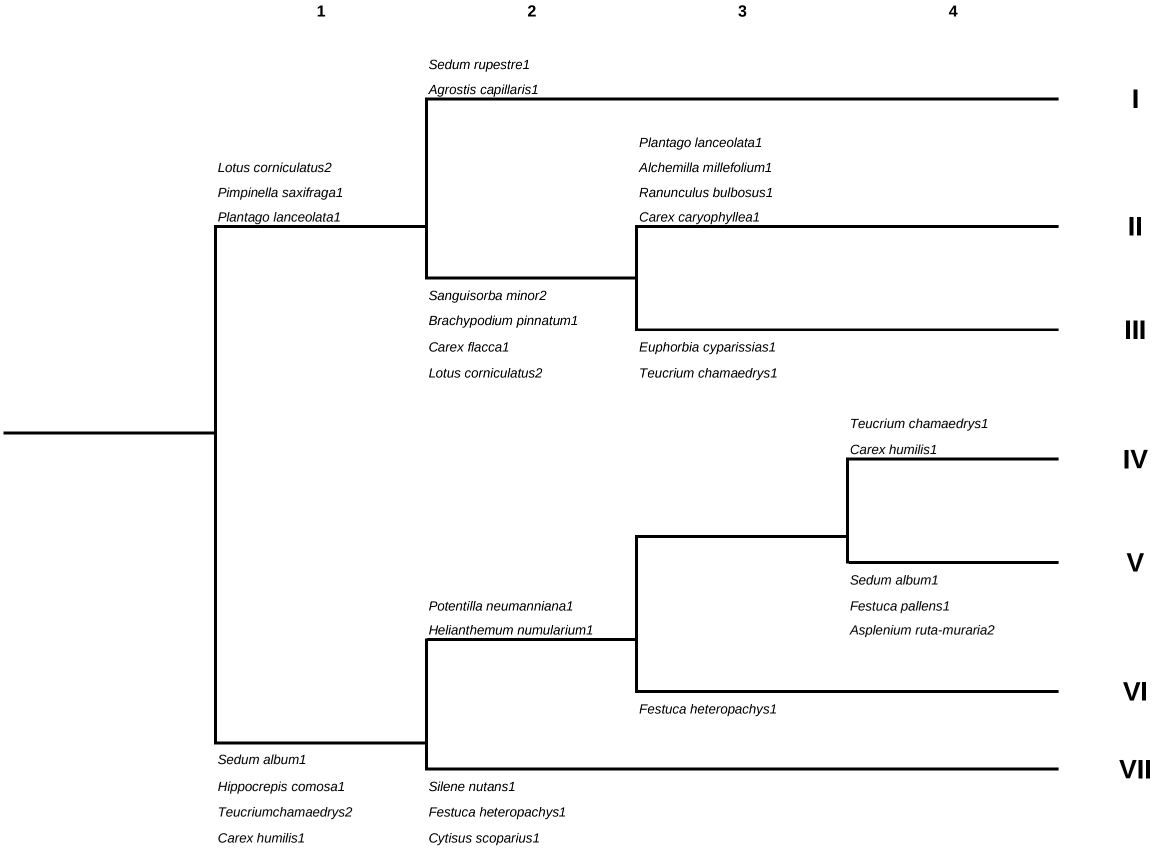

Fig. 1. TWINSPAN classification dendrogram of the calcareous grassland communities of the Calestienne region with their indicator species.

Indicator species of groups I to III were *Lotus corniculatus*, *Pimpinella saxifraga* and *Plantago lanceolata* (Fig. 1).These species are typically mesophilous, so these groups were classified as mesophilous grasslands. Besides the high occurrence of *Mesobromion* species, mesophilous grasslands were characterized by an important proportion of species from the *Molinio-Arrhenatheretea* and the *Trifolion medii* (Table 1). Two mesophilous communities were then identified: the acidic mesophilous grasslands (Group I) and the alkaline mesophilous grasslands (groups II and III). On the other hand, indicator species of groups IV to VII were *Sedum album*, *Hippocrepis comosa*, *Teucrium chamaedrys* and *Carex humilis* (Fig. 1), which are typically xerophilous species. In addition to the presence of *Xerobromion* species, these grasslands were characterized by *Sedo-Scleranthetea* and *Geranion sanguinei* species (Table 1). The xerophilous communities were classified into three different plant communities : the very dry grasslands (group IV), the *Festucion pallentis* grasslands (group V) and the *Koelerio-Phleion* grasslands (groups VI and VII).

Table 1. Synoptic table of the grassland communities identified on the basis of the TWINSPAN classification. Species frequency of occurrence in the different communities (% of relevés with presence of the species).

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Festuco-Brometea							
Potentilla neumanniana	57	50	46	67	79	40	-
Thymus pulegioides	41	42	26	20	7	20	14
Hieracium pilosella	39	22	31	13	32	-	14
Sanguisorba minor	28	88	82	40	68	10	14
Bromus erectus	31	72	57	27	46	-	-
Helianthemum nummularium	24	39	50	67	64	60	14
Festuca gr. ovina	67	44	54	27	4	-	14
Campanula rotundifolia	13	12	36	3	14	-	-
Scabiosa columbaria	2	17	31	6	32	-	-
Koeleria macrantha	24	33	17	11	4	-	-
Teucrium chamaedrys	6	6	41	91	21	-	-
Brachypodium pinnatum	4	54	76	39	11	-	-
Euphorbia cyparissias	15	6	47	26	-	-	-
Hippocrepis comosa	2	1	20	69	50	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Mesobromion							
Pimpinella saxifraga	17	60	47	4	7	-	-
Carex flacca	6	44	59	13	-	-	-
Leontodon hispidus	2	26	27	1	-	-	-
Carex caryophyllea	11	38	8	1	4	-	-
Carlina vulgaris	2	6	10	-	4	-	-
Anthyllis vulneraria	2	10	8	4	-	-	-
Ranunculus bulbosus	17	50	15	-	-	-	-
Centaurea scabiosa	6	23	10	1	4	-	-
Genista tinctoria	7	28	15	-	-	-	-
Linum catharticum	2	35	41	-	-	-	-
Medicago lupulina	7	23	22	-	-	-	-
Ononis repens	2	19	6	-	-	-	-
Primula veris	2	11	10	-	-	-	-
Prunella laciniata	2	3	5	-	-	-	-
Genistella sagitallis	4	3	3	-	-	-	-
Polygala comosa	-	2	17	1	-	-	-
Polygala vulgaris	-	18	7	9	-	-	-
Epipactis atrorubens	-	1	7	1	-	-	-
Gymnadenia conopsea	-	1	7	-	-	-	-
Picris hieracioides	-	2	8	-	-	-	-
Plantago media	-	23	12	-	-	-	-
Galium pumilum	-	15	29	1	-	-	-
Cirsium acaule	-	15	18	-	-	-	-
Ononis spinosa	-	2	1	-	-	-	-
Trifolium montanum	-	4	3	-	-	-	-
Platanthera bifolia	-	3	-	-	-	-	-
Platanthera chlorantha	-	2	2	-	-	-	-
Euphrasia nemorosa	-	1	1	-	-	-	-
Carex tomentosa	-	-	3	-	-	-	-
Bunium bulbocastanum	-	1	2	-	-	-	-
Gentianella germanica	-	-	3	-	-	-	-
Gentiana cruciata	-	1	-	-	-	-	-
Ophrys insectifera	-	-	4	-	-	-	-
Calamagrostis epigejos	-	1	-	-	-	-	-
Genista pilosa	-	1	-	-	-	-	-
Anacamptis pyramidalis	-	1	-	-	-	-	-
Gentianella ciliata	-	-	2	-	-	-	-
Onobrychis viciifolia	-	1	-	-	-	-	-
Ophrys apifera	-	3	1	-	-	-	-
Orchis militaris	-	1	1	-	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Xerobromion							
Melica ciliata	-	-	-	29	46	50	14
Aster linosyris	-	3	1	30	4	20	14
Cotoneaster integerrimus	-	-	-	3	7	10	-
Sesleria caerulea	2	1	18	50	54	-	-
Allium sphaerocephalon	-	1	1	40	4	-	-
Arabis hirsuta	-	1	1	13	7	-	-
Dianthus carthusianorum	4	-	-	9	4	-	-
Allium oleraceum	-	2	3	10	11	-	-
Carex humilis	-	-	2	63	-	-	-
Stachys recta	-	-	-	17	-	-	-
Thlaspi montanum	2	1	3	14	-	-	-
Globularia bisnagarica	-	-	5	11	-	-	-
Pulsatilla vulgaris	-	-	-	7	-	-	-
Veronica prostrata	-	-	-	3	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Molinio-Arrhenatheretea							
Hypericum perforatum	24	19	33	9	4	20	43
Leucanthemum vulgare	11	39	15	-	-	40	14
Lotus corniculatus	26	74	70	7	11	-	-
Taraxacum officinale	4	8	33	4	7	-	-
Centaurea jacea	37	45	19	6	4	30	29
Stachys officinalis	4	3	2	14	-	-	-
Achillea millefolium	24	47	8	-	-	-	-
Dactylis glomerata	19	28	10	3	-	-	-
Succisa pratensis	-	16	2	-	-	-	-
Tragopogon pratensis	-	3	4	1	-	-	-
Trifolium medium	2	8	7	-	-	-	-
Trifolium pratense	2	13	2	-	-	-	-
Trifolium repens	9	5	3	-	-	-	-
Trisetum flavescens	6	23	10	-	-	-	-
Knautia arvensis	2	32	22	3	-	-	-
Vicia cracca	-	5	1	-	-	-	-
Vicia hirsuta	9	13	11	-	-	10	-
Vicia sativa	6	6	1	1	-	-	-
Plantago lanceolata	37	61	22	-	-	-	14
Daucus carota	2	32	12	-	-	-	-
Anthoxanthum odoratum	2	8	-	-	-	-	-
Arrhenaterum elatius	15	15	6	-	-	10	-
Potentilla reptans	11	8	5	-	-	-	-
Prunella vulgaris	2	3	7	-	-	-	-
Geranium molle	4	-	-	1	-	-	-
Senecio jacobaea	4	3	8	-	-	-	-
Avenula pubescens	2	18	5	-	-	-	-
Briza media	4	52	22	-	-	-	-
Crepis biennis	2	3	-	-	-	-	-
Dactylorhiza fuchsii	-	3	1	-	-	-	-
Heracleum sphondylium	-	3	-	-	-	-	-
Holcus lanatus	-	4	2	-	-	-	-
Lathyrus pratensis	-	5	1	-	-	-	-
Leontodon autumnalis	-	4	-	-	-	-	-
Molinia caerulea	-	1	-	-	-	-	-
Parnassia palustris	-	1	-	-	-	-	-
Potentilla erecta	-	6	-	-	-	-	-
Rhinanthus alectolorophus	-	5	-	-	-	-	-
Selinum carvifolia	-	1	-	-	-	-	-
Carex panicea	-	4	-	-	-	-	-
Cerastium fontanum	-	5	-	-	-	-	-
Colchicum autumnale	-	1	-	-	-	-	-
Carex tomentosa	-	-	3	-	-	-	-
Silaum silaus	-	-	1	-	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Nardo-Callunetea							
Danthonia decumbens	9	13	3	1	-	-	-
Luzula campestris	6	8	3	-	-	-	-
Veronica officinalis	2	1	2	-	-	-	-
Calluna vulgaris	2	1	-	-	-	-	-
Agrostis capillaris	50	9	4	-	-	-	-
Cuscuta epithymum	2	-	-	3	-	-	-
Hieracium maculatum	2	-	1	-	-	-	-
Deschampsia flexuosa	6	-	-	-	-	-	-
Rumex acetosella	22	-	-	-	-	-	-
Teucrium scorodonia	4	1	1	3	-	20	29
Cytisus scoparius	7	2	3	3	-	10	71
Festuca heteropachys	-	-	-	-	-	100	86
Hieracium sabaudum	-	-	-	-	-	-	14
Hieracium lachenalii	-	2	3	-	-	-	-
Melampyrum pratense	-	-	-	-	4	-	-
Genista pilosa	-	1	-	-	-	-	-
Viola canina	-	1	-	-	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Sedo-Sceranthetea							
Scleranthus annuus	7	-	-	-	-	-	-
Trifolium arvense	22	1	-	1	-	-	-
Cerastium pumilum	6	2	-	-	-	-	-
Dianthus armeria	7	-	-	-	-	-	-
Trifolium campestre	2	-	-	-	-	-	-
Erophila verna	6	3	-	-	-	-	-
Trifolium dubium	19	5	-	-	-	-	-
Veronica arvensis	9	-	-	3	-	-	-
Echium vulgare	13	1	5	3	11	50	14
Sedum acre	2	-	-	1	7	-	-
Lepidium campestre	4	1	-	-	4	10	-
Sedum album	13	-	-	29	86	90	43
Sedum rupestre	52	1	1	7	-	-	14
Acinos arvensis	2	-	4	16	39	-	-
Arabidopsis thaliana	-	-	-	-	-	40	-
Arenaria serpyllifolia	4	7	5	20	50	40	-
Lactuca perennis	-	-	-	1	-	-	-
Lepidium campestre	4	1	-	-	4	10	-
Poa compressa	9	3	12	4	21	20	-
Teucrium botrys	-	-	1	-	7	-	-
Sedum telephium	-	-	-	-	-	60	-
Sempervirum funckii var. aqualiense	-	-	-	-	-	30	-
Thlaspi perfoliatum	-	-	-	1	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Festucion pallentis							
Festuca pallens	-	-	-	-	50	-	-
Dianthus gratianopolitanus	-	-	-	-	7	-	-
Asplenium ruta-muraria	-	-	1	3	54	-	14
Cotoneaster integerrimus	-	-	-	3	7	10	-
Sesleria caerulea	2	1	18	50	54	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Trifolion medii							
Galium mollugo	13	1	8	44	14	30	-
Origanum vulgare	-	32	27	34	25	60	-
Agrimonia eupatoria	2	21	4	-	-	-	-
Astragalus glycyphyllos	-	1	1	-	-	-	-
Brachypodium sylvaticum	-	5	4	-	-	-	-
Calamintha clinopodium	-	-	7	-	-	-	-
Centaurium erythraea	6	5	5	-	-	-	-
Festuca rubra	6	20	1	-	-	-	-
Galium verum	20	35	24	6	4	-	-
Inula salicina	-	-	1	-	-	-	-
Poa pratensis	17	27	18	1	-	-	-
Senecio erucifolius	-	1	1	-	-	-	-
Solidago virgaurea	-	1	4	-	-	-	-
Trifolium medium	2	8	7	-	-	-	-
Viola hirta	2	25	52	19	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Geranion sanguinei							
Anthericum liliago	-	-	3	29	11	10	14
Seseli libanotis	-	-	-	27	43	-	-
Vincetoxicum hirundinaria	-	-	3	27	14	-	-
Rosa pimpinellifolia	-	-	-	13	4	-	-
Polygonatum odoratum	-	-	-	20	-	-	-
Geranium sanguineum	-	-	-	7	-	-	-
Silene nutans	6	-	-	-	4	40	86
Campanula persicifolia	-	-	-	-	-	10	-
Inula conyzae	-	3	6	7	-	-	-
Bupleurum falcatum	-	1	11	14	-	-	-
Aquilegia vulgaris	-	-	1	1	-	-	-
Digitalis lutea	-	-	-	-	-	-	-
Fragaria viridis	2	5	3	-	-	-	-
Lithospermum officinale	-	-	1	-	-	-	-
Silene vulgaris	-	1	-	-	-	-	-

	I	II	III	IV	V	VI	VII
Number of relevés	54	155	153	70	28	10	7
Koelerio-Phleion phleoidis							
Festuca heteropachys	-	-	-	-	-	100	86
Aster linosyris	-	3	1	30	4	20	14
Artemisia campestris	-	-	-	-	-	30	-
Campanula patula	-	-	-	-	-	10	-

The MANOVA showed significant differences (Wilks'Lambda, F = 17.55, NUM df = 60, DEN df = 2415, P < 0.0001) between groups for environmental variables. Also ANOVAs on separate environmental variables were significant (excepted for shrub cover). Subsequent pairwise comparisons are detailed in Table 2. Because of their very low number of relevés, the groups VI and VII were removed from these analyses, and comparisons were made on a qualitative basis. A strong difference was observed between groups II and III and groups IV and V. Relevés from groups II and III were characterized by significantly deeper soils, less steep slopes, lower bare soil percentage and higher herbaceous cover, in comparison with relevés from groups IV and V (Table 2). This typically contrasts mesophilous grasslands (groups II and

III) against more xerophilous grasslands (groups IV and V). According to the analysis of the nutrient level (mN), mesophilous grasslands were characterised by a more nitrophilous flora than xerophilous ones. The position of group I was rather difficult to interpret on the basis of environmental variables alone.

Table 2. Comparisons of mean environmental variables between grassland communities identified on the basis of the TWINSPAN classification. Pairwise comparisons ; Kruskall-Wallis tests : different letters indicate significant differences for α = 0.05. n = number of relevés ; Y = Y Lambert Coordinate ; MHV = Maximum Height of Vegetation ; % Bare soil = Percentage of the relevé covered by bare soil ; % Herbaceous = Percentage of the relevé covered by herbaceous species ; % Shrubs = Percentage of the relevé covered by shrub species ; % Mosses = Percentage of the relevé covered by mosses ; mF = Mean Ellenberg indicator value for soil moisture ; mN = Mean Ellenberg indicator values for nutrient status.

Group	I	II	III	IV	V	VI	VII
n	54	155	153	70	28	10	7
Altitude	195^{a}	189^{a}	223^{b}	217^{b}	172^{a}	158$^{\text{n.t.}}$	206$^{\text{n.t.}}$
Y	104283^{b}	121816^{c}	95125^{a}	95328^{b}	121174^{c}	131977$^{\text{n.t.}}$	132272$^{\text{n.t.}}$
Soil depth	4.14^{ab}	8.9^{c}	4.67^{b}	2.78^{a}	2.26^{a}	2.9$^{\text{n.t.}}$	3.57$^{\text{n.t.}}$
pH	4.99^{a}	6.27^{b}	6.75^{c}	6.61^{bc}	6.47^{bc}	4.88$^{\text{n.t.}}$	4.68$^{\text{n.t.}}$
Slope	14.9^{a}	13.5^{a}	10.7^{a}	22.8^{b}	33.3^{b}	64.5$^{\text{n.t.}}$	30$^{\text{n.t.}}$
MHV	59.9^{a}	81.2^{c}	70.9^{b}	62.4^{ab}	65.9^{ab}	55.9$^{\text{n.t.}}$	55.9$^{\text{n.t.}}$
% Bare soil	16^{b}	8.2^{a}	8.7^{a}	15.3^{b}	41.3^{c}	46$^{\text{n.t.}}$	45$^{\text{n.t.}}$
% Herbaceous	72^{b}	88.3^{c}	84.4^{c}	76.4^{b}	47.1^{a}	52.5$^{\text{n.t.}}$	40$^{\text{n.t.}}$
% Shrubs	1.96	1.96	2.08	0.69	1.25	2.3$^{\text{n.t.}}$	2.07$^{\text{n.t.}}$
% Mosses	29.3^{c}	12.9^{a}	20.8^{bc}	18.8^{ab}	20.8^{bc}	7.6$^{\text{n.t.}}$	20.7$^{\text{n.t.}}$
Species richness	13.2^{a}	20.7^{b}	18.9^{b}	13.1^{a}	11.2^{a}	13.7$^{\text{n.t.}}$	6.9$^{\text{n.t.}}$
mF	3.58^{c}	3.92^{d}	3.77^{c}	3.09^{b}	2.98^{a}	3.28$^{\text{n.t.}}$	3.12$^{\text{n.t.}}$
mN	2.89^{b}	3.19^{c}	3.08^{b}	2.39^{a}	2.13^{a}	2.86$^{\text{n.t.}}$	2.4$^{\text{n.t.}}$

ACIDIC MESOPHILOUS GRASSLANDS (GROUP I)

This group is characterised by *Sedum rupestre* and *Agrostis capillaris* (Fig. 1) and contains an important proportion of species from the *Mesobromion* and few species from the *Xerobromion* (Table 1). It is characterized by the important occurrence of species from the *Nardo-Callunetea* and the *Sedo-Scleranthetea* (Table 1) and species richness (ca. 13 species/m^2) was lower than other mesophilous grasslands (Table 2).

Although it was grouped among typical mesophilous grasslands at the first level of the TWINSPAN division (Fig. 1), acidic mesophilous grasslands differed significantly from groups II and III in terms of environmental conditions (maximum vegetation height, bare soil percentage, and herbaceous cover). They also differ significantly from xerophilous communities (groups IV and V) for slope and are intermediate for soil depth. Their main characteristic is a significantly lower pH. These communities may be found across all the Calestienne region except in the more North-Eastern sites (Fig. 2). Their presence corresponds more to local variations of ecological conditions (embankments on shale with more or less decarbonated soils) than to a biogeographical gradient.

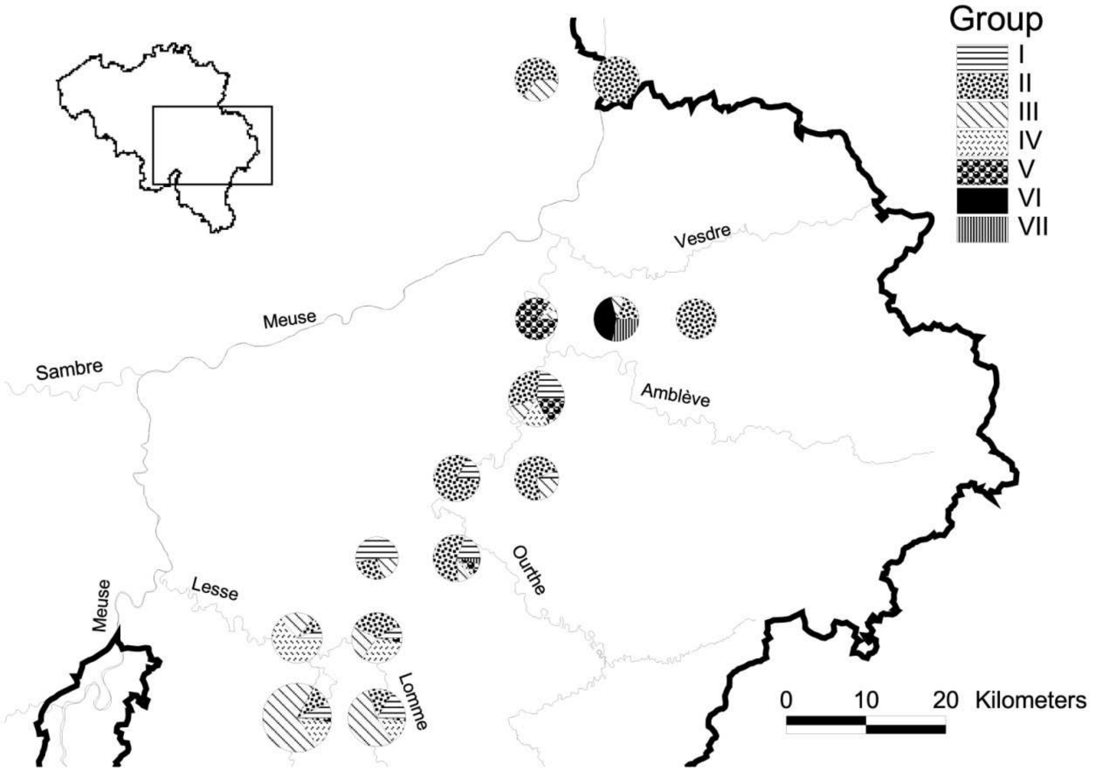

Fig. 2. Study region and proportion of relevés from the different TWINSPAN groups in 10 x 10 kilometres squares. Circle sizes are proportional to the number of relevés in the 10x10 kilometres square.

ALKALINE MESOPHILOUS GRASSLANDS (GROUPS II AND III)

The two alkaline mesophilous communities (groups II and III) correspond to different levels of xericity. Group II represents the typical mesophilous grassland community, characterized by *Plantago lanceolata, Achillea millefolium, Ranunculus bulbosus* and *Carex caryophyllea* (Fig. 1) It also includes many species of the *Molinio-Arrhenatheretea* (Table 1). Group III corresponds to a mesophilous community typical of more xeric environments and was indicated by *Euphorbia cyparissias* and *Teucrium chamaedrys* (Fig. 1). This community is a transition between the *Mesobromion* and *Xerobromion*, as xeric species such as *Carex humilis*, *Globularia bisnagarica* and *Sesleria caerulea* appear and meadow species become scarce (Table 1). Generally, mesophilous grasslands (groups II and III) are well represented in the study region (308 out of 477 relevés) and are characterized by the high abundance of *Bromus erectus* and *Brachypodium pinnatum*. Many typical species of the *Mesobromion* alliance (Wolkinger & Plank 1981) were present in the corresponding relevés, such as *Cirsium acaule*, *Gentianella germanica*, and many orchids (*Ophrys insectifera*, *Ophrys apifera*, *Orchis militaris*, *Anacamptis pyramidalis*). Our relevés revealed a species-rich habitat, with a mean species richness of 20 species/m² and a maximum of 35 species/m². These grasslands developed on gentle slopes or on plateaus with a relatively high soil depth. They exhibit a high vegetation and little bare soil. Contrary to previous the community, the soil pH was high, varying between 6 and 7 (Table 2). The geographic distribution of these communities over the study zone indicates a tendency for dominance of the meso-xerophilous community in the south-western part, which is replaced by the more mesophilous community in the north-eastern part (Fig. 2).

VERY DRY GRASSLANDS (GROUP IV)

This community was characterised by *Teucrium chamaedrys* and *Carex humilis* (Fig.1). Many *Xerobromion* species were found, such as *Allium sphaerocephalon*, *Thlaspi montanum* or *Stachys recta*. This community also contained numerous species of the *Sedo-Scleranthetea*, as *Acinos arvensis* or *Sedum album*, due to a low vegetation cover (76% on average). Its mean species richness was lower than that of the *Mesobromion*, with only 13 species/m².

It occurred on steep slope with a very thin alkaline soil (pH ≈ 7). Bare soil percentage was rather important, while maximum height of vegetation was low (Table 2).

It was mainly located in the southern part of the study region, in the Lesse and Lomme valleys (Fig. 2).

FESTUCION PALLENTIS GRASSLANDS (GROUP V)

This relatively species poor community (11,2 species/m² in average) is characterized by *Festuca pallens*, *Sesleria caerulea, Cotoneaster integerrimus*, and by the very rare *Dianthus gratianopolitanus*. These grasslands occupied calcareous rock cliffs and cracks on very steep slopes.

The *Festucion pallentis* community is very rare in Belgium and has mainly been found in the Ourthe valley (Fig.2).

KOELERIO-PHLEION GRASSLANDS (GROUPS VI AND VII)

Both groups are positively differentiated from the others by the indicator species *Festuca heteropachys* (Fig. 1) and by a higher occurrence of indicator species from the *Koelerio-Phleion* alliance (Table 1). Their species richness was poor to very poor (Table 2).

Mean environmental conditions for these two groups (Table 2) were characteristic of xerophilous communities with superficial soils on steep slopes, supporting open vegetation. These communities, however, differed from the other observed xerophilous communities (groups IV and V) by more acidic conditions (pH ≈ 4,5). They corresponded best to the *Koelerio-Phleion* *phleoidis* alliance. This community is extremely rare in Belgium and mainly occurred in the Amblève valley (Fig.2).

Table 3. Correlation between environmental variables and ordination axes from the CCA. X = X Lambert Coordinate ; Y = Y Lambert Coordinate ; % Herbaceous = Percentage of the relevé covered by herbaceous species ; % Shrubs = Percentage of the relevé covered by shrub species ; % Mosses = Percentage of the relevé covered by mosses ; mF = Mean Ellenberg indicator value for soil moisture ; mN = Mean Ellenberg indicator values for nutrient status.

	Axis 1	Axis 2
Altitude	0.1156	-0.4827
X	-0.1478	0.7444
Y	-0.2063	0.6329
Soil depth	-0.5449	0.2376
PH	0.0221	-0.4287
Slope	0.5153	0.5116
% Herbaceous	-0.5794	-0.3585
% Shrubs	-0.1357	0.1607
% Mosses	0.1397	-0.1263
MF	-0.9237	0.1463
MN	-0.7065	0.2953

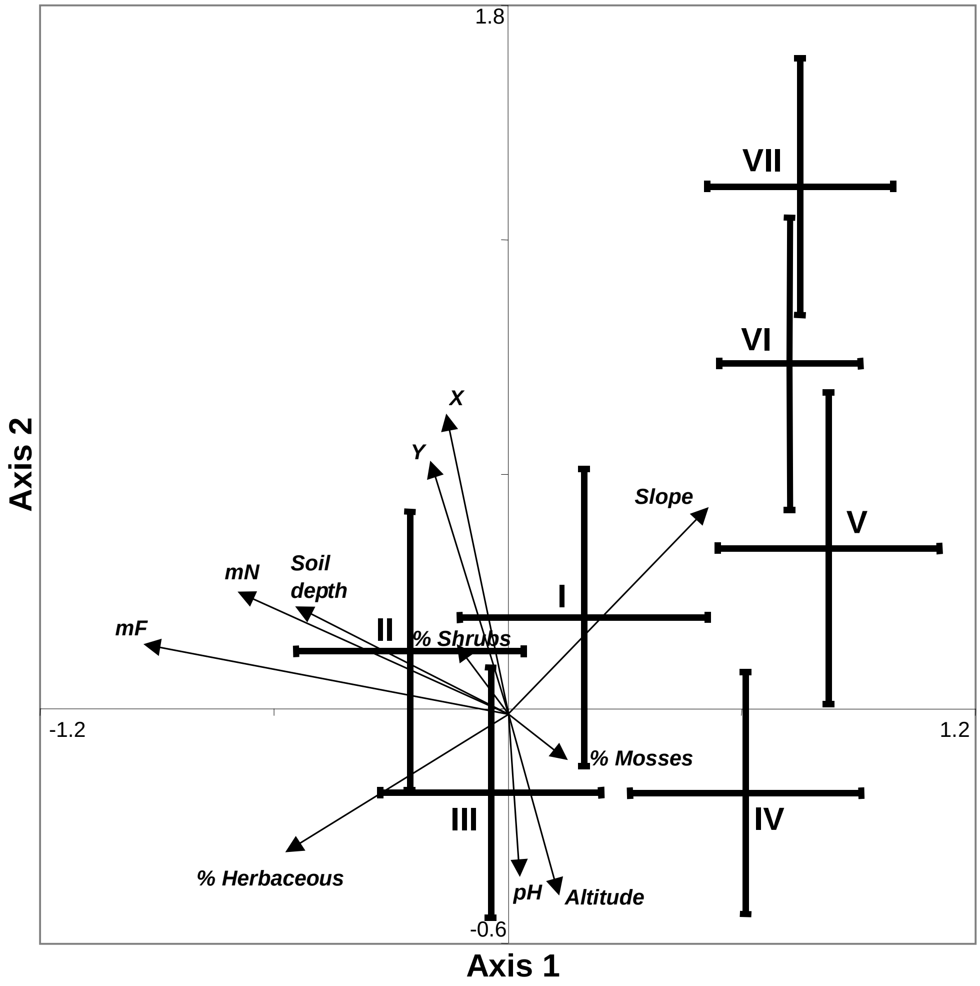

Fig. 3. Position in the CCA ordination graph of communities identified by TWINSPAN. Horizontal and vertical bars represent standard deviation of the positions along the first and second axes of the ordination. Arrows represent environmental variables. X = X Lambert Coordinate ; Y = Y Lambert Coordinate ; % Herbaceous = Percentage of the relevé covered by herbaceous species ; % Shrubs = Percentage of the relevé covered by shrub species ; % Mosses = Percentage of the relevé covered by mosses ; mF = Mean Ellenberg indicator value for soil moisture ; mN = Mean Ellenberg indicator values for nutrient status.

Ordination of relevés and environmental data

The two first axes explained respectively 27.1 % and 15.5 % of the species-environment relation. The ordination analysis confirmed the interpretation based on the TWINSPAN classification (Fig. 3). The first axis was highly correlated to herbaceous species cover, soil depth, soil moisture (mF), soil trophic level (mN), slope, and bare soil percentage (Table 3). The gradient from negative to positive values on axis 1 corresponded to more superficial soils with lower water content and lower nutrient status, steeper slopes and lower herbaceous cover, reflecting the transition from mesophilous to xerophilous grasslands. For the second axis, high correlations were found with most environmental variables although pH exhibited a particularly high correlation. As no significant correlation was found between soil pH and any other environmental factor (Table 4), it can be considered as an independent factor affecting the floristic composition of the studied communities. Biogeographical parameter Y Lambert coordinate, however, exhibited the highest correlation with the second axis, confirming the influence of a south-north biogeographic influence on floristic composition.

Table 4. Between-class correlations of environmental variables. Characters in italics refer to the associated probabilities. MHV = Maximum Height of Vegetation ; % Bare soil = Percentage of the relevé covered by bare soil ; % Herb = Percentage of the relevé covered by herbaceous species ; % Shrubs = Percentage of the relevé covered by shrub species ; % Mosses = Percentage of the relevé covered by mosses ; mF = Mean Ellenberg indicator value for soil moisture ; mN = Mean Ellenberg indicator values for nutrient status.

	Soil Depth	pH	Slope	MHV	% Bare Soil	% Herb	% Shrubs	% Mosses	mF
pH	0.001								
	0.998								
Slope	-0.46	-0.302							
	0.299	*0.511*							
MHV	0.913	0.382	-0.519						
	0.004	*0.397*	*0.232*						
Bare Soil	-0.492	-0.452	0.914	-0.569					
	0.262	*0.309*	*0.004*	*0.183*					
% Herb	0.682	0.449	-0.819	0.741	-0.957				
	0.092	*0.312*	*0.024*	*0.057*	*0.001*				
% Shrubs	0.655	-0.058	-0.212	0.594	-0.269	0.394			
	0.11	*0.901*	*0.648*	*0.159*	*0.56*	*0.382*			
% Mosses	-0.654	-0.398	-0.028	-0.709	0.254	-0.501	-0.485		
	0.111	*0.376*	*0.953*	*0.075*	*0.583*	*0.253*	*0.27*		
MF	0.819	0.065	-0.726	0.777	-0.734	0.799	0.773	-0.362	
	0.024	*0.889*	*0.065*	*0.04*	*0.06*	*0.031*	*0.042*	*0.425*	
mN	0.767	0.028	-0.676	0.701	-0.729	0.79	0.801	-0.369	0.986
	0.044	*0.952*	*0.095*	*0.08*	*0.063*	*0.035*	*0.03*	*0.415*	*<.0001*

Species diversity

Multiple regression analyses of local species richness on environmental variables yielded significant results for all grassland sets considered, explaining between 18.1% and 58.5% of the species richness variation (Table 5). Groups II and III were considered together given that they were just variations of the same community (mesophilous grasslands). Variables included in the models differed from one community to another. Considering all grasslands together, local species richness increased with soil moisture (mF) and decreased with soil nutrient status (mN). Species richness in mesophilous grasslands was also negatively influenced by maximum vegetation height, bare soil percentage, moss cover and positively influenced by shrub cover. Soil pH was a significant factor only for the whole data set. In acidic mesophilous grasslands, species richness was negatively influenced by the moss cover and positively by mF. These two variables explained 38.4 % of the variation. Local species richness in xerophilous grasslands was significantly influenced by geographic location (Y

Lambert coordinate). Grasslands with a more southern location showed higher species richness. *Festucion pallentis* grasslands richness was positively influenced by structure variables (maximum height of vegetation and shrub cover).

Table 5. Optimal multivariate models for the local species richness, considering the whole grassland set and the different TWINSPAN grassland types. Positive relationships are indicated by + and negative by -. Significance is indicated as following : * 0.01 < p ≤ 0.05 ; ** 0.001 < p ≤ 0.01 ; *** p ≤ 0.001. 4thRT Y = fourth root transformation, SQRT Y = square root transformation, *LOG10* Y = logarithmic transformation, Y² = square transformation. X = X Lambert Coordinate ; Y = Y Lambert Coordinate ; % Bare soil = Percentage of the relevé covered by bare soil ; % Herbaceous = Percentage of the relevé covered by herbaceous species ; % Shrubs = Percentage of the relevé covered by shrub species ; % Mosses = Percentage of the relevé covered by mosses ; mF = Mean Ellenberg indicator value for soil moisture ; mN = Mean Ellenberg indicator values for nutrient status.

	Transformation on independant variable	Altitude	X	Y	Soil depth	pH	Slope	Maximum height of vegetation	% bare soil	% Herbaceous	% Shrubs	% Mosses	mF	mN	R²
All grasslands	4thRT Y			-**		+***				+***	+*	-*	+***	-***	18.10%
Mesophilous acidic grasslands (Group I)	SQRT Y											-*	+***		38.40%
Mesophilous grasslands (Groups II, III)	4THRT Y							-***	-***		+**	-***	+***	-**	39.80%
Xerophilous grasslands (Group IV)	$LOG_{10}Y$			-***									+***	-***	58.50%
Calcareous rocks grasslands (Group V)	Y²							+**			+*				33.10%

Discussion

Acidic mesophilous grasslands

A similar grassland type has been described in the Viroin valley by Butaye et al. (2005b) (*Agrostis capillaris-Cytisus scoparius* community). Butaye et al. (2005b) were nevertheless unable to provide a complete description of this community because of the low number of relevés. From our more general survey, we suggest that these grasslands belong to the *Chamaespartio-Agrostidenion* alliance. Such grasslands were often spatially related with *Sedo-Scleranthetea* open grasslands, which could explain the presence of species from this phytosociological class. Despite some affinities with xerophilous grasslands regarding environmental variables, their floristic composition is typically mesophilous. Acidic grasslands have a unique composition because of the simultaneous occurrence of acidic and calcareous species at a one meter square scale. This floristic originality provides a high conservation value to this community.

Alkaline mesophilous grasslands (groups II and III)

These grasslands belong to the *Mesobromion* alliance. Their very high species richness gives them a high conservation value and they are the principal habitat for orchid species. The presence of orchid species is the condition to consider calcareous grasslands as a priority habitat, following the European Directive 92/43/CEE (Habitat Directive). Of the 93 sites that were investigated, about one third contained at least one orchid species. Moreover, some orchids could have been present in the site but did not occur in the relevés. The division between meso-xerophilous and typical mesophilous grasslands was not reported by Butaye *et al.* (2005b) in the Viroin valley. This was maybe due to the absence of a North-South gradient in their survey. Van Speybrouck et al. (1989) reported a similar influence of the North-South gradient in a comparison of calcareous grasslands between the Belgian Meuse and the Lorraine districts.

Very dry grasslands (group IV)

The *Xerobromion* reaches its northern boundary in Belgium, which was confirmed by its sourthern distribution within the study zone (Fig 2). Nevertheless, the classification of these very dry grasslands in this phytosociological alliance can be discussed. Following ROYER (1991), some of them should be classified in the *Teucrio-Mesobromenion* sub-alliance. This sub-alliance is characterised by *Teucrium chamaedrys*, *Globularia bisnagarica* and *Pulsatilla vulgaris,* among others. Nonetheless, following Noirfalise & Dethioux (1984), those species are some

Xerobromion differential species. However that may be, the community is very impoverished as compared to its optimal range in central France (Royer 1982).

Although the classification of this community can be discussed, its conservation value can not. Many species from this community are of peculiar interested for conservation in Belgium. These xerophilous grasslands are not as species rich as the mesophilous ones and therefore the management objectives should be different, in terms of plant species richness.

Festucion pallentis grasslands (group V)

Sites where this community occurred were previously described (e.g. Duvigneaud 1982; Duvigneaud & Saintenoy-Simon 1997). Nevertheless, this survey was the first to characterise its originality. We demonstrated that the floristic composition of this community had specific characteristics compared to the other grassland communities. Most of its typical species such as *Dianthus gratianopolitanus* and *Festuca pallens* are rare and of conservation interested . *Festuca pallens* has his western limit of distribution in the Meuse valley.

Koelerio-Phleion grasslands (groups VI and VII)

The *Koelerio-Phleion phleoidis* alliance was described as a rare habitat in Belgium, and therefore provides a high conservation value to the sites where it occurs (Duvigneaud & Saintenoy-Simon 1988; Duvigneaud & Saintenoy-Simon 1989). A distinction between groups VI and VII could be made as *Xerobromion* species were more represented in group VI (Heid des Gattes) (Table 1). This has traditionally been interpreted as an impoverishment of the flora in the 'Heid de Stinval' as compared to 'Heid des Gattes' (Duvigneaud & Saintenoy-Simon 1988; Duvigneaud & Saintenoy-Simon 1989), and this was confirmed in our study by the lower local plant species richness recorded at the first site (Table 2). To our knowledge, this is the first time that these grassland communities are characterized through direct comparisons at the regional scale and that their particularity is confirmed by a statistical analysis.

Species Richness

The different results concerning species richness were generally in accordance with previous surveys. Bio-geographic influences have been described by different authors (e.g. Van Speybroek et al. 1989; Bruun 2000). These influences are often not easy to explain. In the current study, there was a species decline towards Northern locations. This trends was particularly strong for the *Xerobromion* grasslands (group IV) confirming that these grasslands reach their northern boundary in Belgium (Wolkinger & Plank 1981; Maubert & Dutoit 1995). The pH influence was due to the fact that acidic grasslands exhibited, on average, a lower species richness as compared to other grasslands (Table 2). A higher species richness in alkaline grasslands has also been found by Critchley et al. (2002b). Increasing height of vegetation and shrub cover generally negatively affected local species richness in calcareous grasslands (Bobbink & Willems 1987). Nevertheless, at a larger scale, variation in vegetation structure may provide a higher diversity of microhabitats and promote species richness (Lindborg & Eriksson 2004b). The results found in this study are in accordance with the fact that an increasing fertility of the soil induces a loss in species richness (e.g. Al-Mufti et al. 1977; Marrs 1993; Critchley et al. 2002b).

Appendix

Appendix 1: Name, number of plots, coordinates and location of the different study sites.

Site Name	Number of plots	Site coordinates	Location
Aisne	3	50° 21' 30" N - 5° 33' 54" E	Durbuy
Aye	6	50° 13' 9" N - 5° 18' 18" E	Marche-en-Famenne
Baillonville	6	50° 17' 34" N - 5° 20' 2" E	Somme-Leuze
Bâtis d'Haur	8	50° 6' 4" N - 5° 14' 42" E	Tellin
Belvédère	5	50° 8' 4" N - 5° 12' 11" E	Rochefort
Biernauchamps	6	50° 6' 52" N - 5° 14' 24" E	Rochefort
Bois Niaux	4	50° 6' 13" N - 5° 9' 59" E	Rochefort
Bourdon	3	50° 14' 32" N - 5° 23' 22" E	Hotton
Brochamps	10	50° 5' 55" N - 5° 8' 5" E	Wellin
Brouire	3	50° 6' 18" N - 5° 14' 8" E	Tellin
Carrière de Mont	3	50° 32' 18" N - 5° 47' 55" E	Theux
Carrière de Resteigne	9	50° 5' 23" N - 5° 10' 54" E	Tellin
Carroi-Chenêt	6	50° 5' 48" N - 5° 12' 22" E	Tellin
Chafosse	3	50° 12' 55" N - 5° 16' 57" E	Marche-en-Famenne
Chéfiri	6	50° 5' 49" N - 5° 10' 28" E	Tellin
Chénisse	4	50° 23' 57" N - 5° 30' 40" E	Durbuy
Cocrai	2	50° 11' 54" N - 5° 14' 29" E	Marche-en-Famenne
Comblain-la-Tour	6	50° 27' 28" N - 5° 34' 2" E	Hamoir
Coteau du Tunnel 1	5	50° 45' 41" N - 5° 38' 22" E	Bassenge
Coteau du Tunnel 2	7	50° 45' 34" N - 5° 38' 32" E	Bassenge
Coteau du Tunnel 3	5	50° 45' 39" N - 5° 38' 23" E	Bassenge
Deulin	3	50° 18' 24" N - 5° 23' 19" E	Hotton
Falize	6	50° 28' 58" N - 5° 41' 29" E	Aywaille
Fond des Vaulx	3	50° 13' 48" N - 5° 21' 24" E	Marche-en-Famenne
Fond Saint-Martin	10	50° 8' 3" N - 5° 12' 0" E	Rochefort
Fouyeu	6	50° 20' 56" N - 5° 30' 6" E	Durbuy
Grand Va	3	50° 23' 40" N - 5° 33' 25" E	Ferrières
Grignaux 1	3	50° 6' 50" N - 5° 10' 13" E	Rochefort
Grignaux 2	5	50° 6' 52" N - 5° 10' 11" E	Rochefort
Grignaux 3	3	50° 7' 3" N - 5° 10' 10" E	Rochefort
Grignaux 4	3	50° 7' 6" N - 5° 10' 4" E	Rochefort
Gros Tienne de Lavaux	10	50° 6' 21" N - 5° 5' 57" E	Rochefort
Hamoir	8	50° 25' 30" N - 5° 31' 31" E	Hamoir
Hampteau	3	50° 15' 45" N - 5° 27' 33" E	Hotton
Hazalles	7	50° 19' 50" N - 5° 28' 54" E	Durbuy
Hé des Gattes	9	50° 28' 50" N - 5° 41' 21" E	Aywaille
Heid de Stinval	7	50° 32' 13" N - 5° 41' 50" E	Sprimont
Herbet	6	50° 23' 10" N - 5° 31' 35" E	Durbuy
Hérimont	4	50° 6' 54" N - 5° 10' 20" E	Rochefort
Heyoule 1	3	50° 46' 60" N - 5° 39' 59" E	Bassenge
Heyoule 2	8	50° 46' 47" N - 5° 40' 3" E	Bassenge
Heyoule 3	3	50° 46' 54" N - 5° 39' 58" E	Bassenge
La Soyère	5	50° 5' 13" N - 5° 11' 54" E	Tellin
Les Vevis	3	50° 6' 34" N - 5° 15' 9" E	Rochefort
Logne	6	50° 23' 39" N - 5° 32' 15" E	Ferrières
Lorinchamps	10	50° 5' 45" N - 5° 14' 20" E	Tellin
Maupas	10	50° 6' 37" N - 5° 12' 30" E	Rochefort
Mignées	5	50° 19' 10" N - 5° 28' 28" E	Erezée

Site Name	Number of plots	Site coordinates	Location
Mont	7	50°32' 24" N - 5°48' 7" E	Theux
Mont des Pins	6	50°22' 1" N - 5°31' 11" E	Durbuy
Naurdichamps	3	50°6' 38" N - 5°15' 12" E	Rochefort
Noiseux	5	50°17' 48" N - 5°22' 29" E	Somme-Leuze
Pairées centre	10	50°5' 59" N - 5°11' 4" E	Tellin
Pairées est	5	50°5' 59" N - 5°11' 15" E	Tellin
Pairées sud	5	50°5' 53" N - 5°10' 53" E	Tellin
Pairées ouest	8	50°6' 0" N - 5°10' 20" E	Tellin
Palogne	4	50°23' 50" N - 5°32' 1" E	Ferrières
Parking Han	3	50°7' 42" N - 5°11' 14" E	Rochefort
Petit Herbet	3	50°23' 0" N - 5°31' 5" E	Durbuy
Pierreux/Xhoris	8	50°27' 7" N - 5°35' 25" E	Ferrières
Plome Mohon	5	50°17' 20" N - 5°26' 42" E	Hotton
Prairie Hazalles	3	50°19' 60" N - 5°29' 1" E	Durbuy
Rochers Masbourg-Forrières	3	50°7' 22" N - 5°17' 29" E	Nassogne
Roches Noires	5	50°28' 57" N - 5°34' 29" E	Comblain-au-Pont
Roké	4	50°7' 22" N - 5°9' 8" E	Rochefort
Roptai	4	50°7' 4" N - 5°8' 32" E	Rochefort
Rouge-Croix	10	50°8' 8" N - 5°10' 28" E	Rochefort
Route Bure-Belvaux 1	4	50°6' 33" N - 5°13' 8" E	Rochefort
Route Bure-Belvaux 2	6	50°6' 32" N - 5°12' 43" E	Rochefort
Route Han-Hamerenne	4	50°8' 4" N - 5°11' 41" E	Rochefort
Route Resteigne-Belvaux 1	1	50°6' 1" N - 5°11' 18" E	Tellin
Route Resteigne-Belvaux 2	3	50°5' 57" N - 5°11' 19" E	Tellin
Route Resteigne-Belvaux 3	6	50°5' 51" N - 5°11' 9" E	Tellin
Route Resteigne-Belvaux 4	1	50°5' 49" N - 5°11' 19" E	Tellin
Route Resteigne-Belvaux 5	2	50°5' 44" N - 5°11' 18" E	Tellin
Route Tellin-Wavreille 1	3	50°6' 2" N - 5°13' 53" E	Tellin
Route Tellin-Wavreille 2	3	50°6' 6" N - 5°13' 54" E	Tellin
Saint Remy	9	50°10' 48" N - 5°13' 31" E	Rochefort
Soy-Biron	3	50°19' 14" N - 5°29' 15" E	Durbuy
Spinets 1	2	50°10' 32" N - 5°17' 5" E	Marche-en-Famenne
Spinets 2	9	50°10' 13" N - 5°16' 29" E	Marche-en-Famenne
Sur Tombeux	4	50°25' 55" N - 5°32' 32" E	Hamoir
Tartines	9	50°28' 42" N - 5°35' 25" E	Comblain-au-Pont
Terre Telle	7	50°17' 12" N - 5°27' 11" E	Hotton
Thier Pirard	3	50°28' 30" N - 5°34' 41" E	Comblain-au-Pont
Tienne Moseray	3	50°5' 44" N - 5°10' 43" E	Tellin
Tinaimont 1	4	50°7' 45" N - 5°13' 7" E	Rochefort
Tinaimont 2	2	50°7' 48" N - 5°12' 55" E	Rochefort
Tinaimont 3	2	50°7' 48" N - 5°12' 51" E	Rochefort
Tinaimont 4	4	50°7' 47" N - 5°12' 42" E	Rochefort
Tombe	9	50°47' 37" N - 5°40' 19" E	Bassenge
Verlaine	4	50°23' 55" N - 5°30' 57" E	Durbuy
Viaduc E411	4	50°5' 59" N - 5°7' 33" E	Rochefort
Warre	3	50°21' 53" N - 5°28' 15" E	Durbuy

CHAPITRE 3 : NATURALIZATION AND IMPACT OF A HORTICULTURAL SPECIES, COTONEASTER HORIZONTALIS DECAISNE (ROSACEAE) IN BIODIVERSITY HOTSPOTS IN BELGIUM.

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Abstract

Cultivation of ornamental plant species has been recognized to foster plant naturalization of exotics by counteracting environmental stochasticity and continuously providing propagules. As a preliminary attempt to describe the invasion status of *Cotoneaster horizontalis*, we gathered information on the occurrence of the species at the Belgian level by computing various databases. We assessed the naturalisation status of *C. horizontalis* in Belgian calcareous grasslands, high value habitats recognised as biodiversity hotspots, by characterizing its occurrence, population status, preferred habitats, invaded communities, growth rate and fruiting capacity. We also assessed the impact of the species on calcareous grasslands communities and on individual species.

Cotoneaster occurred in seven of the nine investigated sites, with densities varying from 0.34 to 10 individuals/ha. In the most invaded sites, the high proportion of small individuals suggested an important ongoing colonization process, emphasized by the fact that fruiting capacities were demonstrated for 3-year-old individuals. Invaded habitats were typically Mosan Xerobromion habitats, which are priority Natura 2000 habitats. The presence of *C. horizontalis* was associated with changes in both the structure and composition of the community by decreasing species richness and diversity, and affecting grassland specialist species. These impacts are expected to intensify over time with changing age population and increasing propagule pressure due to close urbanisation.

Introduction

Invasions by exotic species represent a major challenge to biodiversity conservation as they are now considered as being one of the major causes of biodiversity loss worldwide (D'Antonio & Kark 2002; D'Antonio & Meyerson 2002). In many areas, ornamental horticulture has been recognized as the main pathway for plant invasions (Groves 1998; Reichard & White 2001; Kühn & Klotz 2002; Pysek et al. 2002). Importation and cultivation of ornamental species are highly relevant in plant invasion processes as they directly affect the propagule pressure (Dehnen-Schmutz et al. 2007), by influencing both the number of individuals involved in one release event and the number of discrete release events. Moreover, despite the fact that only a small fraction of introduced populations become invasive, cultivation has been recognized to foster plant naturalization by counteracting environmental stochasticity and by continuously providing propagules as repeated immigrations from a donor, contributing to the persistence of the species and the increase of its range (Mack 2000; Kowarik 2003).

Some exotic species are known to invade hotspots of native plant diversity worldwide (Stohlgren et al. 1999; Stohlgren et al. 2001; Pysek et al. 2003). The importance of the phenomenon in such valuable habitats is somehow controversial but in a survey performed in the U.S, Stohlgren et al. (2001) demonstrated at large spatial scale that hotspots of native plant diversity have been far more heavily invaded than areas of low plant diversity. They argued that we cannot expect such hotspots to repel invasions. Exotic plant invasions often have impacts on the resident vegetation by modifying its composition and structure (Levine et al. 2003) and on the ecosystem functioning (Ehrenfeld et al. 2001). Given the variety of possible impacts of exotic species on native vegetation (competition for light and/or nutrients, allelopathic effects, …), such high value habitats might be threatened by the phenomenon of plant invasion. Moreover, comparative studies on impacts at the community-level are surprisingly rare (but see Alvarez & Cushman 2002; Jäger et al. 2007).

Belgium (Western Europe) and neighbouring countries have suffered from invasion by exotic plants, some of which have dramatically increased their range in the last 30 years (Muller 2000; Verloove 2002). Among the exotic species introduced in Belgium, *Cotoneaster horizontalis* (Rosaceae) is a shrubby species originating from West China (Lambinon et al. 2004). The introduction date of *C. horizontalis* as an ornamental species in Belgium is unknown but the first observation in the wild dates back to 1982 (*Harmonia* database 2007). *C. horizontalis* is prostrated, has a rapid lateral expansion and can grow on very thin soils or rocks and on steep slopes. Those characteristics, as well as its red colour in the fall, make the species of particular interest for horticulture, as a cover species for walls or embankments. In a recent survey, we found that 52% of the plant nurseries (n=102) sell *C. horizontalis* in South Belgium (Vanderhoeven unpublished data). Once established, expansion of local colonies may result in extensive smothering of native communities, altering their structure and composition (Crofts &

Jefferson 1999). Ledge, crevice and scree communities may be affected, as well as a range of calcareous grassland communities. Root systems are highly pervasive, often penetrating deeply into crevices in the bedrock. Moreover, *C. horizontalis* is known for causing contact dermatitis (Weller & Ormerod 1996) and might potentially become problematic in case of increasing occurrence. In different parts of England and Wales, *C. horizontalis* induced alterations in the composition and structure of different calcareous grassland communities (Crofts & Jefferson 1999). Infestations have been reported from a variety of substrates including chalk as well as harder Jurassic and Carboniferous limestone. *C. horizontalis* is included in the black list of invasive species in Belgium (Category A2, *Harmonia* database 2007) and is reported to be present in calcareous grasslands.

Calcareous grasslands are among the most species-rich habitats in Western Europe, both at local and regional scales (Willems 2001; WallisDeVries et al. 2002) and are considered as biodiversity hotspots in temperate countries (Prendergast et al. 1993; WallisDeVries et al. 2002). This richness confers them a major conservation interest. Formerly widespread, calcareous grasslands have strongly decreased from the end of the nineteenth century. This phenomenon was particularly dramatic in Belgium (see Adriaens et al. 2006). The major causes for this decrease are the destruction and the fragmentation of habitats due to the abandonment of traditional agro-pastoral practices, to pine plantation and/or urbanisation. As a result of the increasing urbanisation and subsequent ornamental activities in their vicinity, a new pressure might threaten calcareous grasslands in the next few years: invasion by exotic plant species. Given the great interest of calcareous grasslands for nature conservation, we argue that there is a need for a first investigation on the naturalisation of *C. horizontalis* in this habitat in Belgium and on possible impacts of the species.

Data concerning distribution of the species was fragmented. So, as a first attempt to give a complete overview, we gathered information on the occurrence of the species at the Belgian level by compiling various databases and field data. We assessed the naturalisation status of *C. horizontalis* at the local scale of calcareous grasslands by characterizing its occurrence, population status, preferred habitats, invaded communities, growth rate and fruiting abilities. Finally, we considered the impacts of the species at the plant community-level on high value calcareous grasslands and on individual species by comparing species composition and cover in invaded and uninvaded plots. Perspectives for management and control were discussed in the light of the results.

Material and Methods

Study species

Cotoneaster horizontalis (Rosaceae) is a 0.1-0.6 meters height shrubby species. It produces many rose to purple flowers all along the stems. It is insect-pollinated, although self-pollination is possible in other *Cotoneaster* species (East 1940). The fruit is a red, 3-4 mm diameter pome, probably dispersed by birds. *Cotoneaster* berries are highly attractive to blackbirds and other thrushes, which readily disperse them by defecation (Crofts & Jefferson 1999).

Study area

A first assessment of the occurrence of *Cotoneaster horizontalis* in Belgium was performed by compiling different databases: (i) the monitoring of quarries in the Walloon Region (Southern Belgium), compiled observations from 1997 (Remacle 1997-2007); (ii) the inventory of High Biological Interests Sites (HBIS) of the Walloon Region, data from 1993 (Dufrêne 2005); (iii) the atlas flora of Flanders (Northern Belgium) and the Brussels region, compiled observations between 1972 and 2004 (Van Landuyt et al. 2006); (iv) personal field observations (Laboratoire d'Ecologie FUSAGx, unpublished data), observations since 2002.

Secondly, an extensive survey was carried out in calcareous grasslands in the vicinity of Dinant (50°15'30''N – 4°55'E) in Southern Belgium (fig.1). This region is known for the presence of numerous calcareous grasslands and *C. horizontalis* had been recorded in different sites of the region (Dufrêne 2005 and personal observations.). Nine sites were selected in the study area. Some of them were chosen because *C. horizontalis* had previously been recorded ("Montagne de la Croix" and "Champalle") while the others were chosen because of their large area and their high conservation value in the region. All the sites were situated on gentle to steep South to West facing slopes in the Meuse valley or some of its tributaries (Fig.1). In the region, several plant species, like *Helianthemum apenninum, Artemisia alba, Draba aizoides,* reach the northern boundary of their distribution range. The region is moderately urbanised. Most of the investigated grasslands were close to small towns or villages, so the propagule pressure of exotic species might be quite strong due to their presence in private gardens (Fig.1).

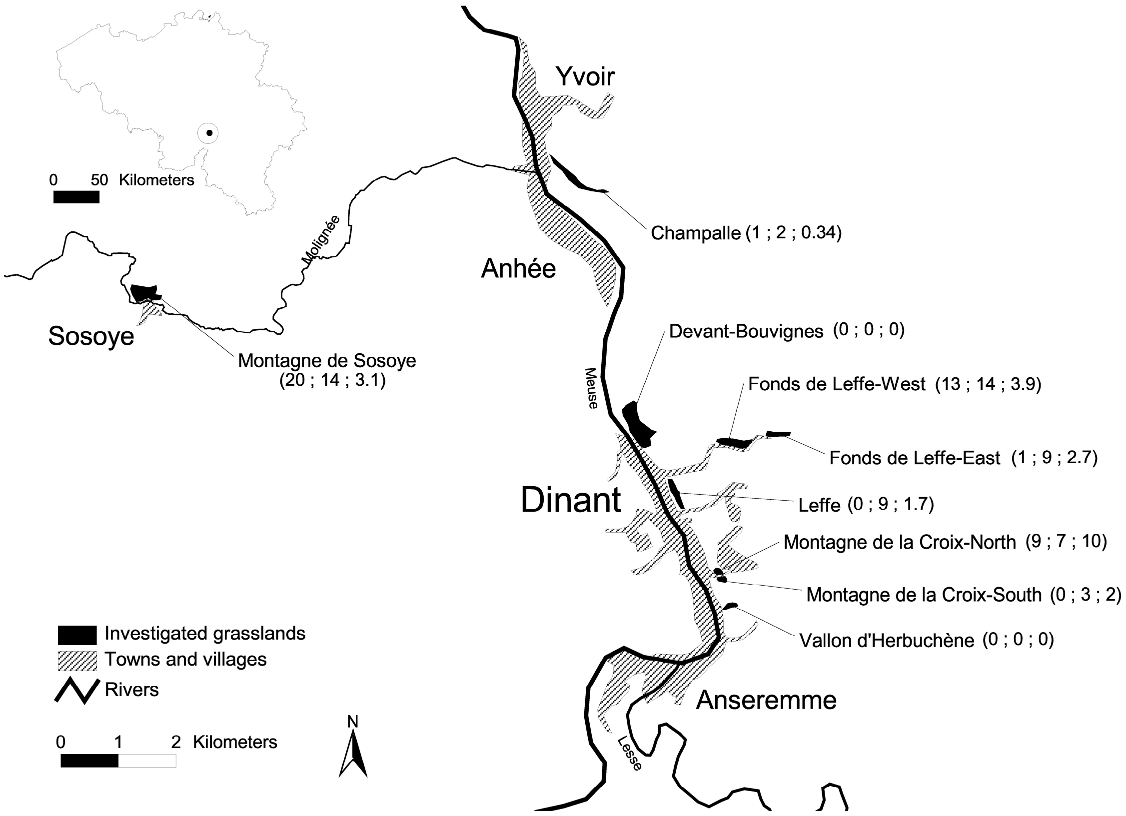

Fig.1: Investigated sites. Cotoneaster counting results are given between brackets, respectively: number of small individuals, number of large individuals, density (number of individuals/ha)

Data collection and analyses

A first field survey took place in mid-November 2006. At that time, *C. horizontalis* had a typical deep red colour, making it very easy to locate. In each site, the number of *C. horizontalis* individuals was counted. Two size classes were considered: small individuals (diameter <100 cm) and large individuals (diameter >100 cm). In accessible sites, the counting was carried out by covering the whole grassland area. In the "Leffe" site, for less accessible areas, individuals were counted from the nearest easily reachable position. On the "Fonds de Leffe West" site, sixteen stem samples were collected from individuals just above ground. Individuals were selected in order to cover habitat and size variability. For each of them, the presence of fruits was noted and the diameter was measured (largest and smallest diameters). Age rings were counted on stem sections.

In order to assess the growth rate, the relationship between the diameter (dependent variable) and the age (number of rings, independent variable) was tested with linear regression. Normality of the residuals was tested by Levene's test and their homoscedasticity by Breuch-

Pagan's test. Box-Cox transformations on the dependent variable were applied using a Minitab Version 14.20 macro (Minitab Inc., 2000) (Palm 2002). In October 2007, another field survey was performed in order to characterise the community and the impact of *C. horizontalis*. As proposed by WALKER & SMITH (1996) and commonly used (Vanderhoeven et al. 2005; Dassonville et al. 2007), the most realistic way to measure the impact of an invader is to compare invaded plots with nearby control plots with similar vegetation, soil, geology, climate and land-use history. For this reason, at three heavily invaded sites ("Montagne de Sosoye", "Fonds de Leffe East" and Montagne de la Croix North"), five *Cotoneaster* individuals were selected. Each of them was used as the centre of a vegetation relevé ("invaded plots", n=15). In the near vicinity of each of those individuals, one or two relevés were realised ("control plots", n=28). In order to test differences in vegetation between invaded and uninvaded sites, ten relevés were made ("uninvaded plots", n=20) in each of the two uninvaded sites ("Herbuchenne" and "Devant-Bouvignes"). All the relevés had an area of 1 m^2 (1m*1m). In each relevé, we recorded: the presence of all visible vascular plants as well as their cover [%], cover of rocks [%], bare soil cover [%], moss cover [%], slope [°] and aspect (eight classes). For each relevé, we estimated the species richness (number of species per square meter) and the Shannon's equitability (Shannon & Weaver 1949). In order to ensure that the three plot types did not differ significantly as to their abiotic conditions, we tested the equality of slopes and soil depths by ANOVA as well as the distribution of the aspects by a χ^2 test. Slopes were log-transformed and soil depths were square root-transformed in order to meet normality and homoscedasticity requirements. As far as the vegetation data is concerned, we carried out two Correspondence Analyses. The first was performed using the cover values of the plant species and the second using only the presence-absence matrix. This provided both quantitative and qualitative approaches to the vegetation data.

For each measurement (cover of rocks, bare soil cover, moss cover, species richness and Shannon's equitability), we used paired t-tests to compare invaded plots and the close control plots of invaded sites. We used Fischer's exact tests to determine whether a species showed a different occurrence in invaded and control plots. For the pre-cited measurements, the invaded sites (control plots) and uninvaded sites were compared by Mann-Whitney tests.

Results

Distribution area

The analysis of the different databases underlined the wide occurrence of *Cotoneaster horizontalis* in Belgium (Fig.2). In the northern part of the country, an important incidence of the species was observed especially in large urban entities (Brussels, Ghent), as well as in the western sandy dunes, another high value habitat (Van Landuyt, pers.com.). In southern Belgium, the most affected zone was the upper Meuse valley, a deep valley showing a high occurrence of steep slopes and rocks. It is worth noting that *C. horizontalis* was less frequent in the eastern part of the country which is characterised by more acidic soil conditions.

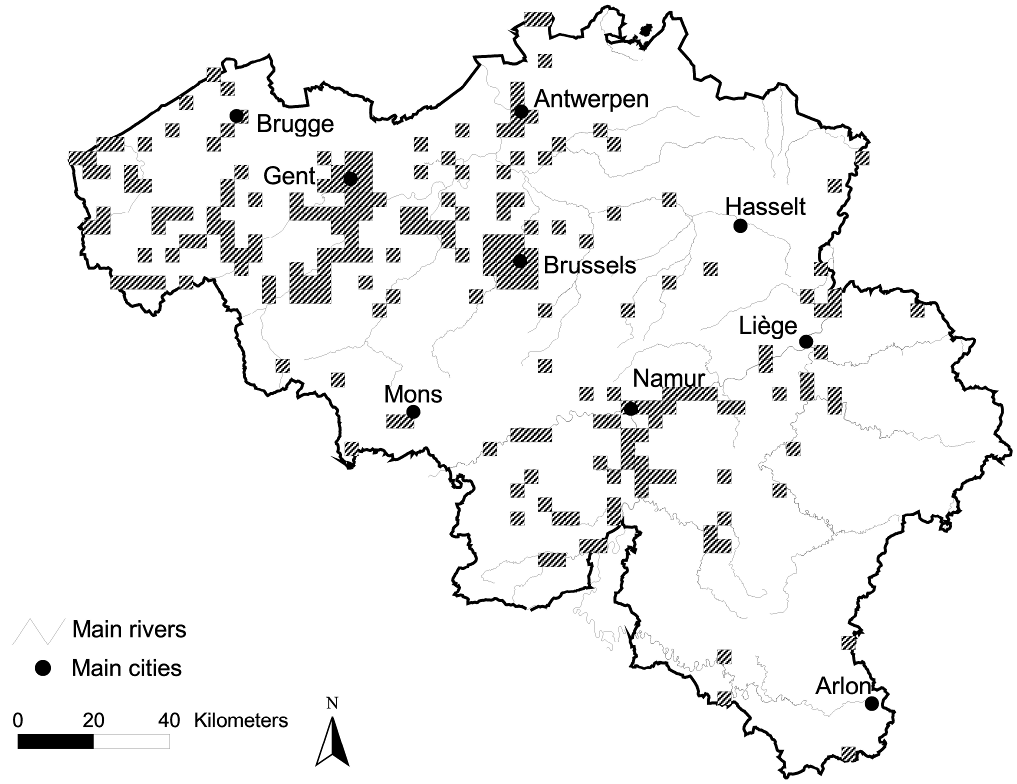

Fig.2::Occurrence of naturalized Cotoneaster horizontalis in Belgium. Striped squares exhibited at least one naturalized individual. (Sources: Remacle 1997-2007; Dufrêne 2005; Van Landuyt et al. 2006, Gembloux Agricultural University-Laboratory of Ecology: unpublished data)

Local invasion status and population structure

A total of 102 *C. horizontalis* individuals were found in the nine calcareous grasslands investigated. Detailed results of observations are given in Fig.1. Only two sites were free of *C. horizontalis* : "Vallon d'Herbuchène" and "Devant-Bouvignes", despite their closeness to infested sites (less than 1 km). In sites colonized by *C. horizontalis*, the individual density (individuals/ha) was variable, ranging from 0.34 ("Champalle") to 10 ("Montagne de la Croix"). Considering all the sites, the mean proportion of small individuals (diameter < 100 cm) was 43% and exhibited an important variation among sites, ranging from 0 to 58.8%. We observed a significant correlation between the number of small individuals and the number of large individuals within a site (R^2=0.578; P=0.047): sites exhibiting the largest number of small individuals ("Montagne de

Sosoye" = 20; "Fond de Leffe-West" = 13) were also those showing the greatest number of large individuals ("Montagne de Sosoye" = 14; "Fond de Leffe-West" = 14).

The 16 individuals collected in "Fond de Leffe-west" were from 2- to 14-year-old and their diameter ranged between 0.3 and 3.25 meters. The largest individual was the oldest one, and the smallest was the youngest. Only 2 individuals (two- and four-year-old) did not exhibit fruits. Fruiting was observed on individuals from three years old and on every individual more than four years old. Diameter was found to be a good predictor of age (R^2 = 0.813, $P < 0.001$). The intercept of the regression was not significant (P=0.663), indicating a constant annual growth from germination. The estimated annual diameter growth (slope of the regression) was 21 cm.

Vegetation and habitat description

Species most frequently observed close to Cotoneaster (i.e. in control plots) were *Allium sphaerocephalon, Brachypodium pinnatum, Bromus erectus, Festuca lemanii, Globularia bisnagarica, Helianthemum apenninum, Helianthemum nummularium, Hieracium pilosella, Hippocrepis comosa, Lactuca perrenis, Pimpinella saxifraga, Potentilla neumanniana, Sanguisorba minor, Scabiosa columbaria, Sedum album, Seseli libanotis* and *Teucrium chamaedrys*. This indicated that Cotoneaster mostly occurred in complex of xeric grasslands and calcareous rocks, along a gradient of rock cover and soil depth (Fig.3). However a few individuals were found at the edges of grasslands or underneath the shrubs with *Prunus spinosa, Salix caprea, Viburnum lantana, Corylus avellana*, and the herbaceous species *Helleborus foetidus, Origanum vulgare, Geranium robertianum* and *Picris hieracioides*. No individuals were found in mesophilous grasslands located on gentle slopes, although such habitats were widespread in some investigated sites, notably at the "Montagne de Sosoye" where *C. horizontalis* was present on steep slopes.

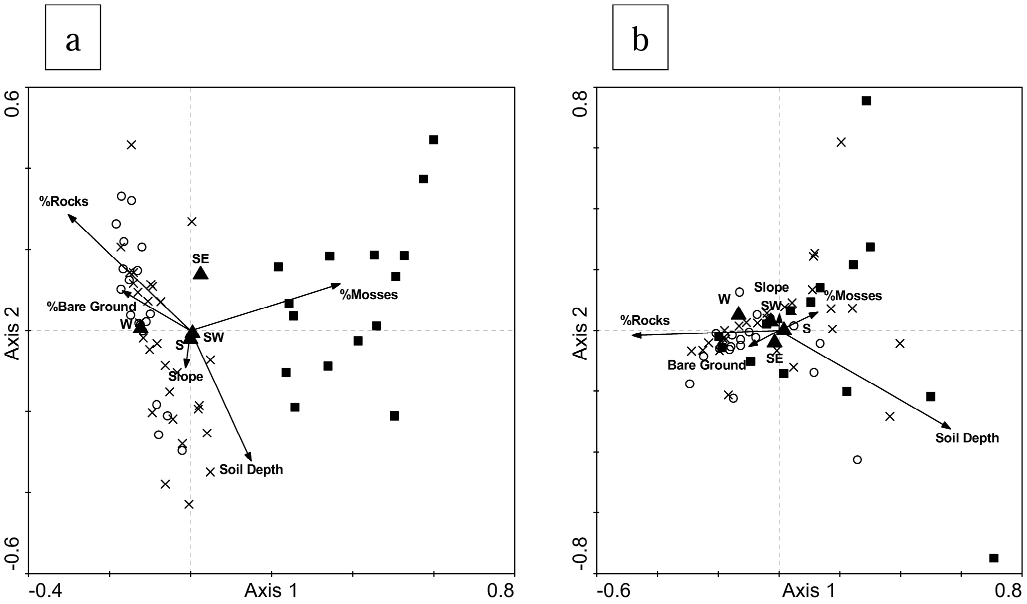

Fig.3: Correspondence analysis ordination graph of plots realised from (a) species abundance data (b) presence-absence data. (○) uninvaded plots, (x) control plots, (■) invaded plots, (→) environmental variables, (▲) Aspects.

The ANOVA did not show any significant difference between invaded plots, control plots of invaded sites and uninvaded plots for slope (*F*=0.01, *df*=2;60, *P*=0.994) or soil depth (*F*=0.08, *df*=2;60, *P*=0.928). The mean values for those measurements were respectively 26° and 4cm. About 87% of the plots were South or Southwest facing. The distribution of the aspects did not differ either ($\chi^2 obs.$=5.99; *df*=6; *P*=0.424).

Impact on the community composition and on the presence of individual species

The Correspondence Analysis showed a high differentiation of the invaded plots along the first axis if species abundance is taken into account (Fig.3a). However this differentiation completely disappeared if their presence/absence was solely considered (Fig.3b). This suggested that rather species cover than species composition differed in invaded plots.

The paired t-tests for comparisons between invaded and control plots showed that if *Cotoneaster horizontalis* is present, we observed a significant decrease in rocky and bare ground cover and an increase in moss cover. We also observed a decrease in both species richness and diversity (Shannon's equitability) (Table 1). Four species were significantly individually affected

(*P*<0.05) by the presence of *C. horizontalis* and four other species marginally (0.1<*P*<0.05) (Table 2). All those species are dry grasslands specialist species.

Table 1: Impact test: comparison (t-paired test) between control and invaded plots. Mean values are given for both plot types.

Tested parameter	Control plots(n=15)	Invaded plots (n=15)	*t*	*P*
Rocks cover	23.1	12	-2.57	0.022
Bare soil cover	13.8	5.5	-2.35	0.034
Moss cover	21.2	43.3	2.66	0.019
Species richness	11.9	8.2	-5.91	<0.001
Shannon's equitability	0.65	0.28	-7.92	<0.001

Comparison between invaded and non-invaded sites

Correspondence Analyses (Fig.3) did not allow to detect any difference between control plots and uninvaded plots, suggesting similar vegetation. Mann-Whitney tests performed on vegetation parameters (cover of rocks, bare soil cover, moss cover, species richness and Shannon's equitability) did not either permit to detect significant differences between invaded and non invaded sites. Those results did not explain the absence of *Cotoneaster horizontalis* at "Herbuchenne" and "Devant-Bouvignes".

Table 2: Comparison of native species occurrence between invaded and control plots. P-value classes associated to the Fischer's exact tests: ***: P<0.001, **: 0.001<P<0.01, *: 0.01<P<0.05, °: 0.05<P<0.1, n is the number of plots considered. Species with P-values >0.1 are not shown.

	Proportion of occupied patches		Comparison invaded/non-invaded spots (Impact)
Species	Invaded	Control	
n	15	28	
Allium sphaerocephalon	0	0.36	**
Bromus erectus	0.6	0.89	*
Festuca lemanii	0.33	0.89	***
Globularia bisnagarica	0.13	0.39	°
Lactuca perrenis	0	0.43	**
Sanguisorba minor	0.6	0.86	°
Sedum album	0.07	0.36	°
Seseli libanotis	0.13	0.39	°

Discussion

Examples of ornamental species able to become naturalized and becoming invasive in Belgium are numerous as illustrated in the *Harmonia* database (2007), currently listing 65 species. Nevertheless, relevant descriptions of the naturalization status of these species are notably missing in the literature. The compilation of the different databases allowed us to highlight that the ornamental *Cotoneaster horizontalis* is widely distributed in Belgium. Moreover, considering that our results do not arise from a complete systematic survey across Belgium, the occurrence is probably still underestimated. Its presence was recorded in urbanised zones like Brussels or Ghent but also in priority habitats for conservation like coastal sandy dunes or dry calcareous grasslands.

The field survey performed during the autumn 2006 confirmed the occurrence of *Cotoneaster horizontalis* in calcareous grasslands of the Belgian Meuse Valley, with seven infested sites out of nine investigated. The density of individuals as well as the proportion of small (and young) individuals varied considerably from site to site, suggesting different histories and dynamics of colonization that will be worth investigating in the future. Particular attention should be paid to the dynamics of populations with a large number of individuals that also showed a relatively high proportion of small individuals, suggesting an important ongoing colonization process. As those small individuals are growing and fruiting, the colonization process is expected to speed up in the following years. On the contrary, sites with a lower occurrence only presented few small individuals, probably resulting from a sporadic colonization process, rather than from an early ongoing colonization process. It should be noted that at the site 'Leffe', the number of small individuals was certainly underestimated. As this site was observed from a distance, we probably minimized the amount of small individuals. In addition, we observed that some three-year-old individuals already reproduced successfully. Fruits are dispersed by birds, which allows long distance dispersal of the species towards non-infested grasslands. Our data stress that the naturalisation process of the species in Belgian calcareous grasslands is effective and that there is a risk of *Cotoneaster* spreading.

The importance of human factors in invasion process has recently been renewed. Biological mechanisms are mostly used to explain exotic species expansion and naturalization processes (Trepl 1984; Pysek & Prach 1993) but as underlined by Kowarik (2003), cultivation, as other anthropogenic mechanisms, should be considered as an important driving force. As stressed by Mack (2000), cultivation counteracts the impact of environmental stochasticity which impedes the development from casual to naturalised populations of exotic species. By growing the species with care under protected conditions, cultivated individuals are indeed sheltered from environmental hazards. Secondary releases resulting from gardens therefore act as a key factor in naturalization processes but also foster population expansion by significantly contributing to long-distance dispersal and consequently overcoming spatial isolation (Kowarik 2003). In the present case, the proximity of gardens and cultivated individuals (personal observations) will most probably intensify the spread of *Cotoneaster horizontalis* in the studied calcareous grasslands. The comparisons we performed between invaded and uninvaded sites did not indicate differences in the community or in abiotic conditions limiting the establishment of *C. horizontalis* in some grasslands. This suggests that differences in invasion history or propagule pressure would explain the absence of *C. horizontalis*.

Habitats invaded by *C. horizontalis* are typically Mosan Xerobromion (Eunis Code E1.2721), which are priority Natura 2000 habitats. Those habitats are biodiversity hotspots both at regional and European scales. They are rare in Belgium, confined to south-facing steep slopes with shallow soils and shelter numerous rare species (Butaye et al. 2005a; Piqueray et al. 2007). A great number of the native species observed in the close vicinity of *C. horizontalis* are threatened in Belgium; seven have a legal status: *Allium sphaerocephalon, Festuca pallens, Globularia bisnagarica, Helianthemum apenninum, Juniperus communis, Lactuca perrenis* and *Orchis sp.*. At Champalle, two other protected plant species, *Aster linosyris* and *Geranium sanguineum*, were also observed in the vicinity of the species thought they were not found in the vegetation relevés. Those observations probably even underestimated the potential impact of *C. horizontalis* on the native flora as the period of investigation did not permit to fully appreciate the floristic conservation value of the sites. Numerous species were not visible anymore in October, among them orchid species (e.g. 15 orchid species known in the very invaded site "Montagne de Sosoye" (Dufrêne 2005)). Impact assessment indicated that the presence of *C. horizontalis* is associated with changes in both the structure and the composition of the community by decreasing species richness and diversity, and directly affecting individual grassland specialists. These impacts are expected to intensify over time with changing population structure.

It is worth noting that none of the vegetation relevés included other invasive plant species, although the invasive *Senecio inaequidens* was occasionally observed in recently restored areas, and the naturalized species *Cheiranthus cheiri* was observed in some rock cracks of investigated sites (pers. obs.). The occurrence of these two species is currently very low but should be monitored in the future.

In addition, an indigenous congeneric species, *Cotoneaster integerrimus* also grows on south facing calcareous rocks in this region. This species shows affinities with numerous species that we found in the vicinity of *C.horizontalis* (Butaye et al. 2005a; Piqueray et al. 2007). The area range of *C.integerrimus* in Belgium, provided by Van Rompaey & Delvosalle (1979), shows many similarities with that of *C.horizontalis*. The sympatry of both species is thus very probable, which implies that the possibilities of hybridization between those two species cannot be excluded. Both species have a basic chromosome number of 17. In Germany, *C. horizontalis* shows triploid and tetraploid individuals while *C. integerrimus* is reported to have di-, tri- and tetraploid individuals (Oberdorfer 1983). To our knowledge, cytotypes are not identified for these two species in Belgium. Hybridization potential would be worth investigating in the future.

Up till now, the description of exotic species invading biodiversity hotspots in Belgium is limited, except for *Rosa rugosa* Thunb. *Rosa rugosa* and *Cotoneaster horizontalis* are both ornamental *Rosaceae* shrubby species widely cultivated close to priority habitats for Nature Conservation. Their dispersal mechanisms by animals allow them a rapid colonization of nutrient-poor habitats with low competition. Indeed, *Rosa rugosa* is another Asiatic shrub invading numerous coastal habitats in NW Europe in recent decades (Bruun 2006; Kollman et al. 2007), including sandy dunes of the Belgian littoral where it forms dense impenetrable thickets. In Belgium, the first observation of this species in the wild dates back from 1934 (*Harmonia* database 2007). In Denmark, the Netherlands and Germany, its distribution and local abundance have markedly increased in the past four decades (Bruun 2005) due to - among others - its high reproductive capacity and seed dispersal by frugivorous birds (Kollman et al. 2007). As a result, species richness and biodiversity of German open dune grasslands were shown to be affected (Grootjans et al. 2002; Isermann 2007; Kiehl & Isermann 2007). Restoration measures included among others re-introduction of traditional management techniques in dunes, such as mowing, grazing and sod cutting, or construction of artificial habitats to compensate for biodiversity loss elsewhere (Grootjans et al. 2002). Although occurring in another kind of high value habitat, this example highlights the potential rapidity of invasion and the necessity to act at early stages of the invasion process to avoid such alarming situations.

In regards to the naturalization and the expansion potential of the exotic horticultural *Cotoneaster horizontalis* in biodiversity hotspots in Belgium, our results would suggest that this species should no longer be commercialised and that it would be worth undertaking intensive management in high value habitats. Alternative species exhibiting the same characteristics of value for gardeners (covering and colour) should be promoted. Nevertheless, due to its large distribution in plant nurseries and its high attractiveness to the public, the feasibility of definitively stopping commercialisation is probably low. It should be combined with campaigns in high risk areas (hotspots zones) to raise public awareness and early management of invasion in calcareous grasslands.

The species forms a very outspread root and rhizome system, so that the pulling up of individuals could induce serious damage to the neighbouring plants, (that were shown to be of conservation value) and should therefore be proscribed. Cutting individuals would probably not allow the eradication of *Cotoneaster*, but, if done regularly (every 3 years), it would limit the development of fruiting individuals. However, resprouting capacity and other biological and phenological traits of the species should be studied further before developing and undertaking coherent and efficient management strategies.

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CHAPITRE 4 : RAPID RESTORATION OF A SPECIES-RICH ECOSYSTEM ASSESSED FROM SOIL AND VEGETATION INDICATORS: THE CASE OF CALCAREOUS GRASSLANDS RESTORED FROM FOREST STANDS.

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Abstract

Calcareous grasslands have long been recognized as biodiversity hotspots in Europe. However, in recent decades these ecosystems have seen rapid decline. In Belgium, more than 100 ha of calcareous grasslands have been restored from oak coppices and pine forests since the 1990s. The aim of the present study was to provide a quantitative assessment of the success of these restoration efforts, using two sets of indicators: one related to soil conditions, the other related to vascular plant communities. Soil conditions were evaluated by comparing soil samples from pre-restoration forest stands, restored grasslands (3 age classes: 2-4 years; 5-8 years, 10-15 years) and reference grasslands. The analysis revealed no significant differences in soil N, P and K contents between pre-restoration forests and restored and reference grasslands. We observed a decrease in the mineralization rate indicators in both pre-restoration forests and recent grassland restorations, which was resorbed in older restorations. Floristic surveys revealed that plant species composition of older restorations was most like reference grasslands. However, some differences in species composition persisted after 15 years. Moreover, a few rare species did not colonise restored grasslands despite a close seed source. Non-recolonization by a set of species expected on calcareous grasslands may be due to dispersal limitation and higher cover by native invasive grasses in restored parcels. These results were discussed in term of implications for management.

Introduction

In the face of destruction and fragmentation of natural and semi-natural habitats, protection and management of the remaining (semi-) natural habitat fragments is no longer sufficient to ensure the long-term viability of all elements contributing to biodiversity. Consequently, habitat restoration has become a necessity to recreate functional ecological networks (Jongman & Pungetti 2004). This approach serves to restore patches of degraded or destroyed habitat by accelerating or skipping successional stages. This is accomplished by directing ecosystem development towards a specific community structure or ecosystem type (Jordan et al. 1987; Bakker & Berendse 1999). However, habitat restoration can be cost prohibitive and efforts to restore natural communities must demonstrate their success in reaching target ecosystems (Fagan et al. 2008). Hence, quantitative indicators are needed to evaluate the efficiency of restoration practices and to improve future restoration projects (e.g. Holl & Cairns 2002; Samu et al. 2008). Ideally, these indicators may address different components of the restored ecosystem.

Habitat restoration is of vital importance in temperate semi-natural calcareous grasslands, and has received considerable attention in recent years. Those ecosystems are considered one of the richest in biodiversity in temperate regions (Prendergast et al. 1993; WallisDeVries et al. 2002) and the most species-rich plant communities in the world at a small scale (<10m^2) (Willems 2001). Once widespread over Europe, calcareous grasslands have undergone dramatic fragmentation since the end of the nineteenth century. Following the abandonment of the traditional agro-pastoral systems that were responsible for their extension, these communities have been replaced by arable land, trees plantations, or have undergone spontaneous encroachment and succession to forest communities (Poschlod & WallisDeVries 2002). The maintenance and enhancement of calcareous grassland networks is now recognized as a priority in European biodiversity conservation policies, as reflected in the Habitat Directive 92/43/EEC.

Restoration success depends primarily on two factors, the reformation and maintenance of suitable environmental conditions and the recolonization capacity of target species (Perrow & Davy 2002; Piqueray & Mahy 2009). Soil conditions in calcareous grasslands are characterized by low soil fertility, and nutrient transfer historically occurred through traditional grazing practices (Willems 2001). A negative relationship between soil fertility and calcareous grasslands species richness has been demonstrated (e.g. Al-Mufti et al. 1977; Janssens et al. 1998) as well as the harmful consequences of intentional soil nutrient enrichment (Willems et al. 1993; Bobbink et al. 1998; Jacquemyn et al. 2003a). On arable lands, attempts to restore soils to historically low fertility levels was shown to be the major constraint for the recovery of calcareous grassland plant communities (Hutchings & Stewart 2002; Walker et al. 2004). However, to our knowledge, soil nutrient status has never been studied in calcareous grasslands restored from forest stands. Green (1972) stressed that natural succession should lead to global eutrophication of any habitat. Hurst and John (1999a) showed nitrogen enrichment in calcareous grassland during the first step of encroachment by the native invasive grass *Brachypodium pinnatum* in England. Data from other grassland types indicate that afforestation can induce different changes in soil conditions. On one hand, it often induces a reallocation of major nutrients (N, P) from mineral soil to litter (Ross et al. 1999; Farley & Kelly 2004), as well as changes in the form of these nutrients (Farley & Kelly 2004; Chen et al. 2008). After clear-cutting, litter decomposition can increase the nutrient release (Ouro et al. 2001). On the other hand, shrub and tree colonization can induce modifications to the soil micro-climate, and subsequently cause changes in soil microbial activity responsible for the mineralization of soil organic matter. This can lead to modifications in soil carbon stock, acidity and C:N ratio (Thuille & Schulze 2006). Therefore, we argue that mineralization indicators (e.g. pH, C:N ratio, and Fe), as well as fertility indicators (e.g. N, P, and K) need to be considered to assess the restoration of soil conditions. Decreasing pH and subsequent increasing Fe availability could be consequences of a lower mineralization rate (Bonneau & Souchier 1979).

In plant community recovery efforts, species richness has been widely used as an indicator of restoration efficiency (e.g. Willems 2001; Lindborg & Eriksson 2004b; Cristofoli et al. 2009). However, it is only a crude indicator of successful restoration (Mortimer et al. 1998; Kiehl et al. 2006). An ecosystem may be species-rich, but the species may be non-representative of the native community. Therefore, restoration success should be evaluated against a reference habitat, such as a local reference site (Society for Ecological Restoration International Science & Policy Working Group 2004; Ruiz-Jaen & Aide 2005; Piqueray & Mahy 2009).

In Belgium, over 90% of calcareous grasslands have been lost (Bisteau & Mahy 2005a; Adriaens et al. 2006). A large proportion were afforested with *Pinus sylvestris* and *P. nigra* at the end of the 19th century (Vandermotten & Decroly 1995). Other areas experienced a natural process of succession following grazing abandonment and were progressively replaced by oak woodlands. To stop this decline, approximately 100 ha of calcareous grasslands have been restored in Belgium over the last 15 years. All restored sites derived from pre-forests or 40-100 year old forest established on ancient calcareous grasslands. Restoration protocols included tree and shrub clearing followed by sheep and goat grazing (André & Vandendorpel 2004; Graux 2004). Restoration of species-rich grasslands from secondary forest or pre-forest ecosystems was studied in different parts of Europe (Kiefer & Poschlod 1996; Zobel et al. 1996; Pärtel et al. 1998), but rarely in Western Europe (Hutchings & Stewart 2002).

In this paper we combined soil condition and plant community data with the aim of assessing the success of calcareous grasslands restoration from afforested sites in Belgium. Using indicators computed from these two datasets, we compared restored sites with calcareous grassland reference sites and forest stands equivalent to pre-restoration conditions and addressed the following questions: 1) Do restoration actions lead to the reestablishment of native calcareous grassland plant communities; 2) How does soil of restored sites compare to soil of reference grasslands and pre-restoration sites?

Material and Methods

Study sites

The study area was located in two Belgian regions: the Viroin Valley, and the Lesse and Lomme Valleys (Table 1), both located in Calestienne, a narrow Devonian limestone strip running southwest to northeast. Both regions support large areas of calcareous grasslands, and are considered the core areas for calcareous grassland conservation in Belgium. Although several grassland communities occurred within the study area (see Butaye et al. (2005b) and Piqueray et al. (2007) for communities description), we focused on the most widespread community : *Mesobromion* calcareous grasslands. A total of 12 sites (six per region) where grassland restoration had occurred were selected for the study (Table 1). Selected sites were restored half from pine stands and half from oak coppices. Pine stands were up to 100-year-old *Pinus nigra* or *P. sylvestris* plantations. Dense shrub oak coppices were mainly formed with *Prunus spinosa*, *Crataegus monogyna* and *Corylus avellana*, with intermingled scarce trees of *Quercus robur*. Historical maps, aerial pictures and field surveys were used to delimit three parcel types: 1) *reference grasslands*, i.e. calcareous grasslands known to have existed for more than two centuries. They harbour the typical local calcareous grassland vegetation and are considered as the reference ecosystem for restoration. 2) *restored parcels*, i.e. former grasslands that were afforested and were then subject to forest clearings with subsequent management (mainly grazing) with the aim of restoring grasslands. Trees and shrubs were exported from the parcels, but tree stumps remained. 3) *pre-restoration forest stands*, i.e. forests established on former calcareous grasslands. Both reference grasslands and pre-restoration forest stands were adjacent to restored parcels, on similar topographic situations. The time passed since restoration (in years) was known for each restored parcel. Restored parcels were chosen to equally cover three age classes since restoration: 2-4 years, 5-8 years and 10-15 years. These classes were mainly defined due to field constraints, e.a. first year was omitted as many uncertainties remained on the location of last clearcuts location at the beginning of our project. Each site comprised at least one parcel of each type. In the "Tienne des Vignes" and "Montagne au Buis" sites, two restored parcels originated from the same forest stand but were restored at different times, these restored parcels had thus the same reference forest stands and reference grasslands. In addition, at "Les Pairées" and "Abannets" sites, two restored parcels were of the same age but originated from two different forest stands (Pine and Oak). In summary, we sampled 8 pre-restoration forests parcels, 12 restored parcels (four per age class) and 8 reference grassland parcels (Table 1).

Table 1: Sampled parcels in the study region, name of the site and age classes (forests are pre-restoration forests; grasslands are reference grasslands). Forest type and age since restoration are respectively given for pre-restoration forests and restored parcels.

Name	Localisation	Region	Age class	Forest stand	Real age
Tienne des Vignes	50°06’N – 5°10’E	Lesse and Lomme	Forest	Pine	/
Lorinchamps	50°06’N – 5°14’E	Lesse and Lomme	Forest	Oak	/
Tienne d'Aize	50°07’N – 5°09’E	Lesse and Lomme	Forest	Oak	/
Les Pairées	50°06’N – 5°11’E	Lesse and Lomme	Forest	Pine/Oak	/
Niémont	50°06’N – 4°42’E	Viroin	Forest	Pine	/
Montagne-aux-Buis	50°05’N – 4°34’E	Viroin	Forest	Oak	/
Rivelottes	50°05’30”N – 4°40’E	Viroin	Forest	Pine	/
Abannets	50°04’30”N – 4°34’E	Viroin	Forest	Pine/Oak	/
Tienne des Vignes	50°06’N – 5°10’E	Lesse and Lomme	2-4 years	/	2 years
Lorinchamps	50°06’N – 5°14’E	Lesse and Lomme	2-4 years	/	2 years
Tienne des Vignes	50°06’N – 5°10’E	Lesse and Lomme	5-8 years	/	8 years
Tienne d'Aize	50°07’N – 5°09’E	Lesse and Lomme	5-8 years	/	8 years
Les Pairées pine	50°06’N – 5°11’E	Lesse and Lomme	10-15 years	/	10 years
Les Pairées oak	50°06’N – 5°11’E	Lesse and Lomme	10-15 years	/	10 years
Niémont	50°06’N – 4°42’E	Viroin	2-4 years	/	2 years
Montagne-aux-Buis North	50°05’N – 4°34’E	Viroin	2-4 years	/	4 years
Rivelottes	50°05’30”N – 4°40’E	Viroin	5-8 years	/	5 years
Montagne-aux-Buis South	50°05’30”N – 4°34’E	Viroin	5-8 years	/	7 years
Abannets pine	50°04’30”N – 4°34’E	Viroin	10-15 years	/	15 years
Abannets oak	50°04’30”N – 4°34’E	Viroin	10-15 years	/	15 years
Tienne des Vignes	50°06’N – 5°10’E	Lesse and Lomme	Grassland	/	/
Lorinchamps	50°06’N – 5°14’E	Lesse and Lomme	Grassland	/	/
Tienne d'Aize	50°07’N – 5°09’E	Lesse and Lomme	Grassland	/	/
Les Pairées	50°06’N – 5°11’E	Lesse and Lomme	Grassland	/	/
Niémont	50°06’N – 4°42’E	Viroin	Grassland	/	/
Montagne-aux-Buis	50°05’N – 4°34’E	Viroin	Grassland	/	/
Rivelottes	50°05’30”N – 4°40’E	Viroin	Grassland	/	/
Abannets	50°04’30”N – 4°34’E	Viroin	Grassland	/	/

Soil analysis

Five soil samples were collected in each parcel, i.e. pre-restoration forests, restored parcels, and reference grasslands. Each sample consisted of a bulk soil sample collected systematically with a 2 cm diameter auger within a 1 m^2 quadrat. The minimum total soil volume collected was 100 cm^3 per quadrat. The soil was thin at all study sites (circa 10 cm); therefore the entire soil layer, from ground surface to bedrock, was collected. Soil depth was measured at the four corners of the quadrat. Mean soil depth was estimated for each soil sample. In forests and reference grasslands, quadrats were randomly localized. In restored grasslands, samples were collected nearby randomly selected tree stumps. Positioning quadrats in the close vicinity of tree stumps ensured that the sample was not situated in a small remnant of the former grassland. Soil samples were sieved (< 2 mm and one fraction < 0.5 mm) after air-drying.

Total nitrogen content was estimated following the modified protocol of the Kjeldahl method described by Bremner & Mulvaney (1982). Available phosphorus was determined using a modified version of Olsen & Sommers (1982). Phosphorus was extracted by shaking 5 g of soil with 100 ml of 0.5N $NaHCO_3$, at pH of 8.5, for 30 minutes. Carbon black was added to clear filtrates. After filtering, a 5 ml aliquot of extracted phosphorus was diluted in a 100 ml volumetric flask with 10 ml 1.5% $(NH_4)_6Mo_7O_{24}.4H_2O$ and distilled water. The sample was coloured blue by adding 0.5 ml of 1% $SnCl_2$, and phosphorus content was measured by spectrophotometry at 560 nm. Total organic content was measured following the Springer-Klee method, i.e. a hot sample oxidation with $K_2Cr_2O_7$ and titration of oxidant excess with 0.1N $(NH_4)_2Fe(SO_4)_2.6H_2O$. Mobilizable fractions of K and Fe were determined after extraction with 1N CH_3COONH_4-EDTA. The soil: solution ratio was 1:5, and the extraction solution pH was buffered at 4.65. Elements were measured in the remaining extraction solution by ICP-OES (VARIAN Vista MPX). Soil pH was estimated with a Hellige pH-indicator.

A preliminary data analysis (results not shown) indicated variation among sites with significant mean differences for all soil variables. To reduce site effect, and to assess the effect of restoration as a function of the reference ecosystem, i.e. reference grasslands, soil data were transformed as follows:

$$X_{ij} = X_{0,ij} - X_{ref,j,}$$

where X_{ij} is the transformed value of the considered soil parameter in quadrat *i* in site *j*, $X_{0,ij}$ is the initial value of the soil parameter in quadrat *i* in site *j*, and $X_{ref,j,}$ is the mean value of the soil parameter over all quadrats of the reference grassland in site j. In order to avoid pseudo-replication the average soil values of each parcel were considered for analyses.

We computed MANOVA (Wilks'lambda) in order to test for an overall difference among mean transformed value for the different soil parameters among the different types of parcels: pre-restoration forests, restored parcels of different ages (three classes) and reference grasslands. Thereafter, single response of each soil parameter was tested with a one-way ANOVA followed by post-hoc Fisher pairwise comparisons tests. By definition, the mean value for reference grasslands was zero. A canonical discriminant analysis was computed in order to illustrate patterns of variation in soil conditions among parcel types.

Floristic analysis

Floristic surveys and species cover (%) were recorded in 20 quadrats (1 m²) in each restored parcel. Quadrats were located on cardinal directions one meter away from five randomly selected tree stumps. In addition, eighty quadrats were sampled in the reference grasslands and eighty other in pre-restoration forests (equally distributed among regions and then among grasslands/forests of each region). Only herbaceous and shrub species (<2m height) were considered. Species cover values were log-transformed for analyses. In order to avoid pseudo-replication the average species cover of each parcel were considered for analyses.

Detrended Correspondence Analysis (DCA) was performed to examine similarities in species composition among parcels (reference grasslands, restored parcels of different age classes, i.e. 2-4 years, 5-8 years and 10-15 years, pre-restoration forests). We computed an Multiple Response Permutation Procedure (MRPP) with 10000 permutations in order to compare Bray-Curtis distances between and within age classes. In case of significant test, pairwise comparison of age classes was peformed. We tested if plant species composition differed among parcel types by identifying indicator species using the indicator value method INDVAL (Dufrêne & Legendre 1997). Significant indicator species were detected for each parcel type following 999 Monte Carlo permutations, at P=0.05. In order to compare plant community structure from a more ecological perspective, species were classified into five ecological groups based on phytosociological alliances (Bisteau & Mahy 2005b) : (1) species of calcareous grasslands (characteristic of the *Festuco-Brometea* or *Sedo-Scleranthetea*), that was divided in two sub-groups : native invasive grass species (NIG) (*Brachypodium pinnatum* and *Bromus erectus*) and other grassland species; (2) ruderal species (i.e. characteristic of the *Artemisietea*, *Plantaginetea* and *Epilobietea*); (3) meadow species (characteristic of the *Molinio-Arrhenatheretea*); (4) edge species (characteristic of the *Trifolio-Geranietea*); and (5) woodlands and forest species (characteristic of the *Querco-Fagetea*). Shrubs were considered separately. Mean values for species richness, species evenness, shrub cover, bare ground cover and total cover of species belonging to each ecological groups (in %) were compared among parcel types with one-way ANOVAs followed by post-hoc Fisher pairwise comparisons tests. Shrub cover, bare ground cover, edge species cover, other grassland species cover and ruderal species cover were log-transformed, and grassland species cover and forest species cover were Asin-transformed to improve normality and homoscedasticity.

Finally, canonical correspondence analysis (CCA) was used to assess the influence of soil parameters on the representation of ecological groups within parcels.

DCA and CCA were performed using CANOCO 4.5 (ter Braak & Smilauer 2002). MRPP was performed using 'Vegan' R-package (Oksanen et al. 2008). MANOVA was conducted unsing the 'Stats' R-package. Canonical discriminant analysis was performed using 'Candisc' R-package (Friendly 2007) ANOVAs were conducted with MINITAB 15 (Minitab Inc. 2007). Nomenclature followed Lambinon et al. (2004).

Results

Soil analysis

Reference grassland soil parameter values are provided in Table 2. Reference grassland pH was, on average, slightly acidic (Table 2), but a high standard deviation reflected large variation for this parameter, with values ranging from 4.5 to 8.5. Pearson correlations revealed that N, P, K, C and pH transformed values exhibited inter-correlations (Table 2). Fe and C:N were independent from each other soil parameters. The first axis of the canonical discriminant analysis explained 79,5% of soils parameters variation. It was mainly linked to mineralization indicators (pH, Fe, C:N) (Fig.1). It discriminated Pre-restoration forests and 2-4 years restorations with positive coordinates, corresponding to lower pH, higher C:N, higher Fe content, from 10-15 years restorations and reference grasslands with negative coordinates.

Table 2: Mean values and standard deviation of soil parameters in reference grasslands and Pearson correlations between transformed soil parameters. Correlation p-levels are given when significant: ***:$p<0.001$; **:$0.001<p<0.01$; *:$0.01<p<0.05$.

	N (%)	P (mg/100g)	K (ppm)	C (%)	Fe (ppm)	C:N ratio	pH	Soil depth (cm)
Mean	0.575	0.963	15.97	7.26	15.15	12.7	5.7	8.19
SD	0.119	0.263	5.22	1.4	2.96	1.4	1.2	2.74
pH	0.566**	0.615***	0.166	0.395*	-0.205	-0.279		n.c.
C:N	-0.039	0.039	-0.053	0.344	0.360			n.c.
Fe	0.102	-0.167	0.198	0.272				n.c.
C	0.913***	0.576**	0.490**					n.c.
K	0.542**	0.198						n.c.
P	0.614***							n.c.

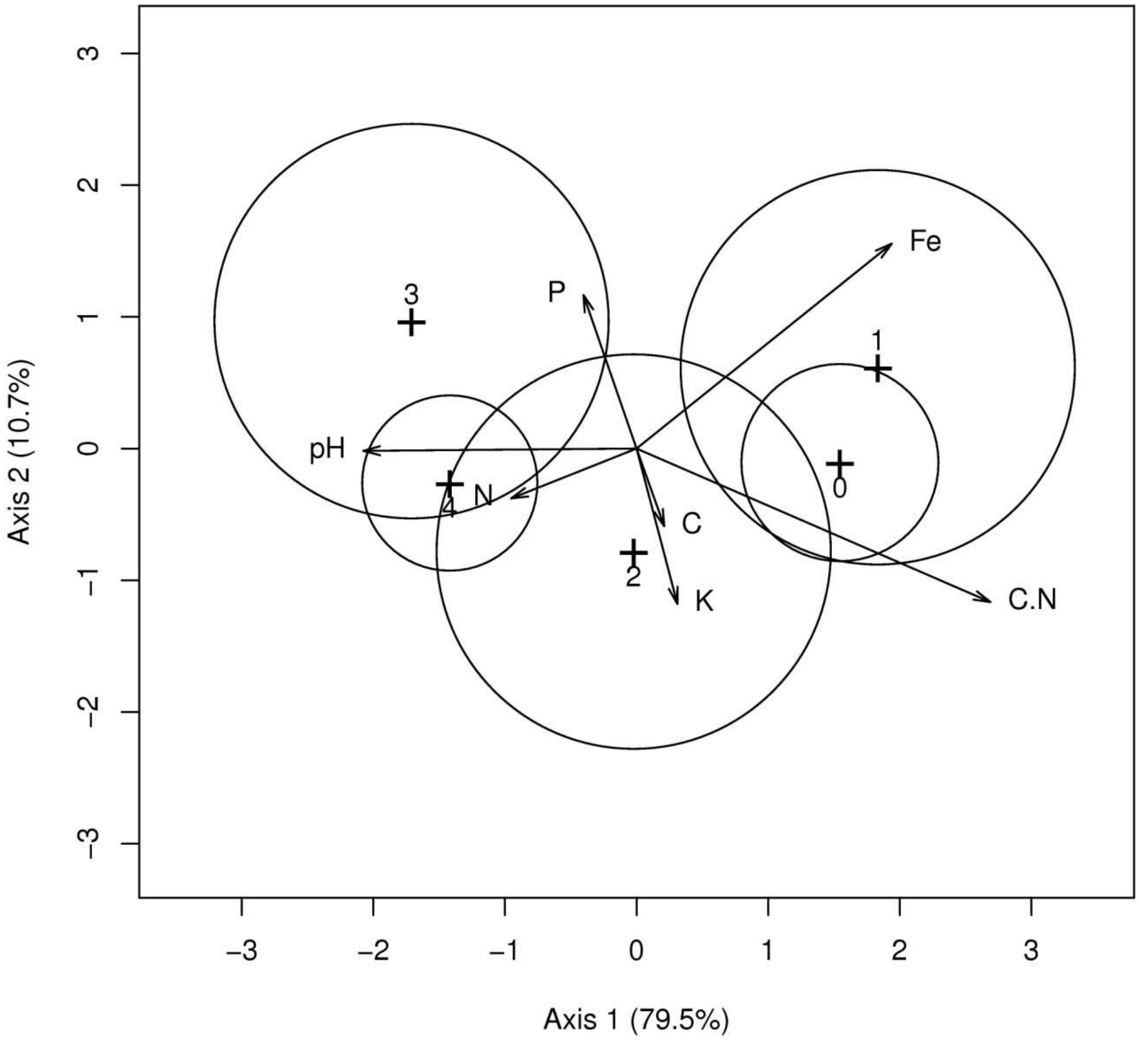

Figure 1: Canonical discriminant analysis ordination plot (axes 1 and 2) of soil parameters in pre-restoration forests (0) , restored parcels (1=2-4 years;2=5-8 years;3=10-15 years) and reference grasslands (4). Mean coordinates and 95% confidence ellipses are plotted.

Comparison of transformed soil parameters revealed marginal differences among parcels types (MANOVA, Wilks' Lambda=0.154, P=0.058). The mean transformed values for N, P, K, C and soil depth did not differ significantly among parcel types (Table 3). However, significant differences for C:N ratio were detected. A significant positive difference was observed for C:N ratio between pre-restoration forest, restored parcels 2-4 and 5-8 years old, and reference grasslands. Older restorations (10-15 years old) did not exhibit a significant difference relative to reference grasslands. Only marginal differences were found for pH, with lower pH in pre-restorations forests and young restorations and the highest values in older restorations.

Althought decreasing Fe content were observed from pre-restoration forests to reference grasslands, this trend was not significant.

Table 3: Mean differences for soil parameters between reference grasslands, pre-restoration forests and restored grasslands of different age classes. (-) indicates a deficiency, (+) indicates a surplus. *p-values* are the result of ANOVA. Different letters indicate significant differences.

	Pre-restoration forests	**Restored grasslands**			**Reference grasslands**	***p-value***
		2-4 years	5-8 years	10-15 years		
N (g/100g)	- 0.050	- 0.026	+ 0.016	+ 0.002	0	0.660
P (mg/100g)	- 0.012	+ 0.032	+ 0.032	+ 0.111	0	0.941
K (ppm)	+ 0.376	- 0,7	+ 0,765	- 0.63	0	0.811
C (g/100g)	- 0.054	+ 0.463	+ 0.909	- 0.04	0	0.882
Fe (ppm)	+ 3.95	+ 5.76	+ 2.84	+ 2.77	0	0.107
C:N	+ 1.32 ^{a}	+ 1.35 ^{a}	+ 1.40 ^{a}	- 0.093 ^{b}	0 ^{b}	0.016
pH	- 0.5	- 0.6	+ 0.3	+ 0.5	0	0.053
Soil depth (cm)	+ 0.66	+ 1.78	- 0.91	- 1.46	0	0.153

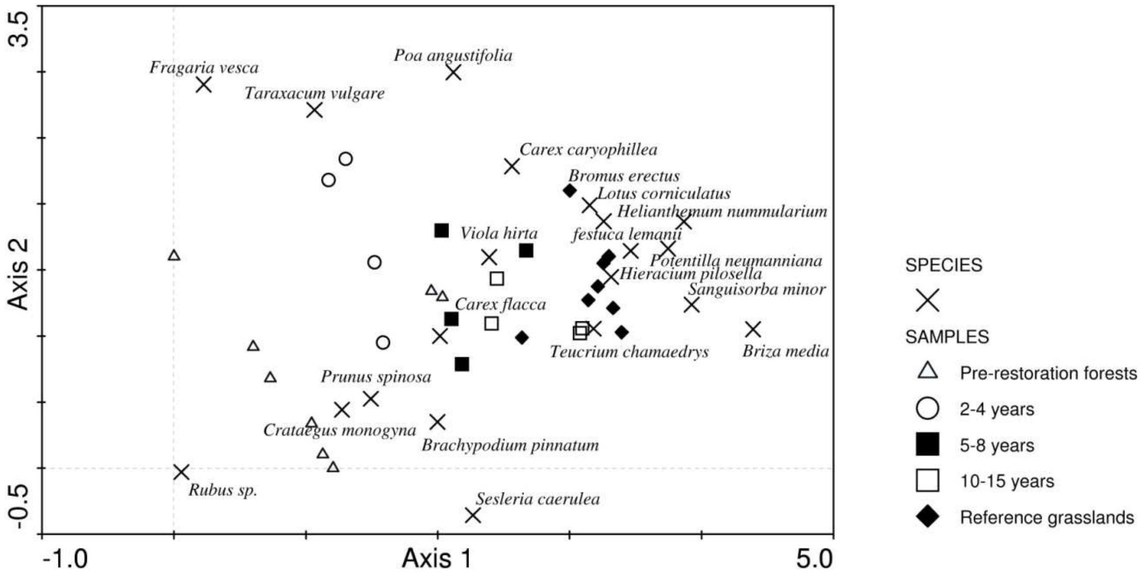

Figure 2: DCA ordination scatter plot (axes 1 and 2) of species composition in pre-restoration forests, reference and restored calcareous grasslands of different age classes. Species with weight >10% were plotted.

Floristic analysis

A total of 210 species was found among our 400 vegetation samples. The first two DCA axes explained respectively 13.6% and 6,9% of the species variation among parcels (Fig. 2). The positions of parcels on axis 1 indicated the clear influence of restoration age with younger restorations (2-4 years) closer to pre-restoration forest and older restorations (10-15 years) closer to reference grasslands. MRPP showed that Bray Curtis distances estimated on species composition among age classes were significant (*A*=0.139, p<0.001). Pairwise comparisons indicated significant mean Bray Curtis distance among all age class, excepted between 5-8 years and 10-15 years restorations (Table 4). Distance between age classes showed that the older a restoration was, the closer to reference grasslands it was. However, the oldest restoration and the reference grasslands were still significantly different in their species composition. Pre restoration forest samples are rather distant from every other age class. Axes 1 and 2 of the CCA respectively explained 21.1% and 13.7% of the ecological group composition variation among parcels. CCA opposed shrubs, forest and ruderal species to grassland and edge species on axis 1 (Fig. 3). This axis was correlated with high N content and pH and with low C:N ratio and Fe content. Axis 2 of the CCA discriminated meadow species that were linked to the higher K contents.

Table 4: Bray-Curtis mean distance between relevés of the different age classes. MRPP p-levels are given when significant: ***:p<0.001; **:0.001<p<0.01; *:0.01<p<0.05.

		Pre-restoration forests	**Restored grasslands**		
			2-4 years	5-8 years	10-15 years
Restored grasslands	2-4 years	0.70*			
	5-8 years	0.71**	0.62**		
	10-15 years	0.73**	0.66*	0.51	
Control grasslands		0.81***	0.74**	0.59*	0.52*

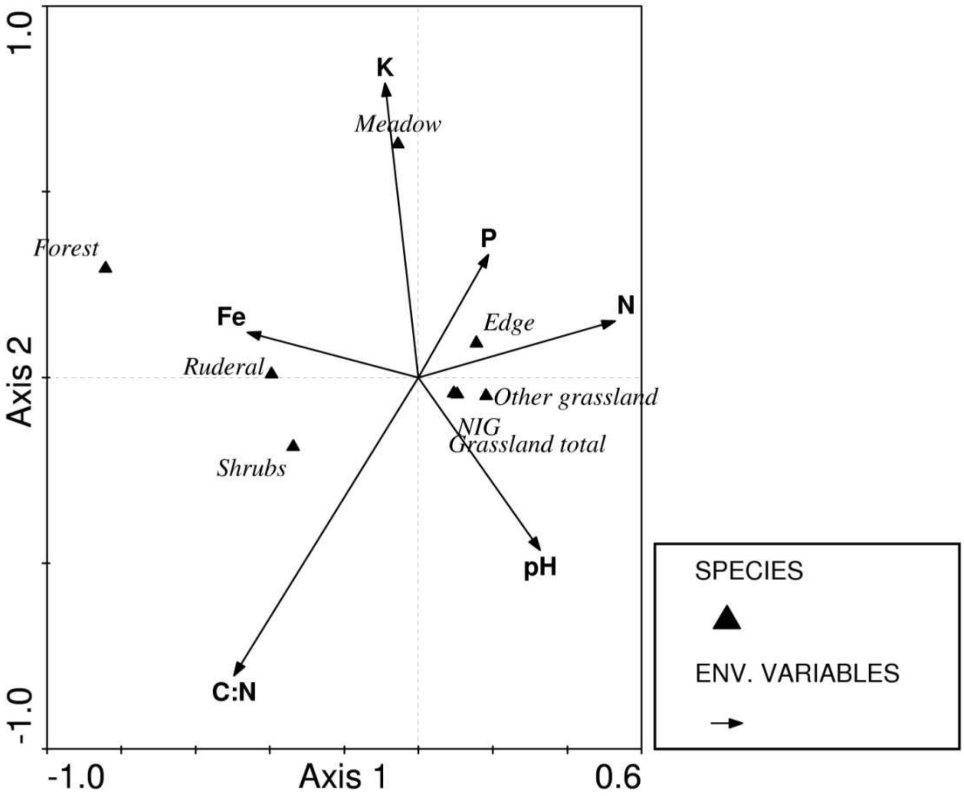

Figure 3: CCA ordination scatter plot (axes 1 and 2) of species ecological groups along soil parameters gradients.

ANOVA showed significant differences for vegetation parameters among age classes, except for the mean cover of meadow species (Table 5). Post hoc comparisons indicated that pre-restoration forests displayed lower species richness than all other parcel types. Species richness tended to increase with increasing age of restored parcels. The highest mean species richness was observed in reference grasslands but was not significantly different from 10-15 years and 5-8 years old restoration. Evenness was significantly higher in reference grasslands than in all other parcel types; while no significant difference was observed among pre-restoration forests and restored parcels. The mean cover of grassland species increased with the age of restored parcels and was not significantly different between 10-15 years old restoration and reference grasslands (Table 5). However, this was mainly due to the overdevelopment of NIG as significant differences was found between reference grasslands and restored parcels for the mean cover of other grassland. NIG cover was the lowest in the forests and in 2-4 years old restored parcels. Older restoration parcels had the highest mean cover of NIG, but the difference with reference grasslands was not significant. Bare ground displayed an inverse pattern, with a minimum cover observed in oldest restorations. In oldest restorations, grassland species tended to replace ruderal species, better represented in recent restoration parcels. Pre-restoration forests were mainly characterized by a relatively high shrub cover.

Shrub cover tended to lower with increasing restoration age, 10-15 years old restored parcels being not significantly different from reference grasslands.

Table 5: Comparisons of vegetation parameters mean values between reference grasslands, pre-restoration forests and restored grasslands of different age classes. *p-values* are the result of ANOVA. Different letters indicate significant differences.

	Pre-restoration forests	**Restored grasslands**			**Reference grasslands**	***p-value***
		2-4 years	5-8 years	10-15 years		
Species richness	6.3^{a}	15.0^{b}	16.8^{bc}	18.9^{bc}	20.3^{c}	<0.001
Evenness	0.57^{a}	0.60^{a}	0.58^{a}	0.58^{a}	0.70^{b}	0.021
Grassland species cover	24.6^{a}	37.8^{ab}	62.0^{bc}	72.9^{c}	73.6^{c}	<0.001
NIG cover	20.0^{a}	16.0^{a}	41.7^{b}	39.7^{b}	28.7^{ab}	0.046
Other grassland species cover	4.6^{a}	21.8^{b}	20.3^{b}	33.2^{c}	45.0^{d}	<0.001
Edge species cover	0.9^{a}	5.0^{b}	4.3^{b}	4.1^{b}	6.6^{b}	0.008
Ruderal species cover	3.8^{a}	22.1^{b}	6.5^{ab}	1.2^{a}	1.2^{a}	0.001
Forest species cover	0.4^{b}	0.8^{b}	0.1^{a}	0.1^{a}	0.0^{a}	0.001
Meadow species cover	2.2	1.6	1.9	2.3	54.7	0.547
Shrubs cover	22.0^{c}	12.4^{bc}	8.7^{bc}	5.2^{ab}	2.3^{a}	0.005
Bare ground cover	51.8^{c}	15.9^{b}	2.8^{a}	2.4^{a}	7.0^{ab}	<0.001

Based on the INDVAL analysis, ten species were significant ($P<0.05$) indicators of pre-restoration forests, consisted mainly in shrubs (60%). Thirty-eight species were significant indicators of the 2-4 years class, with 45% ruderal taxa. Ten species were significant indicators of the 5-8 years class. Ruderal species were well represented (33%), but grassland species were also present (27%). Fourteen and 41 species were significant ($P<0.05$) indicators of the 10-15 years and reference grasslands, respectively. In both groups, grassland species were prominent (71 and 76%, respectively). Further information concerning indicator species identity can be found in Appendix A. Nineteen species, mainly characteristic of calcareous grasslands, were present in reference grasslands but not in restored parcels (*Anthericum liliago*, *Carex humilis*, *Cuscuta epithymum*, *Globularia bisnagarica*, *Himanthoglossum hircinum*, *Koeleria macrantha*, *Lathyrus pratensis*, *Melampyrum pratense, Myosotis ramosissimus, Ophrys apifera, Plantago media, Prunella laciniata*, *Pulsatilla vulgaris*, *Ranunculus bulbosus*, *Sorbus torminalis*, *Thlaspi perfoliatum*, *Trifolium montanum, Valerianella dentata*, *Veronica prostrata*). Only few grassland species were found in pre-restoration forests.

Discussion

How does soil of restored sites compare to reference grasslands and pre-restoration sites?

In this study we used fertility and mineralization indicators for comparing soil characteristics among pre-restoration situations, restored ecosystems and reference grasslands. Globally our results pointed to low differences between pre-restoration and reference ecosystems, which is encouraging for calcareous grasslands restoration from afforested sites. However, differences in mineralization indicators were more pronounced than differences in fertility indicators.

Soil fertility level is a major factor for successful restoration, as typical calcareous grasslands are characterized by low nutrient levels (Grime 1979; Critchley et al. 2002b). Soil nutrients contents observed in reference grasslands in the present study were congruent with the literature (Wells & Cox 1993; Janssens et al. 1998; Critchley et al. 2002a). Our results pointed out low trophic conditions for all reference sites, with low concentrations of total nitrogen and available phosphorus concentrations. Neither pre-restoration forest, i.e, parcels afforested < 100 years ago, nor restored grasslands exhibited significantly higher nutrient levels compared to adjacent reference grasslands. Our results contrast with the patterns reported about restoration of formerly arable lands (Walker et al. 2004), and are encouraging for calcareous grasslands restoration from afforested sites. However, some authors stressed the fact that grassland afforestation increased nutrient availability rather than nutrient content (Farley & Kelly 2004; Chen et al. 2008). Further investigations are thus needed to study the different nutrient forms in restored and reference grassland soils. Also, some more synthetic indicators such as standing crop may be used as a complement to this study in order to confirm the equivalent nutrient status between restored and reference grasslands.

Multivariate analysis of soil characteristics pointed to a general pattern of differentiation between pre-restoration forests/young restorations and old restoration/reference grasslands, mainly due to mineralization indicators. However, only C:N ratio showed significant difference among the parcel types and discriminated reference grasslands and old restorations from other parcel types. The lack of significant differences between old restorations and reference parcels suggests that mineralization status of restored grasslands can be restored rapidly despite initial differences. In our study, pH of calcareous grassland was slightly acidic (pH=5.7). This was due to the fact that only the upper soil layer could be analysed due to soil thinness. In this layer, intense root activity and the organic matter decomposition tends to acidify the soil solution (Mengel & Kirkby 1987). pH tended to be lower in pre-restoration forests and young restorations as compared to reference grasslands. The lower pH and higher C:N ratio in pre-restoration forests and recently restored parcels as compared to reference grasslands, may be due to a lower mineralization rate in forests, leading to a pH decrease.

Do restoration actions lead to the reestablishment of native calcareous grassland plant communities?

Species richness has been widely used as a biological indicator of restoration efficiency in calcareous grasslands. We observed increasing species richness with increasing restoration age, which was congruent with similar studies addressing calcareous grassland restoration (Zobel et al. 1996; Dzwonko & Loster 1998; Lindborg & Eriksson 2004b). However, species richness cannot be used as a unique measure of successful restoration. Restoration can only be considered a success when referenced against target ecosystems, and sites are effectively colonized by species characteristic of those ecosystems, that establish communities with similar structure. Based on indicators of composition and structure of plant communities, our results indicated a rapid evolution of restored grasslands communities towards reference grasslands characteristics, but still with significant differences.

Floristic similarity between reference and restored grasslands increased with the age of restoration. Observed plant species succession was similar to von Blanckenhagen and Poschlod (2005) description. Firstly, ruderal species developed, and then grassland species increased without however reaching cover exhibited in reference grasslands. Contrary to Dzwonko and Loster (Dzwonko & Loster 2007), who showed high shrub cover in ca. 10 years restored sites, we observed decreasing values of shrub cover in old restorations. This could be explained by the fact that, in our sites, shrubs were regularly mechanically cut in addition to grazing. The proportion of calcareous grassland species increased with the age of the restored patch. It suggests that a large proportion of native calcareous grassland species were able to colonize restored sites within a period of 15 years, which is encouraging for future restoration actions. However, we cannot exclude the possibility that this rapid restoration success was partly due to initial conditions of the oldest restored patches. Parcels restored first may have been less degraded at the onset of the restoration efforts. Therefore, some native grassland species may have been established prior to restoration, surviving in small calcareous grassland refugia. It has been shown by Schrautzer et al. (2009) that vegetation prior to restoration could influence restoration success. Nevertheless, our relevés revealed rather few grassland species in pre-restoration forest stands. Moreover most of them were found only in the site "Les Pairées", that was one of the oldest to be restored.

Despite a general tendency towards plant communities restoration, we noted two important differences between restored and references grasslands. First, we observed a lower evenness in old restored sites. This was likely due to the dominance of native invasive grasses (NIG, indicator of plant community structure) in these areas. Second, nineteen species present in reference grasslands did not recolonize restored sites (indicator of plant composition). A similar situation was described in Central Europe grasslands (Kiefer & Poschlod 1996). Fifteen of them are typical grassland species, which represent 24% of the grassland species found in our study. Among the missing species, ten are listed in the regional Red Data Book (Saintenoy-Simon et al. 2006): *Anthericum liliago*, *Carex humilis*, *Cuscuta epithymum*, *Globularia bisnagarica*, *Himanthoglossum hircinum*, *Ophrys apifera, Pulsatilla vulgaris*, *Trifolium montanum, Veronica prostrata* and *Valerianella dentata*. Except the last, all are typical calcareous grassland species. Furthermore, INDVAL analysis revealed that many other species remained better representated in reference grassland (Appendix 1), especially some species described as typical of ancient grasslands (Karlík & Poschlod 2009).

In restoration from arable fields, abiotic conditions were identified as a major constraint for plant communities restoration (Walker et al. 2004). In our study, differences in soil conditions were only revealed in pre-restoration forests and young restorations compared to reference grasslands. In older restorations, soil parameters were similar to these of reference grasslands. Therefore, soil parameters considered in this study were likely not the cause for the difference observed in species composition between old restorations and reference grasslands.

Plant community structure in oldest restored grasslands may be a constraint for the establishment of some typical grasslands species. High cover of graminoid species, such as *Brachypodium pinnatum*, and low bare ground availability observed on restored sites, particularly old sites, could lower availability of microsites for germination and seedling emergence of most sensitive grassland species (Kotorova & Leps 1999; Zobel et al. 2000). Higher cover of graminoid species in restored sites may also modify competition regimes as compared to reference grasslands. It is generally admitted that graminoid species control in restored calcareous grasslands requires higher grazing pressure than in ancient grasslands (Dutoit & Alard 1996b; Verbeke & Lejeune 1996; Delescaille 2000). The increased native invasive grass cover observed in restored patches can be due to insufficient grazing pressure. A possible explanation for such low grazing pressure is that most grazed sites include both restored and ancient grasslands, forming a unique operational grazing unit. Therefore managers often choose to under-graze the restored parcels rather than over-graze the ancient non-degraded grasslands. Additional control of native invasive grasses following restoration may be necessary to promote native calcareous grassland species recolonization. Management measures may include summer mowing of restored parcels in addition to grazing (Green 1980; Burger 1984; Bobbink & Willems 1991).

Dispersal limitation is an alternative, non-exclusive, hypothesis we did not test in the present study to explain that a set of typical species did not recolonize restored grasslands, even after 15 years. Habitat colonization by plant species may result from both spatial and temporal (seed bank) propagule dispersal. Temporal dispersal is limited in calcareous grasslands as most target species present a transient seed bank (Kalamees & Zobel 1997; Bisteau & Mahy 2005b). Moreover, most calcareous grassland species are characterized by low spatial autonomous dispersal capacities (Stampfli & Zeiter 1999). Nevertheless, restored parcels in our study were adjacent to reference grasslands, and most target species occurred within a few meters from restored site. In addition, migrating sheep flocks were used to graze restored sites among calcareous grassland patches in the study regions. Sheep are effective dispersal agents as they can bring large amounts of seeds in their fleece and dung (Fischer et al. 1996; Hellström et al. 2003; Couvreur et al. 2004a). Reintroduction of sheep grazing after clear-cutting is of chief importance for the reclamation of the calcareous grassland species pool (Poschlod et al. 1998). Conditions were therefore optimal for dispersal of grassland species into restored patches. Further research should examine if non-recolonizing species and effectively recolonizing species differ in their dispersal traits.

Conclusions and implication for restoration

Assessment of success of calcareous grassland restoration from forest stands, with soil and plant community indicators, point to a rather rapid success. Several indicators revealed that vascular plant communities tended to restore rather rapidly. However, differences still persist between 10-15 years reference grasslands and oldest restored areas. The latter had a lower evenness and were not colonized by a set of typical grassland species. This differences in plant composition persisted despite very similar soil conditions between pre-restoration forests and reference grasslands and full restoration of soil conditions in oldest restored grasslands. Higher native invasive grasses cover and lower bare ground availability in old restored parcels may be factors limiting the establishment of typical grassland species.

To ensure full success of restoration, management strategies, such as additional mowing, should aim at limiting native invasive grasses development and insure sufficient (similar to reference grasslands) bare ground availability in restored areas. Also, directed sowing of target species may help to counteract species difficulties at colonizing restored areas.

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Appendix

Appendix A: Occurrence of species in the five age classes. First value is the number of relevés where it occurred (total number of relevés is 80 in each class). Second value is the mean abundance [%] in relevés where it occurred. Only significant indicators were listed. Age class for which they were indicator is in bold and underlined.

Species	Pre-restoration forests	Restored grasslands			Reference grasslands	Ecological group
		2-4 years	5-8 years	10-15 years		
Anthericum liliago	0/-	0/-	0/-	0/-	**9/2.1**	Edge
Anthyllis vulneraria	0/-	2/1	4/2	2/0.5	**8/0.9**	Grassland
Arenaria sepyllifolia	0/-	**9/1**	0/-	0/-	2/0.8	Grassland
Avenula pubescens	1/0.5	1/1	5/1.2	**40/0.8**	16/0.8	Meadow
Brachypodium pinnatum	52/26.8	40/32	79/33.4	**80/36.1**	77/21.4	Grassland
Briza media	0/-	0/-	3/2	26/2.1	**45/3**	Grassland
Bromus erectus	11/19.2	0/-	22/31.8	19/15	**30/24.1**	Grassland
Campanula rotundifolia	1/0.5	9/1	27/0.5	**48/0.6**	36/0.6	Grassland
Carex caryophyllea	1/10	13/10.4	15/6.1	35/2.4	**44/2.6**	Grassland
Carex flacca	23/10.1	58/15.8	56/8.6	**78/13**	57/7.1	Grassland
Carex humilis	0/-	0/-	0/-	0/-	**10/12.3**	Edge
Carlina vulgaris	0/-	0/-	1/1	1/0.5	**4/0.8**	Grassland
Carpinus betulus	10/0.9	**12/1.7**	3/0.7	0/-	3/0.7	Shrub
Catapodium rigidum	0/-	**4/0.6**	0/-	0/-	0/-	Grassland
Centaurea scabiosa	0/-	2/0.5	6/0.9	24/1.6	**24/1.9**	Grassland
Centaurea jacea	0/-	2/0.5	4/1.5	38/2.7	**38/3**	Meadow
Cerastium fontanum	0/-	**6/1.3**	0/-	1/0.5	0/-	Meadow
Cerastium pumilum	0/-	0/-	3/1	0/-	**6/0.5**	Grassland
Cirsium acaule	0/-	0/-	3/6.7	9/1.3	**13/4.3**	Grassland
Cirsium arvense	0/-	**19/1.3**	1/1	0/-	0/-	Ruderal
Cirsium vulgare	0/-	**37/2.6**	3/0.8	0/-	0/-	Ruderal
Clematis vitalba	**13/13.2**	16/1.1	10/5.4	1/0.5	1/1	Shrub
Convolvulus arvensis	0/-	1/0.5	**4/5.8**	0/-	0/-	Ruderal
Cornus mas	**6/2**	0/-	0/-	0/-	0/-	Shrub
Cuscuta epithymum	0/-	0/-	0/-	0/-	**5/0.5**	Grassland
Cytisus scoparius	0/-	**9/0.7**	0/-	0/-	0/-	Shrub
Danthonia decumbens	0/-	1/0.5	0/-	3/0.7	**6/2.5**	Grassland
Daucus carota	0/-	**10/0.6**	0/-	0/-	5/0.8	Ruderal
Echium vulgare	0/-	2/1.5	**5/4.5**	0/-	0/-	Ruderal
Eupatorium cannabinum	0/-	**10/5.3**	4/3.1	0/-	0/-	Ruderal
Euphorbia amygdaloides	0/-	2/1.5	**9/0.7**	8/0.6	0/-	Forest
Euphorbia cyparissias	7/1.4	**28/2.7**	24/1	24/1.6	24/1.4	Grassland
Festuca lemanii	0/-	9/32.9	13/5.2	42/11.6	**68/5.2**	Grassland
Festuca rubra	**12/11**	0/-	4/1.6	0/-	2/37.5	Meadow
Fragaria vesca	17/4	**61/16.7**	43/3.9	2/2.5	10/1.3	Ruderal
Fragaria viridis	0/-	0/-	0/-	**32/1**	16/7.1	Grassland
Fraxinus exelsior	**10/2.1**	3/1	2/0.5	0/-	1/1	Shrub
Galium aparine	6/0.7	**19/0.6**	1/0.5	0/-	0/-	Ruderal
Galium mollugo	4/0.6	**13/4.2**	5/0.7	0/-	1/1	Ruderal
Galium pumilum	4/0.5	3/2.8	43/0.9	**66/1.3**	52/0.7	Grassland
Galium verum	0/-	1/0.5	12/3.5	22/1.1	**24/3.6**	Edge

Species	**Pre-restoration forests**	**Restored grasslands**			**Reference grasslands**	**Ecological group**
		2-4 years	5-8 years	10-15 years		
Genista tinctoria	0/-	1/0.5	8/0.8	**18/2.6**	13/2.8	Grassland
Genistella sagittalis	0/-	1/0.5	0/-	25/1.2	**22/2.3**	Grassland
Geranium robertianum	3/0.7	**5/1.4**	0/-	0/-	0/-	Forest
Geum urbanum	4/1.8	**11/0.8**	2/0.5	0/-	0/-	Ruderal
Hedera helix	**7/0.6**	3/0.5	0/-	1/0.5	0/-	Forest
Helianthemum nummularium	2/1.3	4/1	38/3.3	43/1.7	**54/8.1**	Grassland
Hieracium pilosella	0/-	5/1.2	17/4.6	0/-	**28/4.7**	Grassland
Hippocrepis comosa	0/-	14/2.2	11/0.6	1/5	**18/4**	Grassland
Hypericum perforatum	0/-	35/1.2	**43/0.7**	32/0.5	35/0.7	Ruderal
Inula conyzae	0/-	**14/1.6**	9/2	0/-	3/2.2	Edge
Knautia arvensis	0/-	1/1	4/1.8	17/1.3	**18/3.1**	Meadow
Koeleria macrantha	0/-	0/-	0/-	0/-	**24/1.6**	Grassland
Lapsana communis	1/1	**16/1.9**	0/-	0/-	0/-	Ruderal
Leontodon hispidus	0/-	1/0.5	10/1.8	**24/0.9**	16/4.2	Grassland
Leucanthemum vulgare	0/-	1/5	3/1.3	0/-	**5/3.2**	Meadow
Ligustrum vulgare	4/2	0/-	**9/5.3**	1/0.5	1/0.5	Shrub
Linum catharticum	0/-	4/0.5	31/0.8	**45/0.6**	29/0.5	Grassland
Lotus corniculatus	0/-	25/1.2	41/1.4	53/1.5	**60/1.6**	Grassland
Medicago lupulina	1/0.5	4/0.8	3/2.8	3/0.5	**12/0.7**	Grassland
Mercurialis perrenis	1/1	**4/7.3**	0/-	0/-	0/-	Forest
Mycelis muralis	3/1	**8/0.5**	4/0.6	0/-	0/-	Forest
Myosotis arvensis	1/0.5	**12/1.4**	5/1	0/-	3/1.5	Ruderal
Myosotis ramosissima	0/-	0/-	0/-	0/-	**5/0.5**	Grassland
Ononis repens	0/-	0/-	1/0.5	2/2	**5/1.1**	Grassland
Origanum vulgare	0/-	**15/4.1**	4/4.5	0/-	6/1.7	Edge
Picris hieracioides	0/-	**10/3.7**	5/3.2	0/-	0/-	Ruderal
Pimpinella saxifraga	0/-	4/0.5	**45/0.7**	39/0.6	34/1	Grassland
Pinus sylvestris	0/-	0/-	1/0.5	0/-	**5/0.9**	Shrub
Plantago lanceolata	0/-	10/1.4	0/-	11/0.6	**28/2**	Meadow
Plantago major	0/-	**7/1.4**	0/-	0/-	0/-	Meadow
Plantago media	0/-	0/-	0/-	0/-	**9/1.8**	Grassland
Plathantera chlorantha	2/0.8	0/-	**18/0.9**	10/0.7	4/1.4	Grassland
Poa nemoralis	**6/2.6**	2/10.3	0/-	0/-	0/-	Forest
Poa pratensis	0/-	22/6.8	**31/3.4**	24/3.8	15/8.4	Edge
Poa trivialis	4/3.1	**6/4.4**	2/0.5	0/-	0/-	Meadow
Polygala comosa	0/-	2/0.5	7/0.6	**16/0.6**	6/0.6	Grassland
Polygala vulgaris	0/-	11/1.5	**13/2.1**	0/-	10/0.8	Grassland
Potentilla neumanniana	1/0.5	12/0.9	27/0.7	33/0.9	**58/2.5**	Grassland
Primula veris	4/1.1	**10/3.6**	6/2	2/10.3	3/0.7	Grassland
Prunella laciniata	0/-	0/-	0/-	0/-	**4/1.1**	Grassland
Prunus avium	**3/0.8**	0/-	0/-	0/-	0/-	Shrub
Prunus spinosa	41/3.5	32/5.8	43/4.8	**61/4.3**	22/4.6	Shrub
Pulsatilla vulgaris	0/-	0/-	0/-	0/-	**5/4.1**	Grassland
Ranunculus bulbosus	0/-	0/-	0/-	0/-	**13/1.8**	Grassland

Species	**Pre-restoration forests**	**Restored grasslands**			**Reference grasslands**	**Ecological group**
		2-4 years	5-8 years	10-15 years		
Ranunculus repens	0/-	**6/0.7**	0/-	0/-	1/0.5	Meadow
Rosa arvensis	**8/9.2**	0/-	0/-	0/-	0/-	Shrub
Rosa canina	13/4.1	20/1.5	**29/1.1**	19/0.6	7/0.7	Shrub
Rubus idaeus	1/35	**6/2.6**	0/-	0/-	0/-	Shrub
Rubus sp.	38/28.1	**65/7.3**	18/8.1	8/0.9	0/-	Shrub
Sanguisorba minor	0/-	12/0.9	30/1.7	49/4	**68/5.4**	Grassland
Scabiosa columbaria	0/-	0/-	3/2.7	36/0.8	**36/2.6**	Grassland
Senecio jacobaea	0/-	**7/2**	1/3	0/-	2/0.8	Ruderal
Sesleria caerulea	9/10.2	4/5.5	**27/6.6**	17/4.5	24/9	Grassland
Solidago virgaurea	0/-	0/-	1/1	**20/1.7**	1/4	Ruderal
Sonchus arvensis	0/-	11/0.7	**19/0.9**	4/0.5	1/0.5	Ruderal
Sonchus asper	0/-	**14/0.5**	0/-	0/-	0/-	Ruderal
Stachys officinalis	3/0.7	0/-	2/1	**11/3.2**	8/4.5	Grassland
Stellaria media	**4/2.1**	0/-	0/-	0/-	0/-	Ruderal
Taraxacum sect. Ruderalia	18/2	**62/4.4**	37/2.8	25/1.1	16/1	Ruderal
Teucrium chamaedrys	3/3.8	2/0.8	43/3.8	44/1.2	**56/3.7**	Grassland
Thlaspi perfoliatum	0/-	0/-	0/-	0/-	**3/0.5**	Grassland
Thymus preacox	0/-	0/-	0/-	2/0.5	**9/1.3**	Grassland
Thymus pulegioides	0/-	4/21.5	5/2.4	4/1.6	**23/1.5**	Grassland
Torilis japonica	0/-	**7/1.1**	3/0.7	0/-	0/-	Ruderal
Tragopogon pratensis	0/-	0/-	**6/1.2**	0/-	2/0.5	Meadow
Trifolium dubium	0/-	0/-	**10/2**	0/-	0/-	Ruderal
Trifolium montanum	0/-	0/-	0/-	0/-	**6/1.8**	Grassland
Trifolium repens	0/-	**6/1.1**	2/2.8	0/-	1/0.5	Meadow
Trisetum flavencens	0/-	2/0.8	**19/5.1**	0/-	4/5.6	Meadow
Verbascum thapsus	0/-	**5/1**	0/-	0/-	0/-	Ruderal
Veronica arvensis	0/-	6/0.8	0/-	0/-	**8/0.5**	Ruderal
Veronica officinalis	0/-	**10/1.8**	1/1	0/-	0/-	Grassland
Vicia cracca	0/-	**5/0.6**	1/0.5	0/-	0/-	Ruderal
Vicia sativa	0/-	4/0.9	0/-	0/-	**10/0.7**	Ruderal
Vicia sepium	**2/0.8**	0/-	0/-	0/-	0/-	Edge
Viola hirta	12/1	62/2.5	71/1.8	**80/2.3**	55/2	Edge

CHAPITRE 5 : PLANT SPECIES EXTINCTION DEBT IN A TEMPERATE BIODIVERSITY HOTSPOT: COMMUNITY, SPECIES AND FUNCTIONAL TRAITS APPROACHES

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Abstract

Destruction and fragmentation of (semi-) natural habitats have been identified as the main cause of biodiversity loss worldwide. Plant species may exhibit a slow response to fragmentation, resulting in the formation of an extinction debt in fragmented plant communities. The detection of extinction debt is of prime importance in habitat conservation strategies. We applied two different approaches proposed in the literature to detect extinction debt in Southeast Belgium calcareous grasslands. The first method compared species richness between stable and fragmented habitat patches. The second explored correlations between current species richness and current and past landscape configurations using multiple regression analyses. We subsequently examined results generated by both methods. In addition, we proposed techniques to identify species that are the more likely to support extinction debt and the associated functional traits. We estimated the extinction debt of approximately 28% and 35 % of the total and specialist species richness, respectively. Similar results were obtained from both methods. We identified 15 threatened specialist species under the current landscape configuration. It is likely the habitat landscape configuration no longer supports the requirements of these species. We showed that non-clonal species are most threatened, as well as taxa that cannot persist in degraded habitats and form only sparsely distributed populations. We discussed our results in the light of other studies in similar habitats and the overall implications for habitat conservation.

Introduction

Changes in land use are responsible for destruction and fragmentation of natural and semi natural habitats and have been identified as the primary cause of biodiversity decline (Balmford et al. 2005). The consequences of fragmentation include suitable habitat loss for plant and animal species, progressive habitat isolation among remaining habitat patches and increased edge effects at the expense of interior habitats (Andrén 1994; Fahrig 2003). The rate of species extinction or colonization can be altered due to the effects of these influences on remnant habitat, and consequently affect species richness (Levins 1970; Hanski 1998). Species may almost immediately respond to fragmentation, but a time lag in the response (relaxation time) may also occur, creating an extinction debt: a situation in which following environmental changes, populations and metapopulations are still present in a habitat patch, even if the population/metapopulation is expected to go deterministically extinct (Tilman et al. 1994; Hanski & Ovaskainen 2002; Kuussaari et al. 2009). As a result, the current species patch occupancy patterns may overestimate the carrying capacity of the present landscape and lead to underestimating the current threat level of the species in fragmented habitats (Hanski & Ovaskainen 2002; Adriaens et al. 2006). Therefore, it has recently been recognized that when developing conservation programs based on spatial patterns of species richness, the history of the study landscape must be investigated (Poschlod et al. 2008; Karlík & Poschlod 2009) and detection of extinction debt is of prime importance (Cousins 2009).

Despite increased attention in recent years, the number of studies that directly test for extinction debt in fragmented plant communities remains limited (Lindborg & Eriksson 2004a; Berglund & Jonsson 2005; Adriaens et al. 2006; Helm et al. 2006; Piessens & Hermy 2006; Vellend et al. 2006; Cousins et al. 2007; Ellis & Coppins 2007; Lindborg 2007). Cousins (2009) notes that any consideration of the landscape history itself has rarely been considered in ecological studies at a landscape scale. Moreover, the outcomes of extinction debt studies are variable, even for similar habitats. For example, different authors have evaluated natural and semi-natural grasslands in different regions of Western Europe. Lindborg and Erikson (2004a) found evidence for an extinction debt in semi-natural grasslands of Sweden. Helm et al. (2006) reported that on the basis of results obtained from the Alvar grasslands in Estonia, extinction debt should be general in temperate grasslands. However, in respective studies in Belgium and Sweden, Adriaens et al. (2006) and Cousins et al. (2007) detected low evidence for extinction debt in calcareous semi-natural grasslands. Studies conducted at different sites are expected to generate different results due to species specific regional pools and/or differences in land use history and fragmentation processes (Adriaens et al. 2006; Cousins et al. 2007). However, in addition to ecological considerations, appropriate quantitative methodologies should be implemented, particularly for comparisons among studies and to interpret data and draw reliable conclusions (Tremlová & Münzbergová 2007). Furthermore, special attention should be given to the compatibility among different statistical approaches. Two main methods have been formulated to evaluate extinction debt: 1) a comparison of habitats highly impacted by fragmentation versus relatively stable habitats (Berglund & Jonsson 2005; Helm et al. 2006; Vellend et al. 2006); and 2) test for a relationship between present day species richness and historical landscape configuration (Lindborg & Eriksson 2004a; Adriaens et al. 2006; Helm et al. 2006; Piessens & Hermy 2006; Cousins et al. 2007; Ellis & Coppins 2007; Lindborg 2007). Tests to determine if different methodological approaches result in the same or similar conclusions in site-specific situations have not been reported (but see Helm et al. 2006).

Because response to fragmentation is species-dependant (Lindborg 2007; Mildén et al. 2007), identifying species at increased risk of extinction in the near future is vital for developing conservation programs. Managers must identify individual species that require urgent action due to the threat of extinction. The relationships between species traits, landscape attributes and species distribution has been a topic of many studies (e.g. Dupré & Ehrlén 2002; Kolb & Diekmann 2005; Tremlová & Münzbergová 2007; Römermann et al. 2008) and has recently been extended to relationships with past landscape structure. These studies have served to test if different functional groups possess different sensitivity to extinction debt (Adriaens et al. 2006; Lindborg 2007). Investigations of this nature are of heuristic value because they are able to generalize beyond particular case studies.

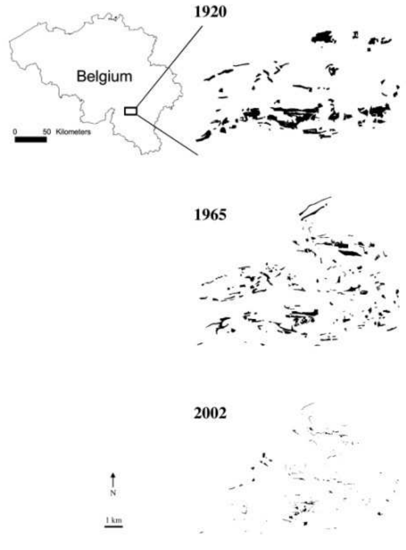
Among temperate plant communities, semi-natural calcareous grasslands rank as one of the most species-rich and are therefore considered biodiversity hotspots (Prendergast et al. 1993; WallisDeVries et al. 2002). These grassland ecosystems are well suited for studies of extinction debt because they (1) are delimited from other habitat types; (2) are highly fragmented; and (3) harbour species-rich plant communities with a high proportion of specialist species. These habitats suffered a high fragmentation process in the past due to abandonment of a traditional agro-pastoral system and resulting encroachment due to natural succession, re-forestation, and urbanization or transformation into arable lands (Poschlod & WallisDeVries 2002). A number of recent studies have examined the effects of landscape spatial structure on grassland species richness, and at least four studies have explicitly tested for extinction debt in fragmented semi-natural grasslands (Lindborg & Eriksson 2004a; Adriaens et al. 2006; Helm et al. 2006; Lindborg 2007). Hence, semi-natural grasslands are currently the best habitat for comparative studies.

In the present study, the following objectives were addressed: (i) test the occurrence of an extinction debt in South-East Belgium calcareous grasslands applying two different approaches proposed in the literature; (ii) compare the results generated by both methods; (iii) identify the species that are the more likely to support the extinction debt; and (iv) identify the traits that make the species more likely to support the extinction debt.

Methodology

Study site

The study site (approximately 25 km^2) is located in the Lesse Valley, Calestienne region of southern Belgium (50°05′ to 50°09′ N; 5°06′ to 5°15′ E; alt. 150 to 250 m) (Fig. 1). The Calestienne region is a 5 km-width belt of Devonian limestone hills and plateaus, SW-NE oriented The site matched the proposed Site of Community Importance (pSCI) BE35038, delineated as part of the Natura 2000 network according to the European Directive 92/43/EEC. Calcareous grasslands developed on Devonian limestone hills and plateaus under traditional agro-pastoral practices, primarily sheep herding. Due to abandonment of traditional agriculture in the 19th century and re-forestation, calcareous grassland ecosystems have dramatically declined in the region (Bisteau & Mahy 2005b; Adriaens et al. 2006). Calcareous semi-natural grasslands are presently located in well-delimited patches surrounded by forests, intensively managed meadows or arable lands. Calcareous grassland management has not occurred at the study site from the 1920s until the end of the 1990s. At this time, experimental grazing in enclosures was reintroduced in a few sites (Delescaille 1999; Delescaille 2002). In 2003 (concomitant with our floristic survey) a management regime of a migrating sheep flock was introduced to the study site. Therefore, the influence of the recent management was probably negligible in our study.



Date	Number of patches	Grasslands total area (ha)	Patch area (ha) Mean	Patch area (ha) SD	Patch connectivity Mean	Patch connectivity SD
1920	96	448	4.67	8.86	62.05	28.60
1965	195	353	1.81	3.25	35.29	10.69
2002	190	59	0.31	0.59	5.38	2.40

Fig.1: Evolution of calcareous grassland network and landscape parameters between 1920 and 2002.

Present and past landscape configuration

All calcareous grassland patches included in the study site were localized on the basis of recent aerial photographs and field surveys in 2002 and digitised in a GIS (ArcView 3.2, ESRI 2000) Extent of calcareous grasslands was reconstructed for 1920 and 1965 using historical detailed topographical maps (scale 1:20,000) and aerial photographs for 1965 (Belgium National Geographic Institute). The 1920 period roughly corresponds to the end of shepherding in Belgium and the beginning of the rapid decline of calcareous grasslands (Delescaille 2005). For each time period (1920, 1965 and 2002), area and connectivity of calcareous grasslands patches were calculated in reference to the corresponding landscape configuration. Patch area was directly derived from GIS data. Current patch connectivity was estimated by the IFM index derived from Hanski (1994), using edge-to-edge distances between all patches. This metric takes into account distances to (no multiplication factor was used for the distance) and the area of all possible source populations in the landscape (Moilanen & Nieminen 2002).

Vascular plant species were recorded in all calcareous grassland patches during spring and summer 2002 and 2003. All the patches were dominated by *Mesobromion* calcareous grasslands, which is the main calcareous grasslands community in the study site (Piqueray et al. 2007). Specialist species of Mesobromion grasslands were identified on the basis of recent community classifications (Royer 1991; Butaye et al. 2005b; Piqueray et al. 2007). Nomenclature followed Lambinon et al. (2004).

Data analysis

COMMUNITY EXTINCTION DEBT

Extinction debt was estimated with two different methods, in relationship to 1920 and 1965 landscape structure independently, for total and specialist species richness. Only current grassland patches that occurred on all historical documents were retained for analysis. The first method was based on a comparison between "low loss" grasslands (less than 80% loss in area since the considered historical date) and "high loss" grasslands (more than 80% loss in area since the considered historical date). The limit of 80% loss was chosen to make our analysis comparable to other studies (Helm et al. 2006). High loss and low loss patches were determined independently in reference to 1920 and 1965. For each historical date, only high loss and low loss patches that covered approximately the same range of surfaces were retained. To estimate the magnitude of the extinction debt, we compared two linear regression models, built independently for high loss and low loss patches, where current species richness was explained by current patch metrics. The comparisons were made independently for 1920 and 1965. We built both models with the following requirements: the same independent variable (area or connectivity), identical transformations, the absence of non-normality or heteroscedasticity of residuals, and a

P-value for the regression test as low as possible. The independent variables to include in the models (area or connectivity) were selected based on regression's coefficient of species richness on patch metrics. Because we used only current patch metrics as independent variables, it is likely this method provided an underestimate in the possible extinction debt in low loss patches (Helm et al. 2006). Different transformations on dependent and independent variables were tested in order to improve normality and homoscedasticity of residuals using Minitab macro (Palm 2002). Non-parallelism was tested between regression lines of the two models (General Linear Model, GLM). Non-parallelism would indicate a significant interaction between the independent variable (patch metrics) and the low loss/high loss factor, i.e. in such a case, species richness would not react in the same way to patch metrics for low loss and high loss patches. Perfect parallelism between both straight regression lines was forced by removing the interaction effect. We tested for an extinction debt by testing for the significance of the difference between the y-intercepts of the regression lines of the two models (see Draper & Smith 1998 for more details). This difference provided a measurement (number of species) of the mean extinction debt over all high loss patches in reference to low loss patches.

The second method was based on the more widespread approach of explaining current species richness by present and past patch metrics (independent variables) with multiple regression analysis. A preliminary analysis revealed the prominent effect of current patch area on species richness. In this context, we specifically searched for past-related metrics improving the model that explained species richness with current patch area. In addition to current and past area/connectivity, the proportion of patch area loss between each historical date (1920, 1965) and present was considered as an additional independent variable. Included in the same model than current patch area, this variable tests if, for a given current area, patches that loss more area in the past are currently richer, and therefore exhibit an extinction debt. We used the best-subset method to select independent variables that, in addition to current patch area, significantly influenced current species richness. A measurement of the extinction debt was computed as the mean species surplus due to past-related metrics. Best subset models were built for 1920 and 1965 independently. All analyses were performed using Minitab 15 (Minitab, Inc.)

SPECIES SENSITIVITY TO EXTINCTION DEBT AND TRAITS INFLUENCE

To identify specialist species that contribute most probably to the extinction debt, we tested for a significant difference between the current occurrence (% of patch colonized) of each specialist species in patches that were historically larger than the largest current patch (4,061 ha) and current occurrence in patches that were historically smaller than this size, using an unilateral Fisher's Exact Test (FET) (Siegel 1956). Species were considered sensitive to extinction debt when the *P*-value of the test was less than 0.05.

With this method, we made the hypothesis that species significantly associated with historically large patches (larger than currently) do not meet anymore their habitat requirements in the current landscapes and thus support an extinction debt. Only specialist species were tested due to their high conservation value and low ability to establish in other habitat types. In addition, for use in further analyses, we computed the exact power of FET related to the highest possible association with historical large patches, given the species occurrence and the number of large and small patches for a given date. This approach resulted in an estimate of the ability of FET to detect the sensitivity of a species to extinction debt with a given occurrence in the landscape. It was named *Safeness* and was computed using a Javascript applet (Schoenfeld 2007).

We subsequently evaluated traits to explain plant association to historically large patches (former area > 4.061 ha). We considered a set of characters with different implications for survival in fragmented habitats i.e. dispersal, establishment and persistence (see Table 1 for details of traits) (Weiher et al. 1999; Poschlod et al. 2000). Trait values were derived from the BIOPOP database (Poschlod et al. 2003; Jackel et al. 2006), with the exception of persistence in degraded habitats that were taken from Poschlod et al. (1998)(Table1). We assumed the following: a species can exhibit several dispersal types (e.g. an anemorochorous species can also be zoochorous); and a species that can persist in a late habitat successional stage is also able to persist in the earlier stages (e.g. a species that can persist in forest can also persist in pre-forest and in young fallows). The dispersal type and persistence in degraded habitat traits were therefore defined as “non-exclusive” (Table 1). The traits were broken down into as many nominal traits as were available values. The trait “only present in grazed grasslands” was eliminated because we interpreted it as the exact inverse of “persistent in young fallows”.

Table 1: Trait selection for the species-approached extinction debt analysis (BIOPOP - see Jackel et al. 2006)

Trait	Values	Variable type	Data sources
Branching species	1:True, 2:False	Nominal	BIOPOP
Canopy height	Value [m]	Numeric	BIOPOP
Clonal growth	1:True, 2:False	Nominal	BIOPOP
Dispersal type	1:Epi- or endo-zoochory, 2:Meteorochory, 3:Unspecialized	Nominal, non-exclusive	BIOPOP
Dormancy	1:True, 2:False	Nominal	BIOPOP
Flowering window	Value [months]	Numeric	BIOPOP
Evergreen	1:True, 2:False	Nominal	BIOPOP
Autofertility	1:True, 2:False	Nominal	BIOPOP
Strict autogamy	1:True, 2:False	Nominal	BIOPOP
Presence of mycorhyze	1:True, 2:False	Nominal	BIOPOP
Nitrogen fixing ability	1:True, 2:False	Nominal	BIOPOP
Eaten by animals	1:True, 2:False	Nominal	BIOPOP
Releasing height	Value [m]	Numeric	BIOPOP
Seed bank	1:transient, 2:short-term persistent, 3:long-term persistent	Nominal	BIOPOP
Seed mass	Value [mg]	Numeric	BIOPOP
Seedling emergence	1:spring, 2:autumn, 3:all year	Nominal	BIOPOP
SLA	Value [mm^2/mg]	Numeric	BIOPOP
Persistence in degraded habitats	1:Occurring only in grazed sites, 2:Still occurring in young fallow sites (recently abandoned), 3:Still occuring in older fallow sites (pre-forest stages), 4: still occurring in very old fallow sites (forest stages)	Ordinal, non-exclusive	Poschlod et al., 1998
Life form	1:Chamaephyte, 2:Hemicryptophyte, 3:Therophyte; 4:Geophyte	Nominal	BIOPOP
Leaf disposition	1:Rosette, 2:Semi-rosette, 3:Regularly distributed	Nominal	BIOPOP
Alpha-density	Value [/]	Numeric	
Beta-density	Value [/]	Numeric	

Population size has been considered of primary importance for survivorship in a fragmented landscape (Henle et al. 2004; Leimu et al. 2006) as well as a species capacity to form large populations in a given habitat area. In order to characterize these attributes, we computed two density indices. From the dataset presented in Piqueray et al. (2007), we extracted 227 1-m^2 vegetation relevés derived from 46 patches in the study site. We computed the alpha-density of a species as the mean cover in the 1-m^2 relevés where the species occurred and the beta-density as the mean proportion of occupied 1-m^2 relevés in the sites where the species occurred (Table 1).

For each species we measured its association with historically large patches (ALP) = $p_L/p_L+ p_S$, where p_L was the occurrence of the species in patches historically larger than the currently largest patch and p_S was the current occurrence of the species in patches historically smaller than the currently largest patch. ALP was computed with 1920 and 1965 as historical dates and only the highest value was retained. ALP values ranged from 0 to 1 for species that were found only in historically small and historically large patches, respectively. We tested for the relationship between ALP and plant traits by linear regression (numeric traits) or ANOVA (nominal/ordinal traits). ALP was arcsin-transformed to improve its normality. *Safeness* was introduced as weight in the analyses to down weight rare species (for which ALP is very sensitive to an accidental presence) and very abundant species (that are indifferent by definition) and increase the weight of intermediate occurrence species. We tested independence among traits that influence significantly ALP with chi-square tests (nominal vs. nominal traits), ANOVA (numeric vs. nominal traits) or regression analysis (numeric vs. numeric traits).

Results

Landscape configuration and species richness

The current landscape supported 190 calcareous grassland patches that clearly originated from a fragmentation process (Fig. 1). Total habitat area decreased by 87% within c.80 years with a higher rate of decrease during recent decades (21% loss from 1920 to 1964; 83% loss from 1964 to 2002). Mean patch area collapsed from 4.67 ha (range: 0.012- 45.27) in 1920 to a current 0.26 ha (range: 0.001-4.061); a decrease of 93 %. From 1920 to 1964, the patch area decrease was the result of the fragmentation of large patches, in addition to a total loss of habitat, as indicated by a doubling in the number of patches. In contrast, between 1964 and 2002, the number of patches remained stable but total area was reduced. Mean patches connectivity decreased from 62.05 in 1920 to 5.36 in 2002. A total of 492 species were identified with 46 grassland specialists.

Community extinction debt based on 'low loss' – 'high loss' method

Forty-four patches (28 high loss and 16 low loss) were conserved for analysis in the 1965 models and 22 (14 high loss and 8 low loss) for the 1920 models. The corresponding ranges of area were respectively 0.048 – 2.794 ha and 0.222 – 2.162 ha. In relation to 1920 average area loss in high loss and low loss patches was respectively 96% and 48%; in relation to 1965, it was respectively 92% and 44%. Simple linear regression between current species richness and current patch metric for each set of patches (low loss and high loss in reference to each historical dates) indicated a predominant effect of current area on species richness compared with connectivity (Table 2). Only in one case was the connectivity significantly related to species richness: for the 1920 high loss patches (r = 0.54; P = 0.048) but the relationship (r-value) was comparable to area (r = 0.46; P = 0.098). Hence, area was the independent variable selected to build the regression models. In all cases, parallelism between the regression models testing the influence of area on species richness for "low loss" and "high loss" patches was accepted.

Table 2: Coefficient of linear regression between current species richness (total and specialist species richness) and current patch spatial metrics (area and connectivity) for patches that have retained > 20% of their area (low loss) and patches that have retained < 20% of area (high loss) in reference to two historical dates (1920, 1965) : * : 0.05>P>0.01; ** : 0.01>P>0.001; *** : P<0.001). n is the number of patches.

Species richness	**Patch metrics**	**1920**		**1965**	
		high loss (n=14)	low loss (n=8)	high loss (n=28)	low loss (n=16)
Total	area	0.53	0.93***	0.64***	0.65**
	connectivity	0.49	-0.54	0.08	0.42
Specialist	area	0.46	0.87**	0.57**	0.63**
	connectivity	0.54*	0.03	0.37	0.40

A significant extinction debt in high loss patches was demonstrated for total species richness and for specialist species whatever the historical dates (1920, 1965). *P*-values of the regression models indicated a more important extinction debt for specialist species. Mean extinction debt per high loss patch for specialist species was 6.68 and 6.06 species, respectively for 1920 and 1965, corresponding to 34.3% and 35.1% of the mean specialist species richness per patch (Table 3, Fig. 2). Mean extinction debt per high loss patch for total species richness was 26.5 species with 1920 as reference date (27.3% of the total species richness). A logarithmic transformation of species richness and a square root transformation of area were performed for the 1965 total species richness model. Extinction debt was therefore estimated as a percentage of species present in the patch : 27.2% corresponding to a mean extinction debt of approximately 21 species.

Table 3: Extinction debt evaluation for 1920 and 1965, using a method based on the comparisons of regression models between area and current species richness for patches that have retained > 20% of their area (low loss) and patches that have retained < 20% of area (high loss) in reference to two historical dates (1920, 1965). For eah model : n is the number of patches, R^2 is the model determination coefficient with the associated p-value in brackets. The parallelism test provides the p-value generated for the test between both straight regression lines. The extinction is measured as the difference between y-intercepts of the two models. Values in brackets are the confidence interval at a 95% level and P is the p-value associated with the analysis for the difference in intercepts.

Species richness	**Date**	**Loss level**	**R^2**	**Parallelism test**	**Extinction debt**
Total	1920	low loss (n=8)	86.3% (*P*=0.001)	*P*=0.341	26.51 species (2.72-50.30) *P*=0.032
		high loss (n=14)	28.1% (*P*=0.051)		
	1965	low loss (n=16)	51.9% (*P*=0.002)	*P*=0.914	27.2% (7.5-46.9) *P*=0.006
		high loss (n=28)	50.0% (*P*<0.001)		
Specialist	1920	low loss (n=8)	74.8% (*P*=0.006)	*P*=0.218	6.68 species (1.74-11.62) *P*=0.011
		high loss (n=14)	21.1% (*P*=0.098)		
	1965	low loss (n=16)	40.0% (*P*=0.009)	*P*=0.502	6.06 species (2.43-9.69) *P*=0.002
		high loss (n=28)	31.6% (P=0.002)		

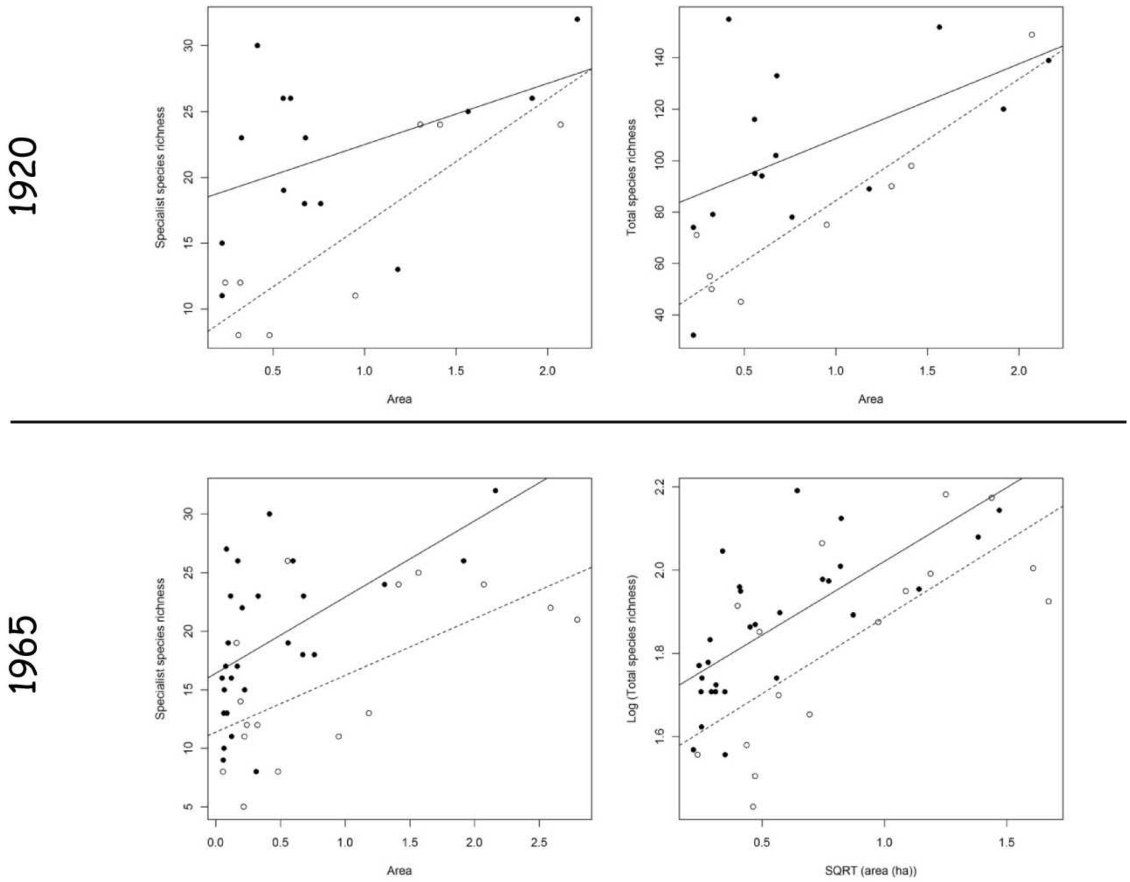


Figure 2: Relationship between total and specialist species richness and patch area for "high loss patches" (full points, plain lines) and "low loss patches" (empty points, dotted lines), for 1920 and 1965.

Community extinction debt based on multiple regression with current and patch spatial metrics

Multiple regressions between species richness (total and specialist) and patch current / historical metrics were performed on the 54 patches present on all cartographic data (Table 4). No significantly debt was found for total species richness in reference to 1920, as no past-related metrics was able to improve the model built with current patch area. This was different from the results found with the previous method. In addition to current area, past-related patch metrics explained a significant part of current species richness in the three other cases (total species richness in reference to 1965 and specialist species richness in 1920 and 1965) suggesting the existence of an extinction debt. Current specialist species richness was influenced by 1920 patch connectivity and area (estimated extinction debt: 5.99 species). Both current total and specialist species richness were influenced by area loss (i.e. the area percentage lost by a patch between the historical date and present) since 1965. For a given current area, species richness increased with the percentage of area loss since this period. No other past-related patch metrics were included in the regression models for specialist species in reference to 1965. Extinction debts were 23.27 species and 7.06 species for total species richness and specialist species richness respectively.

Table 4: Extinction debt evaluation for 1920 and 1965, using a multiple regression of current species richness to current and past metrics (area and connectivity) and the area loss. Global fit provided the R^2 and p-value of the multiple regression analysis. Partial P is the effect of each independent variable. Extinction debt is the partial contribution of past linked variables (past metrics and area loss in bold). Values in brackets are the 95% confidence intervals.

		Model			
Species group	**Date**	Global P	Independant variables	Partial P	**Extinction debt**
All species	1920	$R^2=0.51$, $P<0.001$	Current area	<0.001	No extinction debt
	1965	$R^2=0.55$, $P<0.001$	Current area **Area loss**	<0.001 **0.048**	23.27species (0.23-46.3)
Specialist species	1920	$R^2=0.48$, $P<0.001$	Current area **Past connectivity** **Past area**	<0.001 **0.020** **0.030**	5.99 species (0.82-11.2)
	1965	$R^2=0.54$, $P<0.001$	Current area **Area loss** Current connectivity	<0.001 **0.003** 0.003	7.06 species (2.48-11.64)

Species sensitivity to extinction debt and traits influence

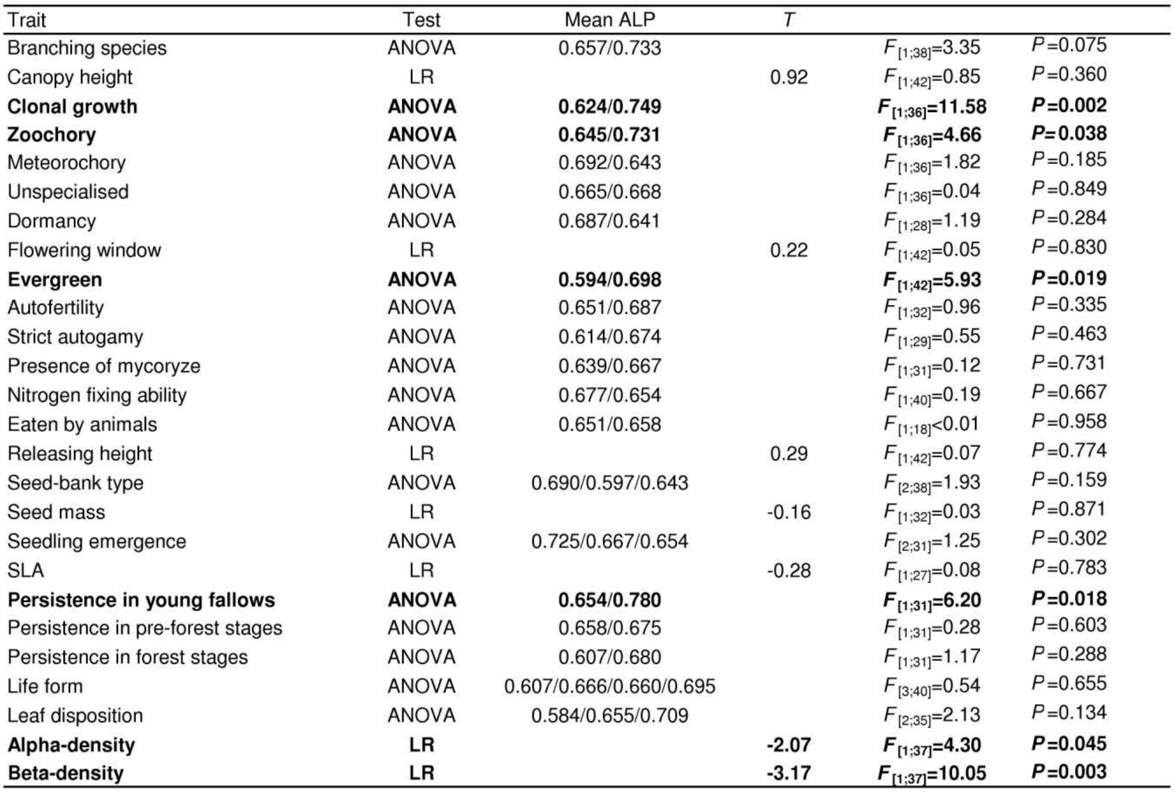
Sensitivity to extinction debt were measured on the 54 patches present on all cartographic data. From the 46 specialist species, 44 were present in at least one of those patches. Following our approach, 15 species sensitive to extinction debt (at least in reference to one historical date) were identified (Table 5). Eight and nine of them presented a significantly higher current occurrence in patches larger than 4.061 ha, in 1920 and 1965 respectively. Two of them (*Centaurea jacea* and *Linum catharticum*) presented a significantly higher current occurrence in patches larger than 4.061 ha for both time periods. A few other species (e.g. *Carlina vulgaris*, *Gentianella germanica*, *Centaurium erythrea*, and *Polygala comosa*) also exhibited a strong association to historically large grasslands, although the probability this correlation was due to chance was greater than 0.05 (Appendix 1).

Table 5: List of specialist species significantly sensitive to extinction debt (at least in reference to one historical period) and their occurrence (% presence) in patches that were larger or smaller than the currently largest patch (large patch and small patch, respectively) at two historical dates (1920, 1965). n is the number of patches.P is the p-value associated with the Fisher Exact Test (FET) testing for a difference in occurrence between historically large and small patches. Species were considered sensitive to extinction debt at P<0.05.

Species	**1920**			**1965**		
	Occurrence in small patch (n=11)	Occurrence in large patch (n=43)	(FET) P	Occurrence in small patch (n=31)	Occurrence in large patch (n=23)	(FET) P
Anthyllis vulneraria	0.27	0.40	0.351	**0.23**	**0.57**	**0.012**
Bromus erectus	**0.64**	**0.93**	**0.025**	0.84	0.91	0.353
Carex flacca	**0.36**	**0.79**	**0.010**	0.65	0.78	0.215
Centaurea jacea	**0.09**	**0.60**	**0.003**	**0.35**	**0.70**	**0.013**
Centaurea scabiosa	0.36	0.63	0.108	**0.45**	**0.74**	**0.032**
Galium pumilum	0.36	0.53	0.250	**0.39**	**0.65**	**0.048**
Gentianella ciliata	0.00	0.14	0.236	**0.03**	**0.22**	**0.044**
Gymnadenia conopsea	**0.00**	**0.33**	**0.024**	0.19	0.35	0.167
Linum catharticum	**0.27**	**0.67**	**0.019**	**0.45**	**0.78**	**0.014**
Pimpinella saxifraga	**0.27**	**0.88**	**<0.001**	0.71	0.83	0.254
Primula veris	0.45	0.60	0.287	**0.42**	**0.78**	**0.008**
Prunella laciniata	0.18	0.37	0.205	**0.23**	**0.48**	**0.049**
Ranunculus bulbosus	**0.18**	**0.53**	**0.037**	0.32	0.65	0.016
Sanguisorba minor	**0.82**	**1.00**	**0.038**	0.97	0.96	0.823
Trifolium montanum	0.09	0.23	0.280	**0.10**	**0.35**	**0.027**

The magnitude of ALP (a measure of the association with historically large patches) was significantly associated with six of the 26 traits examined : clonality, zoochory, evergreeness, high alpha and beta densities, persistence in young fallow (Table 6). Species that exhibited at least one those traits were less associated to historical large patches (lower ALP) than species not possessing those traits, and, hence, less sensitive to extinction debt due to patch area reduction following habitat fragmentation. Associations between these six traits are shown in Table 7. Evergreeness was shown to the more independent traits, and was only correlated to persistence in young fallows ($\chi^{2}_{[1]}$=4.30; *P*=0.038). Beat-density was associated to all other traits, except evergreeness.

Table 6: Relationships between traits and sensitivity to extinction debt (measured as the highest ALP - Association to historically Large Patches- detected for each species). Significant differences fro mean ALP were tested with Anovas for nominal and ordinal traits. Linear regressions (LR) between ALP and traits values were used for numeric traits. Mean ALP are estimated for each classes of non-numeric traits (see Table 1 for order). T are the T-values for traits from linear regressions. Traits significantly associated to ALP are in bold.



Trait	Test	Mean ALP	*T*		
Branching species	ANOVA	0.657/0.733		$F_{[1;38]}$=3.35	P=0.075
Canopy height	LR		0.92	$F_{[1;42]}$=0.85	P=0.360
Clonal growth	**ANOVA**	**0.624/0.749**		$\boldsymbol{F_{[1;36]}}$**=11.58**	$\boldsymbol{P}$**=0.002**
Zoochory	**ANOVA**	**0.645/0.731**		$\boldsymbol{F_{[1;36]}}$**=4.66**	$\boldsymbol{P}$**=0.038**
Meteorochory	ANOVA	0.692/0.643		$F_{[1;36]}$=1.82	P=0.185
Unspecialised	ANOVA	0.665/0.668		$F_{[1;36]}$=0.04	P=0.849
Dormancy	ANOVA	0.687/0.641		$F_{[1;28]}$=1.19	P=0.284
Flowering window	LR		0.22	$F_{[1;42]}$=0.05	P=0.830
Evergreen	**ANOVA**	**0.594/0.698**		$\boldsymbol{F_{[1;42]}}$**=5.93**	$\boldsymbol{P}$**=0.019**
Autofertility	ANOVA	0.651/0.687		$F_{[1;32]}$=0.96	P=0.335
Strict autogamy	ANOVA	0.614/0.674		$F_{[1;29]}$=0.55	P=0.463
Presence of mycoryze	ANOVA	0.639/0.667		$F_{[1;31]}$=0.12	P=0.731
Nitrogen fixing ability	ANOVA	0.677/0.654		$F_{[1;40]}$=0.19	P=0.667
Eaten by animals	ANOVA	0.651/0.658		$F_{[1;18]}$<0.01	P=0.958
Releasing height	LR		0.29	$F_{[1;42]}$=0.07	P=0.774
Seed-bank type	ANOVA	0.690/0.597/0.643		$F_{[2;38]}$=1.93	P=0.159
Seed mass	LR		-0.16	$F_{[1;32]}$=0.03	P=0.871
Seedling emergence	ANOVA	0.725/0.667/0.654		$F_{[2;31]}$=1.25	P=0.302
SLA	LR		-0.28	$F_{[1;27]}$=0.08	P=0.783
Persistence in young fallows	**ANOVA**	**0.654/0.780**		$\boldsymbol{F_{[1;31]}}$**=6.20**	$\boldsymbol{P}$**=0.018**
Persistence in pre-forest stages	ANOVA	0.658/0.675		$F_{[1;31]}$=0.28	P=0.603
Persistence in forest stages	ANOVA	0.607/0.680		$F_{[1;31]}$=1.17	P=0.288
Life form	ANOVA	0.607/0.666/0.660/0.695		$F_{[3;40]}$=0.54	P=0.655
Leaf disposition	ANOVA	0.584/0.655/0.709		$F_{[2;35]}$=2.13	P=0.134
Alpha-density	**LR**		**-2.07**	$\boldsymbol{F_{[1;37]}}$**=4.30**	$\boldsymbol{P}$**=0.045**
Beta-density	**LR**		**-3.17**	$\boldsymbol{F_{[1;37]}}$**=10.05**	$\boldsymbol{P}$**=0.003**

Table 7: Relationship between traits significantly associated to species sensitivity to extinction debt (measured with ALP). Relationship between two nominal traits, one nominal and one numeric trait and two numeric traits was respectively tested by chi-square tests (χ^2[degree of freedom]), Anova (F[degrees of freedom]) and regression (Pearson's r[n=number of observations]). P is the p-value for each test, significant values are in bold. (+) for chi-square tests indicates that the presence of one trait significantly promoted the presence of the other trait. (+) for Anova indicates that the presence of the nominal trait significantly increased the value of the numeric trait.

	Clonal growth	Zoochory	Evergreen	Persistence in young fallows	Alpha-density
Zoochory	$\chi^2_{[1]}$=4.27 **P=0.039 (+)**				
Evergreen	$\chi^2_{[1]}$=2.188 P=0.139	$\chi^2_{[1]}$=0.006 P=0.938			
Persistence in young fallows	$\chi^2_{[1]}$=2.167 P=0.141	$\chi^2_{[1]}$=0.204 P=0.652	$\chi^2_{[1]}$=4.30 **P=0.038 (+)**		
Alpha-density	$F_{[1;33]}$=2.55 P=0.120	$F_{[1;34]}$=2.00 P=0.167	$F_{[1;37]}$=0.24 P=0.626	$F_{[1;30]}$=2.38 **P=0.133**	
Beta-density	$F_{[1;33]}$=14.00 **P=0.001 (+)**	$F_{[1;34]}$=15.50 **P<0.001 (+)**	$F_{[1;37]}$=0.05 P=0.832	$F_{[1;30]}$=8.32 **P=0.007 (+)**	r=0.509 **P=0.001**

Discussion

Extinction debt in South-East Belgium calcareous grasslands

The probability that a plant community suffers from an extinction debt depends on the time since the habitat was altered, the extent of habitat fragmentation and the nature of the alteration (Kuussaari et al. 2009). It is hypothesized that 1) extinction debt is most likely to exist in landscape where habitat fragmentation occurred recently, and, 2) extinction debt would be paid off faster in landscapes with large reduction of habitat (Hanski & Ovaskainen 2002; Kuussaari et al. 2009). Some support was provided to the last hypothesis by extinction debt studies on semi-natural grasslands in Europe. In landscapes with <10% of grassland remaining, no evidence of extinction debt was detected, in contrast to less fragmented grasslands (Adriaens et al. 2006; Cousins et al. 2007; Cousins 2009).

In contrasts to those previous studies, our results pointed to an elevated extinction debt in a landscape of highly fragmented calcareous grasslands. The extinction debt was estimated at approximately 28% of the total species richness, but was higher for specialist species (34 - 41% based on method and reference date). We reported an important decrease of calcareous grasslands with a loss of 87% of grasslands area in c.80 years. Mean patch area and mean patch connectivity also strongly decreased within this time lag. This spatio-temporal dynamics was very similar to the situation described by Adriaens et al. (2006) for a neighbouring landscape in the same region, where no extinction debt was reported for calcareous grasslands. One reason for differences among the two studies may come from the timing of fragmentation. An important grassland reduction occurred during the last forty years in our study; with a reduction of 83% area as compared to 21% for the 1920-1965 period. With such a recent fragmentation process, extinction det may not be already paid off even in very small isolated remaining grasslands.

A second reason why extinction debt estimations may differ among landscapes with similar history may come from differences in methodology. If an empirical study fails to detect extinction debt, it is important to assess whether adequate methods have been used (Kuussaari et al. 2009). In this study, we used the two more widespread methods proposed to date to test for an extinction debt in communities of fragmented habitat (Kuussaari et al. 2009). Each method holds its own unique challenges. Therefore, it was relevant to the current study that the approaches resulted in the same conclusions: calcareous grasslands in the landscape suffered from an extinction debt. One difference was detected between the two approaches. The regression model did not indicate an extinction debt for generalist species in relation to 1920, while a significant extinction debt (on average 26.51 species) was found under the high loss/low loss model. However, this extinction debt harboured the lowest probability level (P=0.032). Also, the magnitude of the extinction debt by the two methods was very similar.

Although we showed that current area exhibited the main effect on species richness, multiple regressions showed that the variation in species richness that remained unexplained in part was due to patch history, indicating a possible extinction debt in our study site. However, we concluded an extinction debt from the multiple regression approach mainly because we include area loss as an independent variable, in addition to past and current landscape structure. For a given current area, those grasslands that have lost a higher proportion of area in the last 40 years host a higher species richness, which may be interpreted as a legacy of their past spatial structure. If we did not include area loss in the regression models, an extinction debt would not have been detected for 1965, indicating a discrepancy among methods. This is an important point because studies that have reported the absence of extinction debt (Adriaens et al. 2006; Cousins et al. 2007) in highly fragmented grasslands have done so based on the lack of a correlation with past landscape structure and have not taken into account individual patch dynamics.

It was necessary to make the current cartographic data comparable to the historic data. This required eliminating the numerous very small patches (< 0.048 ha) that exist in the current landscape. It is likely that comparable grasslands were present in the past, but historical cartographic documents are not adequate to detect habitat details at this level. Consequently, extinction debt could not be calculated in the very small patches using our present methodologies.

Species sensitivity to extinction debt

Identifying species that have not yet paid extinction debt is important as it may not be a random sample of the habitat species pool. The probability of an extinction debt can depend on the life-history traits of a particular species. However, information is scarce on the influence of species traits on extinction debt (Kuussaari et al. 2009).

We developed a method that mirrors community extinction debt estimations to detect species for which present pattern of occurrence in the landscape is influenced by past configuration of habitats. Specifically we tested whether the current occurrence of specialist species in calcareous grassland patches was significantly associated to grasslands that have been historically larger than the current largest patch. This was the case for 15 specialist species. Among them, *Trifolium montanum* is critically endangered (CR), *Gentianella ciliata* is endangered (EN) and *Gymandenia conopsea* is vulnerable (VU) at a regional scale following IUCN criteria (Saintenoy-Simon et al. 2006). Also, in all case where data were available, those species have been shown to have reduced fitness in small populations. Schleuning et al. (2009) showed that in *Trifolium montanum* not only isolation and population size decreased seed production per individual but also population density. It has also been shown that *Gentianella ciliata* exhibits a reduction in reproductive performance in small populations (Kéry & Matthies 2004) and small populations of this species are more likely to go extinct than large populations. *Primula veris* exhibited lower fitness in small populations (Kéry et al. 2000; Brys et al. 2003). *Centaurea jacea* showed reduced germination rates as well as a smaller survival rate of seedlings in small populations (Soons & Heil 2002).

It is worth noting that several rare species showed high ALP (= association with large patch) values for 1920, and the species rarity rejected the hypothesis that the distribution was due to chance. In other respects, some common species (e.g. *Bromus erectus* and *Sanguisorba minor*) were significantly associated to former large patches although their ALP values were close to 0.5, suggesting indifference (Appendix 1). The high number of observations for these common species may result in statistical significance, which does not always indicate a form of ecological significance.

Our results suggested that species forming scarce populations need larger habitat patches. It is likely that such species are not able to maintain relatively large populations in small habitat patches and are more sensitive to fragmentation. Persistence traits (clonality, evergreeness, persistence in young fallows and density) were shown to make the species less dependent on large habitat patches. These traits were not independent: clonal species and species persistent in young fallows exhibited the highest beta-density. Clonal species are more likely to survive in small habitat patches and are often long-lived and less prone to extinction (Fischer & Stöcklin 1997; Poschlod et al. 2000). Other traits that reduce the risk of extinction, such as seed banks and long-term seed viability (Stöcklin & Fischer 1999; Fréville et al. 2007) could have similar effects, which were not the case in our study. This indicates that species with transient seed bank are not more likely to support the extinction debt in our study landscape. Zoochory also appeared to reduce dependence to large habitat patches. In a large scale study, Römermann et al. (2008) demonstrated that epizoochorous species were underrepresented among declining species. However, because grazing was abandoned almost one century ago in our grasslands, an explanation for this effect is not clear. It is probably an artefact of the link between persistence and dispersal traits. We showed that clonal species are mainly zoochorous; this does not exclude an effect of dispersal ability itself, however it makes difficult to differentiate from clonality. We can therefore conclude that non-clonal, deciduous species that form sparse populations and that are not able to persist in degraded habitats are the most likely to pay an extinction debt. Their current occurrence could just be an heritage of the past landscape configuration.

Implications for conservation

Extinction debt had the greatest affect on remnant areas of a fragmented habitat. However, we argue that the establishment of an extinction debt in a given habitat landscape should be considered a benefit. Most habitats respond slow to fragmentation. Therefore, the identification of an extinction debt enables managers to begin restoration of damaged habitat with adequate time to implement restoration programs. The lag time inherent in an extinction debt is variable and difficult to predict. Vellend et al. (2006) argued that an extinction debt in forest habitat could persist for more than a century. A forest is hardly comparable to that of a grassland, but it is worth noting that Pärtel et al. (1998) and Zobel et al. (1996) estimated that 50-100 years are required to restore a calcareous grassland from a forest stand. Whatever the time necessary, restoration is required for the long-term conservation of fragmented habitats. This study indicated that priority should be given to grassland patches that suffered a high loss in the past. It also indicated that patches larger than the current maximal patch area are needed to maintain a set of specialist species that have not yet paid their extinction debt.

If extinction debt provides a new opportunity to support habitat restoration, it also imposes an undefined deadline. While we wait to launch large-scale restoration programs, the extinction debt payment is in progress.

Acknowledgements

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Appendix

Appendix 1: Specialist species with their occurrence in small and large patches both considered their corresponding ALP (= association with large patches), Sensitivity and Safeness.

	1920			1965			Sensitivity	Safeness
	Occurrence in small patch (n=11)	Occurrence in large patch (n=43)	ALP	Occurrence in small patch (n=31)	Occurrence in large patch (n=23)	ALP		
Allium oleraceum	0.45	0.47	0.506	0.35	0.61	0.632	0.632	1.000
Anacamptis pyramidalis	0.00	0.00	-	0.00	0.00	-	-	-
Anthyllis vulneraria	0.27	0.40	0.592	0.23	0.57	0.715	0.715	1.000
Brachypodium pinnatum	0.82	0.91	0.526	0.87	0.91	0.512	0.526	0.997
Bromus erectus	0.64	0.93	0.594	0.84	0.91	0.521	0.594	0.999
Carex caryophyllea	0.27	0.37	0.577	0.26	0.48	0.650	0.650	1.000
Carex flacca	0.36	0.79	0.685	0.65	0.78	0.548	0.685	1.000
Carex tomentosa	0.09	0.02	0.204	0.03	0.04	0.574	0.574	0.135
Carlina vulgaris	0.00	0.16	1.000	0.16	0.09	0.350	1.000	0.038
Centaurea jacea	0.09	0.60	0.869	0.35	0.70	0.662	0.869	0.999
Centaurea scabiosa	0.36	0.63	0.633	0.45	0.74	0.621	0.633	1.000
Centaurium erythrea	0.00	0.16	1.000	0.06	0.22	0.771	1.000	0.038
Cirsium acaule	0.09	0.21	0.697	0.10	0.30	0.759	0.759	0.998
Euphorbia cyparissias	0.91	0.91	0.499	0.90	0.91	0.503	0.503	0.382
Festuca lemanii	0.82	0.56	0.406	0.55	0.70	0.559	0.559	1.000
Galium pumilum	0.36	0.53	0.595	0.39	0.65	0.628	0.628	1.000
Gentianella ciliata	0.00	0.14	1.000	0.03	0.22	0.871	1.000	0.013
Gentianella germanica	0.00	0.16	1.000	0.10	0.17	0.642	1.000	0.038
Gymnadenia conopsea	0.00	0.33	1.000	0.19	0.35	0.642	1.000	0.792
Helianthemum nummularium	0.91	0.98	0.518	1.00	0.91	0.477	0.518	0.621
Himantoglossum hircinum	0.00	0.07	1.000	0.06	0.04	0.403	1.000	<0.001
Hippocrepis comosa	0.64	0.56	0.467	0.52	0.65	0.558	0.558	1.000
Koeleria macrantha	0.55	0.44	0.448	0.35	0.61	0.632	0.632	1.000
Koeleria pyramidata	0.09	0.02	0.204	0.00	0.09	1.000	1.000	0.135
Linum catharticum	0.27	0.67	0.712	0.45	0.78	0.634	0.712	1.000
Medicago lupulina	0.36	0.44	0.549	0.35	0.52	0.595	0.595	1.000
Neotinea ustulata	0.00	0.02	1.000	0.00	0.04	1.000	1.000	0.016
Onobrychis viciifolia	0.09	0.07	0.434	0.03	0.13	0.802	0.802	0.586
Ononis repens	0.27	0.53	0.662	0.39	0.61	0.611	0.662	0.999
Ophrys apifera	0.00	0.02	1.000	0.03	0.00	0.000	1.000	<0.001
Ophrys fuciflora	0.00	0.00	-	0.00	0.00	-		
Ophrys insectifera	0.00	0.09	1.000	0.06	0.09	0.574	1.000	<0.001
Orchis anthropophora	0.00	0.02	1.000	0.00	0.04	1.000	1.000	0.016
Orchis militaris	0.00	0.02	1.000	0.03	0.00	0.000	1.000	<0.001
Pimpinella saxifraga	0.27	0.88	0.764	0.71	0.83	0.538	0.764	1.000
Plantago media	0.36	0.58	0.615	0.45	0.65	0.591	0.615	1.000
Polygala comosa	0.09	0.35	0.793	0.23	0.39	0.634	0.793	0.925
Potentilla neumanniana	0.91	0.79	0.465	0.84	0.78	0.483	0.483	0.964
Primula veris	0.45	0.60	0.571	0.42	0.78	0.651	0.651	1.000
Prunella laciniata	0.18	0.37	0.672	0.23	0.48	0.679	0.679	1.000
Ranunculus bulbosus	0.18	0.53	0.746	0.32	0.65	0.669	0.746	0.999
Salvia pratensis	0.09	0.00	0.000	0.03	0.00	0.000	0.000	0.016
Sanguisorba minor	0.82	1.00	0.550	0.97	0.96	0.497	0.550	0.621
Scabiosa columbaria	0.55	0.60	0.526	0.48	0.74	0.604	0.604	1.000
Thymus praecox	0.00	0.02	1.000	0.03	0.00	0.000	1.000	<0.001
Trifolium montanum	0.09	0.23	0.719	0.10	0.35	0.782	0.782	0.999

CHAPITRE 6 : EXTINCTION DEBT AND COLONIZATION CREDIT: WHEN BOTH PHENOMENA ARE INTEGRATED.

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Abstract

Calcareous grasslands are among the most species-rich ecosystems in temperate countries. However, these ecosystems have suffered from high fragmentation processes during the last century, which can lead to the origin of an extinction debt in remaining habitat patches. Results at our study site indicated that high loss (area loss since 1965 >80%) old habitat patches exhibited an extinction debt inverse to low loss (area loss since 1965 <80%) old habitat patches. However, human activities also created new habitat patches in the landscape and therefore provided opportunities for calcareous grassland plant species to colonize new sites. This also provided opportunities to study species colonization abilities in the context of habitat restoration. We analyzed species richness in new patches compared to old patches in order to detect colonization credit. We detected the presence of a colonization credit in new patches when using high loss old patches (that exhibit an extinction debt) or all old patches as a reference. However, when the reference was low loss old patches alone (those less likely to exhibit an extinction debt), no colonization credit was detected. In addition, correspondence analyses revealed that species composition in new patches was similar to old patches. These results are encouraging for restoration programs. However, the results indicated that the presence of an extinction debt in reference habitats could lead to inaccurate conclusions in restoration monitoring. Therefore, extinction debt should be considered when choosing reference habitats to evaluate restoration success.

Introduction

Anthropogenic activities are currently the primary drivers of landscape and consequently biodiversity dynamics (Baudry & Tatoni 1993; Jongman 2002). Human-driven landscapes are characterized by destruction and fragmentation of (semi-)natural habitats (Saunders et al. 1991). The risk of extinction is predicted to increase due to habitat fragmentation in remnant populations as a consequence of reduction in population size and/or low colonization rates. Therefore, affects on species diversity are expected (Saunders et al. 1991; Fahrig & Merriam 1994; Young & Clarke 2000). However, landscape dynamics may also result in the origin of new habitat patches with conditions conducive to spontaneous colonization by species from natural or semi-natural habitats (Krüger et al. 2002). New habitat patches may establish naturally in the absence of management programs from road sides (Quintana-Ascencio et al. 2007), abandoned quarries and mining sites (Krüger et al. 2002; Bizoux et al. 2004; Faucon et al. 2010) and forest clearings (Collins et al. 1985), as well as from directed restorations.

Species diversity is largely dependent on spatio-temporal landscape patterns, both at the landscape and the habitat patch scale (Turner et al. 2001; Cousins & Eriksson 2002; Ricklefs 2004). Conservation strategies have used the relationship between species richness and habitat area/connectivity to devise conservation approaches at the landscape and habitat scale (Ellis & Coppins 2007). However, landscape history itself has only recently been considered in ecological studies at the landscape level (Cousins 2009; Kuussaari et al. 2009). Habitat patch fragmentation/creation dynamics may be a rapid process (Adriaens et al. 2006). Alternatively, plant response to environmental change can be slow and a time lag can therefore exist between these processes. As a consequence, dynamic communities that experience habitat destruction and/or creation of new habitat patches may face the two following unbalanced states with regard to patch spatial characteristics: (1) extinction debt (Hanski & Ovaskainen 2002); and (2) colonization credit (Cristofoli & Mahy 2010; Cristofoli *et al.* 2010). These terms are defined as the number of species yet (1) to go extinct in a patch, or (2) to colonize a patch. Both extinction debt and/or colonization credit follow landscape change and continue until species richness reaches equilibrium with the new spatial properties of the patch.

In terms of conserving species richness and habitat restoration, estimates of extinction debt and colonization credit are integral components. On the one hand, the identification of an extinction debt in a given landscape indicates that some species are deterministically fated to go extinct, as current landscape structure does not permit their long-term survival. As a consequence, the restoration of functional habitat networks has become an important goal, and understanding colonization dynamics and processes is a critical component of those efforts (Jongman & Pungetti 2004). Studies aimed at quantifying colonization credit provide information regarding species colonization ability following the creation of new habitat patches (Cristofoli et al. 2009; Cristofoli & Mahy 2010).

An estimate of colonization credit may be challenged when the fragmentation of historical habitats occurs concurrently with the creation of new habitat patches in a landscape. Cristofoli et al. (2010) cautioned that in studying colonization credit (i.e. a reduction or lack in the number of species) in new habitat patches in relationship to reference habitat patches that exhibit an extinction debt (i.e. species richer than expected given their spatial characteristics) may lead to inaccurate conclusions. Both phenomena would tend to create a difference in species richness between new and reference patches. However, the co-occurrence of both phenomena is likely to occur in some landscapes. Consequently, it is necessary to determine how the presence of an extinction debt in reference habitat may influence the results of colonization credit studies and how to differentiate both conditions when they co-occur.

Semi-natural calcareous grasslands rank as one of the most species-rich among temperate plant communities. Therefore, these plant assemblages can be considered local biodiversity hotspots (Prendergast et al. 1993; WallisDeVries et al. 2002). In Belgium, as in many parts of Europe, these habitats suffered extensive effects of fragmentation processes in the past due to abandonment of the traditional agro pastoral system and resulting encroachment, re-aforestation, urbanization and transformation into arable lands (Poschlod & WallisDeVries 2002; Adriaens et al. 2006). In other respects, some human activities (e.g. road verge creation, quarry abandonment, and forest hunting clearings) may provide colonization opportunities for calcareous grassland species. As a consequence of these landscape dynamics, communities hosted by calcareous grasslands may be confronted with unbalanced states resulting from an extinction debt or a colonization credit. Several studies have addressed extinction debt in calcareous grasslands with incongruent results (Lindborg & Eriksson 2004a; Adriaens et al. 2006; Helm et al. 2006; Lindborg 2007). However, to date colonization credit has not been assessed in these habitats. Several calcareous grassland restoration projects have emerged in Europe over the last 15 years (Kiefer & Poschlod 1996; Zobel et al. 1996; Pärtel et al. 1998; Kiehl et al. 2006; Fagan et al. 2008), and it is therefore essential to evaluate species capacity to respond to the origin of new habitat patches in the landscape. However, most restoration programs are rather recent and little can be determined regarding the long-term effects of restoration. However, habitat patches created in the last few to several decades and for which history may be well documented are ideal systems to evaluate the probability of colonization credit in a temporal framework suitable for ecological restoration projects.

The aim of this study was to investigate and assess the consequences of habitat fragmentation and habitat creation on calcareous grassland plant species richness in Belgium. An analysis of calcareous grassland spatial and temporal landscape change over approximately 100 years was assessed and the complex nature (habitat destruction-fragmentation) under a global fragmentation pattern was elucidated. We asked 1) whether fragmented habitat patches harbor an extinction debt; 2) whether newly created patches (since 1965) harbor a colonization credit; and, 3) whether the presence of an extinction debt in reference habitats may influence estimates of colonization credit. The consequences for restoration monitoring success are discussed.

Methodology

Study site

The study site (approximately 25 km²) is located in the Lesse Valley, in southern Belgium (50°05′ to 50°09′ N; 5°06′ to 5°15′ E; alt. 150 to 250m) (Fig. 1). Calcareous grasslands were developed under traditional agro-pastoral practices on Devonian limestone hills and plateaus. Due to abandonment of traditional agriculture in the 19th century, calcareous grasslands have declined dramatically in the region (Bisteau & Mahy 2005b; Adriaens et al. 2006). Semi-natural calcareous grasslands are presently located in small well- delimited patches surrounded by forests and intensive meadow systems or arable lands. Although highly fragmented, the region supports one of the last significant areas of calcareous grasslands in Belgium.

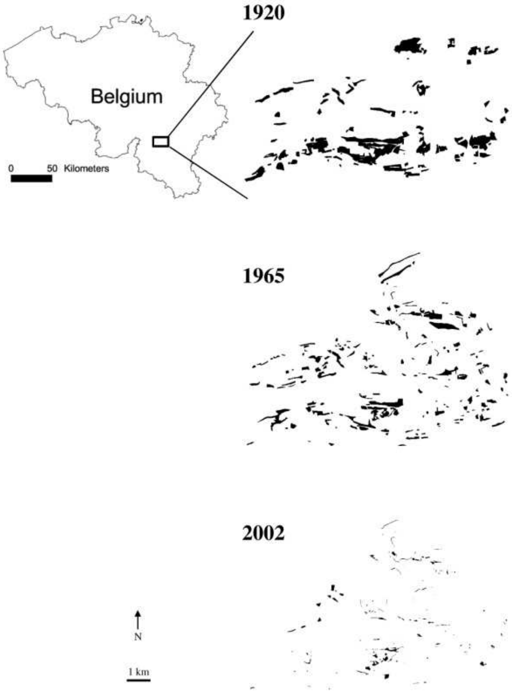

Fig. 1: Localisation of the study site in Belgium and calcareous grassland network at three time periods.

Landscape dynamics and species data

Calcareous grassland patches located in the study site were digitised in a GIS database (ArcView 3.2, ESRI 2000) derived from recent aerial photographs and field surveys. Past calcareous grassland landscape configuration was reconstructed using historical detailed topographical maps for 1920 (Institut Royal Militaire de Belgique, scale 1:20000) and 1965 (Belgian National Geographic Institute, scale 1:10,000) and 1965 aerial photographs (Belgian National Geographic Institute, scale 1:20,000). The 1920 period corresponds to the end of shepherding and the onset of a rapid decline of calcareous grasslands in Belgium (Delescaille 2002). Current and historical patch areas were directly derived from the GIS. Current and historical patch connectivity was estimated using the IFM index derived from Hanski (1994), using edge-to-edge distances among all patches. The metric applies distances to (no multiplication factor was used for distance) and area of all possible source populations in the landscape (Moilanen & Nieminen 2002) as follows:

$$IFMi=\sum Aj*e^{-dij}$$

where IFMi is the connectivity of the ith patch, Aj is the area of the jth patch, dij is the distance between the ith and the jth patch.

All vascular plant species were recorded in each calcareous grassland patch during spring and summer 2002 and 2003. Calcareous grassland specialist species were identified on the basis of a classification of local grassland communities (Piqueray et al. 2007). We subsequently estimated for each grassland patch the total species richness (Rtot) and the specialist species richness (Rsp). Species nomenclature followed Lambinon et al. (2004).

Extinction debt and colonization credit estimates

Estimates of extinction debt and colonization credit were based on the same method. The method rationale was to compare linear regression models testing species richness as a function of patch configuration (area/connectivity), built independently for two groups with different histories (see below for comparison descriptions) (Helm et al. 2006; Cristofoli et al. 2009). Three patch groups were distinguished:

Low loss old patches (designed as "low loss patches"). Patches present in all cartographic data and area loss since 1965 was lower than 80%. The 80% limit was chosen in order to make this study comparable to Helm et al. (2006). These represent the most stable patches in the landscape over time. Because these patches have been present in the landscape since at least 1965, we postulate they provide the closest to equilibrium for relationships between spatial configuration and species richness.

High loss old patches (designed as "high loss patches"). Patches present in all cartographic data and area loss since 1965 was higher than 80%. Due to recent fragmentation, these patches are more prone to exhibit an extinction debt, i.e. observed species richness was higher than expected at equilibrium with the spatial configuration of the patch.

New patches. Patches created after 1965. Due to recent creation, patches may exhibit a colonization credit, i.e. observed species richness was lower than expected at equilibrium with the spatial configuration of the patch. We recorded the origin of these patches, considering three observed cases (conversion of arable fields, clearing for hunting in forests and creation of new road verges).

In each group of patches, only patches that covered approximately the same surface area were retained. Extinction debt was estimated by comparing regression models for "*low loss patches*" and "*high loss patches*". Extinction debt was estimated in relationship to the 1965 landscape structure only. In this way, both extinction debt and colonization credit were estimated from the same data set. Colonization credit estimates were not reliable for 1920 due to uncertainties regarding the status of new patches for this date (see results).

Colonization credit was estimated for three conditions: 1) Comparisons between "*new patches"* and "*low loss patches*" (patches that are unlikely to exhibit an extinction debt); 2) comparisons between "*new patches*" and "*high loss patches*" (patches that are likely to exhibit an extinction debt); 3) comparisons between "*new patches*" and all old patches ("*low loss patches*" and "*high loss patches*") (patches that may exhibit different levels of extinction debt).

In each comparison, we selected the independent variable for the models. We looked for a compromise so that both models were built with the same independent variable (current area or connectivity). Each variable was given the same transformation; variables did not exhibit non-normality or heteroscedasticity of residuals at a 5% significance level and had a *P*-value for the regression test as low as possible. Different transformations on dependent (Rtot and Rspec) and independent variables (area and connectivity) were tested in order to improve normality and homoscedasticity of residuals using a Minitab macro (Palm 2002). The total species richness models were log-transformed and square root-transformed for richness and area, respectively to improve homoscedasticity and normality of residuals.

Equality of slopes between the regression lines of the two models for each comparison was tested using analyses of covariance (ANCOVA). Nonparallelism indicates that species richness responds differently to patch metrics for two groups of compared patches. In addition, we forced perfect parallelism between regression lines of the two models compared, i.e. two regression lines were built, where slopes equaled the mean slope of the adjusted regression lines. The distance between the two parallel lines was evaluated (ANCOVA), providing an estimate of the extinction debt or the colonization credit, depending on which two models were compared.

In order to detect the influence of the new patches origin (conversion of arable fields, clearing for hunting in forests or creation of new road verges), we used ANOVA to test for differences in patch colonization credit (e.g. difference between species richness predicted by reference model and observed species richness) between patches with different origins, considering "*low loss patches*" as reference.

Colonization credit analyses were performed using Minitab 15 (Minitab, Inc.).

Species richness may result from different species composition in the communities, therefore plant assemblages of each patch class were compared using a detrended correspondence analyses in CANOCO 4.5 (ter Braak & Smilauer 2002). Two independent analyses were computed for total and specialist species.

Results

Spatio-temporal dynamics of calcareous grasslands

Calcareous grasslands experienced severe fragmentation over the last 90 years in the study area (Figs. 1 and 2). From 1920 to 2002, the total area of calcareous grasslands was reduced by 87%. However, over the same time period, the number of calcareous grassland patches increased two-fold. Patch mean area decreased by 93% (1920: mean=4.67ha, SD=8.86ha; 2002: mean=0.31ha, SD=0.59ha). The fragmentation process between 1920 and 1965 was evidenced by an increase in the number of patches (approximately two-fold), and a limited loss in total area (21%). The fragmentation process between 1965 and 2002 was characterized by a decrease in patch area (83% total area loss; 83% mean patch area decrease), while the number of patches remained relatively stable.

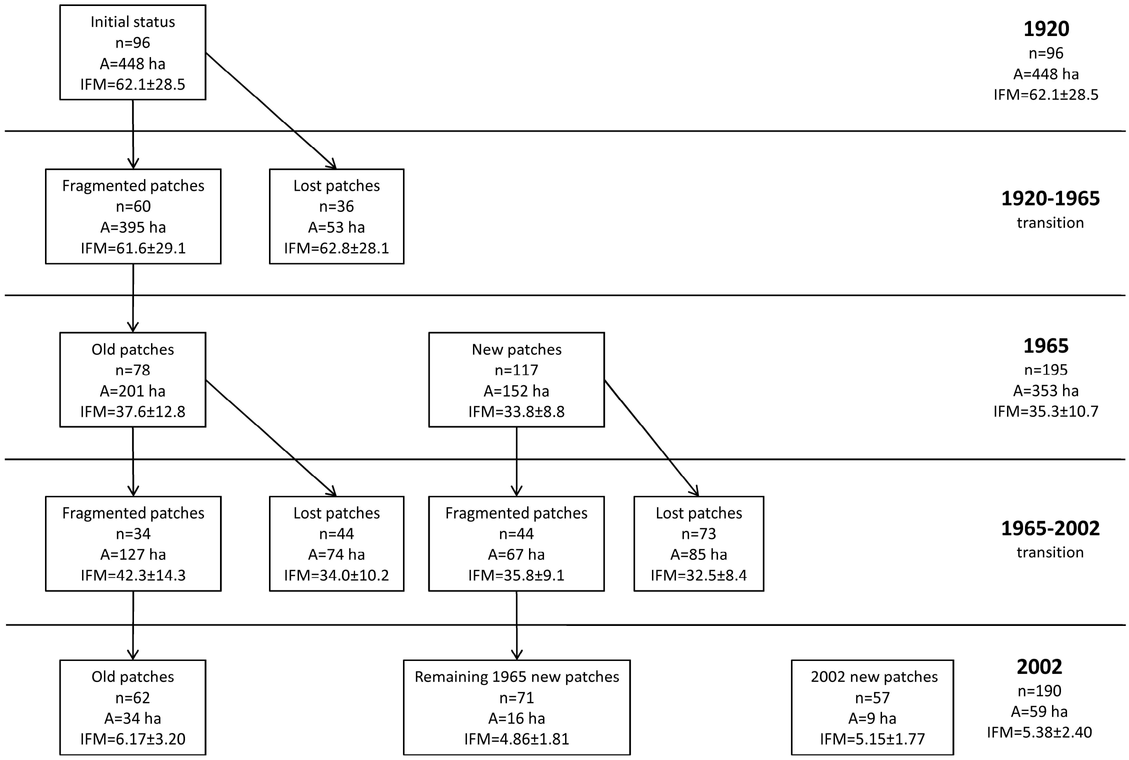

Fig. 2: Calcareous grassland patches dynamics in the study site. n is the number of patches, A is the total area, IFM is the connectivity index.

However, habitat dynamics was by far more complex than simple destruction of historical patches and resulted from a complex combination of destruction and creation of calcareous grassland patches (Fig. 2). In 1920, 96 calcareous grassland patches were evident in the study landscape and occupied a total area of 448 ha. Between 1920 and 1965, 36 (37.5%) were lost. The 60 remaining patches suffered high fragmentation between 1920 and 1965 as they lost approximately half of their total area and were divided into 78 patches. However, in 1965, 117 new patches (a total area of 152 ha) were identified on cartographical documents, which were not present on the 1920 document. We suggest these patches originated from the creation of new calcareous grasslands between 1920 and 1965. However, due to the higher quality of cartographic documents in 1965 (use of aerial photographs), we cannot exclude the possibility that some patches interpreted as calcareous grasslands were already present in 1920, but were not mapped at that time. Therefore, the "new" status of these patches remains unconfirmed. Between 1965 and 2002, 56% of the old patches (i.e. patches that were already present in 1920) were destroyed. Remaining old patches suffered tremendous fragmentation with a 73% reduction of total area and an 83% increase in patch number due to division. Patches created between 1920 and 1965 also suffered the same fragmentation pattern; and 38% of the patches, representing 44% of the total area, were lost between 1965 and 2002. The area of the remaining patches was reduced by 76% and division increased patch number by 38%. However, during the 1965-2002 period, we found the origin of 57 new patches for a total area of 9 ha. The target habitat dynamics evaluated over the past 90 years and within each time period shows a complex mix of new and old patches of different ages over the current landscape. From the total area of calcareous grasslands currently present, 57.6% were already present in 1920, 27.1% were created between 1920 and 1965, and 15.3% were created between 1965 and 2002.

Extinction debt and colonization credit estimates

We limited our estimates of colonization credit to patches that were created between 1965 and 2002 (i.e., patches less than 40 years old), due to the uncertainty regarding the origin of new patches created between 1920 and 1965. Fifty-seven new patches were found between 1965 and 2002, and 17 were retained for credit colonization estimates; 35 were discarded because they occupied an area less than 0.052 ha. These patches were not suitable for comparisons with other groups of patches because comparably small patches were absent. Five new patches were eliminated because they were observed in sites that were calcareous grasslands in 1920, were absent in 1965 and re-appeared in 2002. Their *"new patch"* status was therefore tenuous. The total area covered by the 17 selected "*new patches*" was 8.02 ha (approximately 90% of the area of new patches created between 1965 and 2002). The 17 patches (mean area: 0.472 ha, range: 0.052-2.778 ha) originated from the conversion of arable fields (four patches, 3.34 ha), clearing for hunting in forests (four patches, 2.41 ha) and creation of new road verges (nine patches, 2.27 ha). Sixteen "*low loss old patches*" (mean area: 0.938 ha, range: 0.056-2.794 ha) and 28 "*high loss old patches*" (mean area: 0.413 ha, range: 0.048-2.162 ha) exhibiting a similar range of area were retained for model comparisons.

Table 1: P-values regression between total species richness (Rtot) and specialist species richness (Rsp) and current area and connectivity. (-) indicates a negative relation.

		Area	**Connectivity**
Low loss patches (n=16)	Rtot	0.007	0.106
	Rsp	0.009	0.129
High loss patches (n=28)	Rtot	<0.001	0.693
	Rsp	0.002	0.051
New patches (n=17)	Rtot	0.907	0.338 (-)
	Rsp	0.849 (-)	0.344 (-)

Species richness (Rtot and Rsp) was significantly related to the current area for "*low loss patches*" and "*high loss patches*" (Table 1). However, significant relationships were not detected between current species richness and current patch area for “*new patches*”. Furthermore, no significant relationship between current patch isolation and current species richness, for any group of patches was indicated. Area was the independent variable most influencing patterns of species richness, therefore it was chosen as the independent variable to build regression models between patch metrics and species richness.

Table 2: Estimation and test for the extinction debts and colonization credits. Parallelism is the parallelism test between straight lines. P is the p-value of the ANCOVA.

		Total species richness		Specialist species richness	
		Parallelism	Debt/Cedit	Parallelism	Debt/Cedit
Extinction debt (Chap. 5)		P=0.914	27.20% P=0.002	P=0.502	6.06 species P=0.002
Colonization credit	In relation to *low loss patches*	P=0.046	6.57% P=0.650	P=0.032	1.55 species P=0.429
	In relation to *high loss patches*	P=0.022	26.00% P=0.006	P=0.010	6.23 species P<0.001
	In relation to both *low loss and high loss patches* (pooled)	P=0.050	19.44% P=0.044	P=0.063	4.50 species P=0.008

Parallelism of regression lines between the species richness-area models for “*high loss old patches*” and “*low loss old patches*” was accepted for extinction debt estimates (Table 2). A significant extinction debt for both total and specialist species richness was detected (27.20% and 6.06, respectively, Table 2). Total species richness results are given in percent due to the log-transformation on this variable. The 6.06 specialist species value corresponds to approximately 35% of the mean specialist species richness for “*high loss patches*”.

Parallelism between the species richness-area models for “*new patches*” and “*low loss patches*” or “*high loss patches*” was rejected at the 5% significance level in all cases but one (*P*=0.063) for the colonization credit estimates (Table 2). This absence of parallelism between regression lines for the different models is illustrated in Figure 3. After forcing parallelism, a significant colonization credit was detected when “*new patches*” were compared to “*high loss patches*” (colonization credit = 26.0% and 6.23 species for Rtot and Rsp, respectively) or when new patches were compared to all “*old patches*”, including “*low loss patches*” and “*high loss patches*” (colonization credit = 6.57% and 1.55 species for Rtot and Rsp, respectively). The colonization credit detected when “*new patches*” were compared to “*high loss patches*” was almost equivalent to the extinction debt indicated for “*high loss patches*” (26.00% of total species richness, 6.23 specialist species). In contrast, no colonization credit was detected when “*new patches*” were compared to “*low loss patches*”. Colonization credit did not differ between patches with different origins (conversion of arable fields, clearing for hunting in forests and creation of new road verges), neither for specialist species richness ($F_{[2;14]}$=1.01, P=0.390), nor for total species richness ($F_{[2;14]}$=0.58, P=0.573).

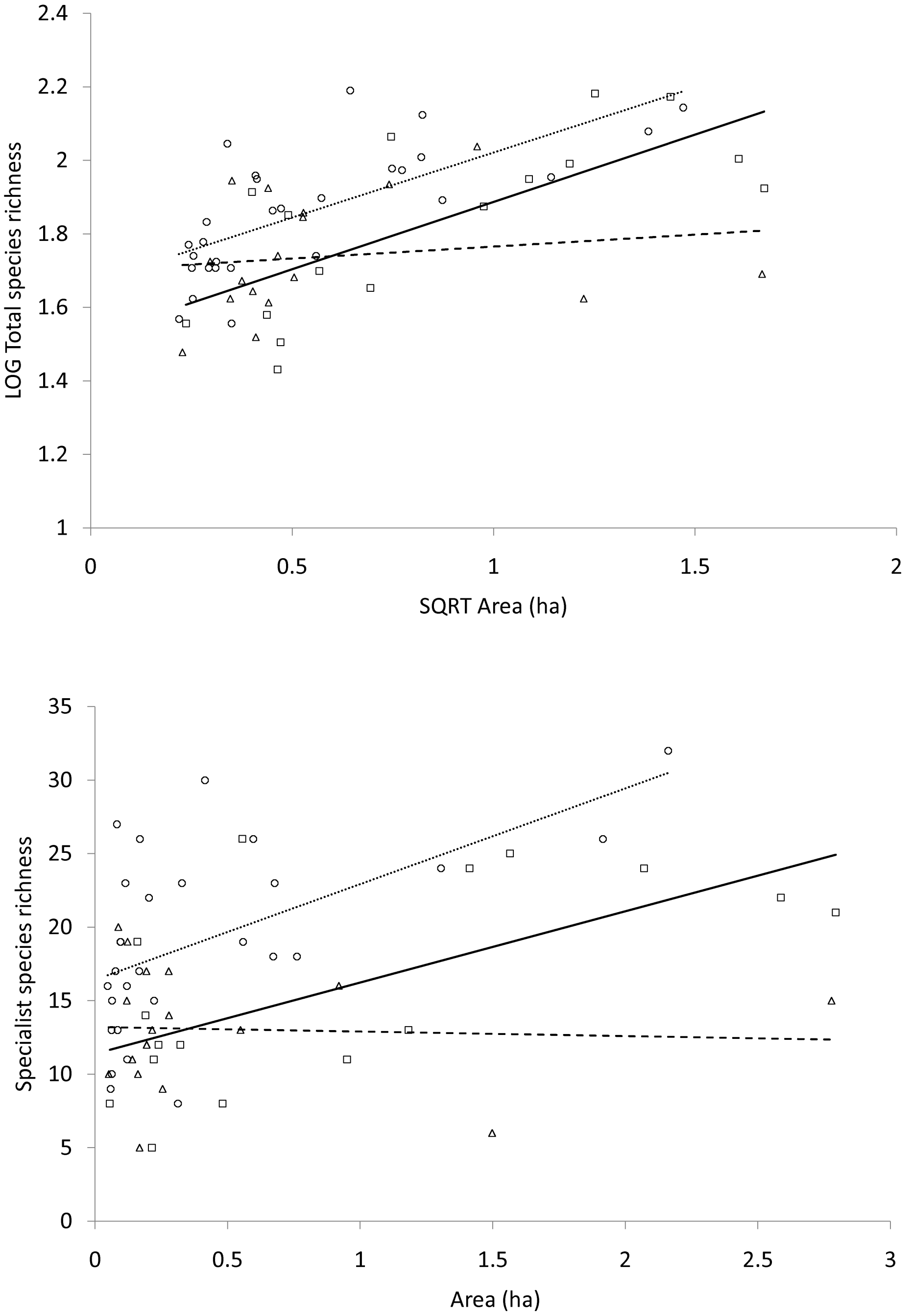

Fig. 3: Relationship between area and (a) total species richness (upper graph) and (b) specialist species richness (lower graph) for the three patch types. Variable transformations are these used for the ANCOVA analyses. Circles and dotted lines are high loss patches, Squares and plain lines are low loss patches, Triangles and dashed lines are new patches.

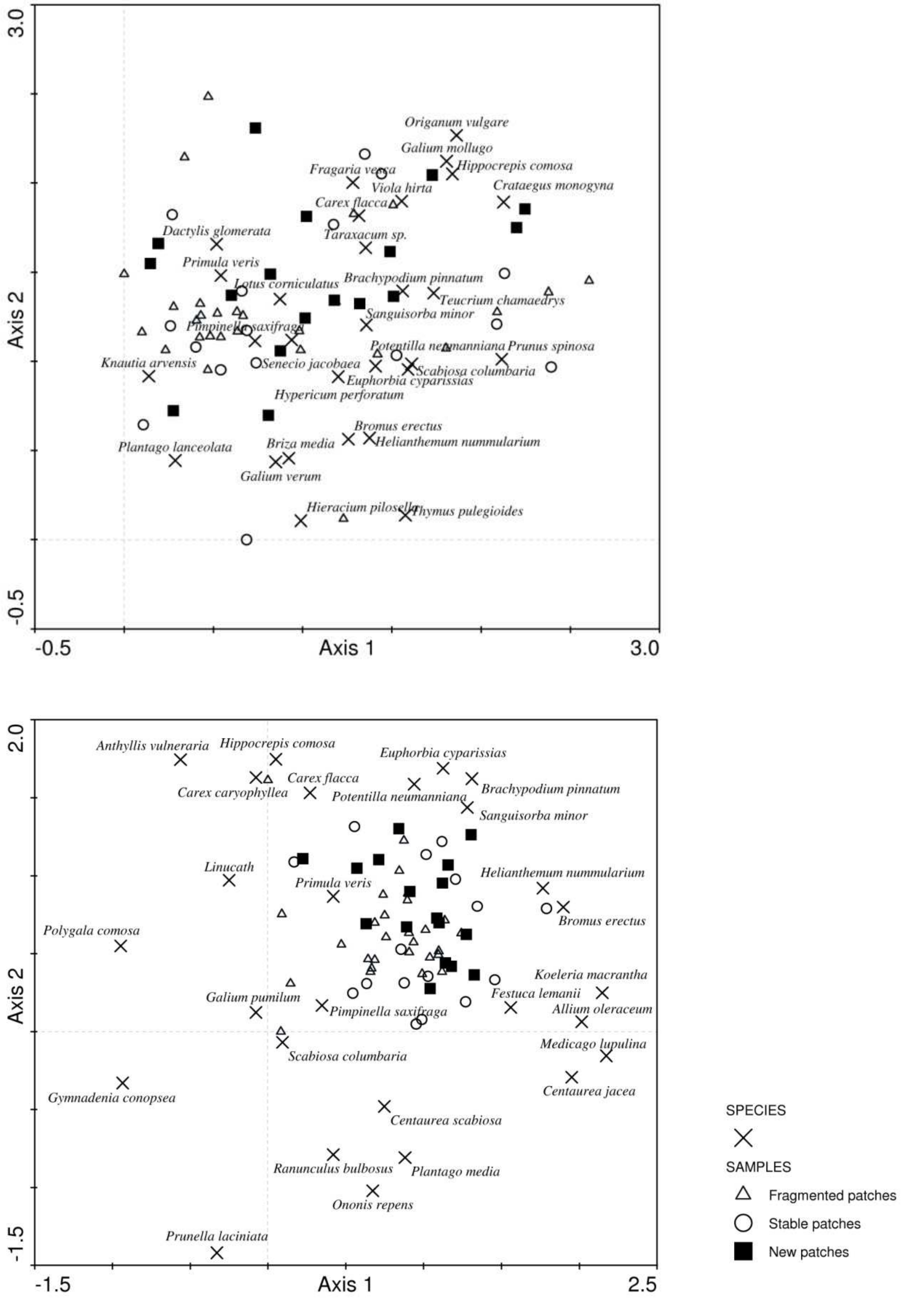

Fig. 4: Detrended correspondence scatter plot of (a) total species composition (upper graph) and (b) specialist species composition (lower graph).

DCA revealed that the species composition of "*new patches*" was comparable to other groups of patches (Fig. 4). The total species composition analysis (Fig. 4a) revealed that "*new patches*" were less represented and placed in the lower-right of the scatter plot, which corresponded to the presence of specialist species (e.g. *Scabiosa columbaria*, *Helianthemum nummularium*, and *Thymus pulegioides*). The same analysis based only on specialist species (Fig. 4b) revealed that species typical for short turf grasslands (e.g. *Plantago media*, *Prunella laciniata*, and *Polygala comosa*) were less represented in "*new patches*".

Discussion

Studies of temporal dynamics in temperate, open, and semi-natural habitats have systematically addressed the general patterns of area decrease and isolation increase resulting from habitat fragmentation (Lindborg & Eriksson 2004a; Adriaens et al. 2006; Helm et al. 2006; Krauss et al. 2010), whereas little attention has been paid to the potential contribution of secondary habitat origins through time (but see Jacquemyn et al. 2003b; Arroyo-Mora et al. 2004b).

Our work demonstrated that in our study landscape, spatial changes in calcareous grasslands resulted from two processes acting jointly: destruction and creation of habitat patches. As a result, target habitats are presently composed of a complex mix of new patches and old patches of different ages. However, the emergence of new patches was not adequate to counteract the fragmentation process. The current level of calcareous grassland fragmentation observed in the present study was among the highest reported in Europe (Krauss et al. 2010). The co-existence of historical fragmented and newly created patches colonized by grassland species may lead to conditions of double disequilibrium.

In the present study, historical patches (>80 years old) represented only 32.6 % of the number of patches currently in the landscape, but nonetheless represented a larger proportion (57.6%) of the current habitat area. These historical patches represent core areas of habitat ecological networks. Our results indicated that historical patches have lost a high proportion of area and are not at equilibrium with their species richness as compared to more stable patches. Consequently, these patches likely support an extinction debt indicated by an average excess of approximately 35% specialist species. Extinction debt was already shown to occur in calcareous grassland landscapes (Lindborg & Eriksson 2004a; Helm et al. 2006). However, in similarly fragment landscapes, previous studies reported the absence of extinction debt (Adriaens et al. 2006; Cousins et al. 2007). The future payment of this extinction debt will most likely lead to the extinction of a proportion of the rarest specialists, and may also seriously affect the metapopulation of other specialists with a decrease in population numbers.

Colonization credit estimates in "*new patches*" differed with reference to historical patches. When habitat patches exhibiting an extinction debt ("*high loss patches*") were included in the analysis, a colonization credit was detected, leading to the conclusion that, on average "*new patches*" had not reached their final species. However, when historical "*low loss patches"* were used as a reference (the most likely to be at equilibrium), a colonization credit was not found within "*new patches".* New patch with different origins (conversion of arable fields, clearing for hunting in forests or creation of new road verges) did not exhibit different colonization credit, suggesting that species richness may recover equally whatever the origin of the patch creation. In restoration ecology, it is widely accepted that restoration success should be assessed in comparison with a reference ecosystem (Holl & Cairns 2002). The choice of an appropriate reference ecosystem is of major importance as it determines the goals for a restoration program. Our results highlight the dangers in using habitats exhibiting an extinction debt as reference habitats for establishing milestones in restoration evaluation and success.

In practice, the identification of an extinction debt calls for management programs that include habitat restoration. A lack of colonization credit detected in newly created patches formerly not dedicated to directed restoration is all the more encouraging for future restoration programs. Our results were unexpected given the current fragmentation of calcareous grasslands in our study area. Increased levels of habitat fragmentation typically prevents diaspore dispersal between isolated patches, and the rescue-effect (Brown & Kodrik-Brown 1977) is less likely to prevent newly established populations from going extinct. Species richness itself is not an accurate indicator of the complete restoration of an ecosystem (Mortimer et al. 1998; Kiehl et al. 2006). An ecosystem may be species-rich, but the species may not be representative of the reference community. Our results found that not only species richness, but also species composition was similar between "*old patches"* and "*new patches*". Only a few original species representative of the expected species composition were absent in "*new patches*", notably those from short turf grassland species.

Nevertheless, it is worth noting that richness-area relationships were significantly different between "*new patches*" and "*old patches*". This was due to the fact that the largest "*new patches*" did not recover their definitive species richness, while smaller new patches recovered. In this context, we cannot conclude the complete absence of a colonization credit. The non-significant area-richness relationship in "*new patches*" suggests that rate of colonization is independent of area. As a consequence, large patches that have to recover higher species richness might exhibit a colonization credit for a longer period of time.

Conclusions

Our data showed that calcareous grasslands created between 1965 and 2002 were as species-rich as old grasslands (created before 1920). However, this conclusion was only valid when reference habitats were low loss patches that did not exhibit an extinction debt. Other studies have demonstrated that not only species richness can be influenced by landscape configuration, but also species density or single species occurrence (Lindborg & Eriksson 2004a;

Cousins 2006). These indicators are often used to assess restoration success; therefore we argue that the occurrence of an extinction debt in current landscapes might influence the results of restoration studies. These criteria should not be further neglected when addressing restoration targets.

Chapitre 7 : Why some species cannot colonize restored habitats? The effects of seed and microsite availability

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Abstract

Restoration was promoted as a conservation strategy to preserve natural and semi-natural habitats. Restoration provided promising results for many taxa, however certain typical species still failed at colonizing restored habitat. Availability of seed and germination microsites were determined as potential limiting factors for site colonization by plant species. Furthermore, processes that break seed dormancy may be absent due to changes in habitat management. We studied three species that failed at colonizing restored calcareous grasslands in Belgium, including *Pulsatilla vulgaris* (Ranunculaceae), *Trifolium montanum* (Fabaceae) and *Veronica prostrata* (Scophulariaceae). The three species were germinated under controlled conditions in the absence of treatment (control). Dormancy breaking indicators were species specific. Cold enhanced the germination of *V. prostrata*. Fire-related treatments (heat shock and smoke exposure) did not enhance germination and were deleterious to *V. prostrata*. Litter cover improved *P. vulgaris* emergence in outdoor containers, but had a negative effect on *V. prostrata*. In the field, *V. prostrata* did not emerge regardless of the conditions, either in the reference (control) or restored grassland. *T. montanum* seedlings were observed in the reference but not restored grassland. Emergence patterns were site dependent, but manual seed addition always promoted emergence. *P. vulgaris* emerged in the reference, and to a lower degree in the restored grasslands. The combination of seed addition and providing microsites for seed germination resulted in enhanced seedling emergence for *P. vulgaris*. Our results suggest that seed and microsite limitations are integrated and cannot be separated. Implications for species conservation are discussed.

Introduction

The last century has shown a decline in natural and semi-natural habitats throughout many parts of Europe. One consequence of habitat fragmentation is a decrease in specialist plant species population size and connectivity, leading to increased risk of extinctions (Leimu et al. 2006). Jongman & Pungetti (2004) proposed habitat restoration as a conservation strategy to reduce the risks imposed by habitat loss and fragmentation. One aim of ecosystem restoration is to create and expand habitat that support species characterizing former plant communities (Society for Ecological Restoration International Science & Policy Working Group 2004). Among semi-natural habitats, calcareous grasslands are of particular interest for nature conservation in Europe, as they represent a priority habitat within the European directive 92/43/EEC. However, in restored grassland communities typical species found in intact communities are often absent (Zobel et al. 1996; Piqueray et al. 2010) .

Species colonization within a site is largely controlled by two factors that limit seedling recruitment, including seed availability within the dispersal area (i.e. the number of seeds reaching the site) and seedling establishment based on microsite availability (i.e. number of microsites suitable for regeneration) (Coulson et al. 2001). These two processes determine if seeds spread over a site and if seeds establish as seedlings once settled on a site. Seedling recruitment is either limited by a lack of seeds or suitable establishment sites. This is usually determined by comparing the effect on emergence by the addition of seeds to a microsite or by site disturbance (Coulson et al. 2001 and references therein). Limited or lack of dispersal, which induces seedling establishment limitations, is a well-known constraint for semi-natural habitat restoration. Indeed, several studies have shown that dispersal is the primary limiting factor to re-establish species populations (e.g. Lee 1993; Crawley & Brown 1995). Seed germination and seedling establishment are crucial stages in the regeneration of plant communities (Grubb 1977). Considering these stages, microsite availability is a vital element in site colonization (Foster 2001; Münzbergová & Herben 2005). Biotic factors, such as inter-specific competition, notably of grass species or litter effects (mechanical interaction, alteration of light and moisture conditions) can be responsible for the lack of microsite availability, reducing seedling survival. Microsite availability can be improved through application of disturbance regimes, which creates regeneration niches or gaps for germination and establishment through vegetation and/or litter effect removal. Several techniques can be applied corresponding to more or less severe disturbance approaches, e.g. harrowing, turf stripping, or sward removal (Zobel et al. 2000; Hofmann & Isselstein 2004; Edwards et al. 2007). A combination of both factors (seed and microsite limitations) can therefore best explain recruitment dynamics in restored populations (Poschlod & Biewer 2005).

In Belgium, over 90% of calcareous grasslands have been lost due to encroachment by shrubs and trees following shepherding abandonment (traditional agropastoral practices) and intentional forestation (Bisteau & Mahy 2005a; Adriaens et al. 2006). To stop further decline, approximately 100 ha of calcareous grasslands have been restored in Belgium over the last 20 years. All restored sites were pre-forest communities comprised of 40-100 year old forests. Restoration protocols included tree and shrub clearing followed by sheep and goat grazing. Restoration has been shown successful in Europe (Willems 2001; Kiehl & Pfadenhauer 2007; Piqueray et al. 2010), and restored habitats globally tended to resemble reference habitats despite some differences in vegetation structure and floristic composition (Piqueray et al. 2010). However, some characteristic species may fail at colonizing restored sites, despite the close vicinity of seed sources (Zobel et al. 1996; Piqueray et al. 2010). Most calcareous grassland species exhibit low spatial and temporal dispersal (seed bank) ability (Kalamees & Zobel 1997; Stampfli & Zeiter 1999; Bisteau & Mahy 2005b). In addition, Zobel et al. (2000) showed that some calcareous grassland species were dependant on microsite availability for recruitment. Moreover, disturbance enhanced the success of seed addition in calcareous grassland restoration (Edwards et al. 2007). In this type of habitat, the influence of fire on germination and microsite availability is particularly important. The indirect effects of fire can lead to a reduction in competition by vegetation removal or a direct effect on seed germination through the actions of heat and smoke (Thomas et al. 2003). Fire was formerly used as a traditional management technique to prevent shrub overgrowth (Kahmen et al. 2002). Currently, this practice is not recommended as a management tool, as comparative studies revealed it was not effective at maintaining traditional calcareous grassland communities (Kahmen et al. 2002; Köhler et al. 2005). However, at the species level, these same studies revealed that some plants benefit from fire treatments and some specialist species present in calcareous grassland seed banks require heat shock to germinate (Bossuyt & Honnay 2008).

In this study, we evaluated germination, and seed and microsite availability in three calcareous grassland species (*Pulsatilla vulgaris*, *Trifolium montanum* and *Veronica prostrata*) that failed at colonizing restored calcareous grasslands despite the close vicinity of seed sources. Our objective was to determine the factors limiting the spread and establishment of these species by addressing the following questions: (i) do the study species produce viable seed and do the seeds require treatments (cold, heat shock and smoke exposure) to germinate?; (ii) do microsite (presence of litter and/or disturbance) conditions influence seedling emergence?; (iii) does seedling emergence differ between restored and control calcareous grasslands?; and (iv) can seed addition improve seedling emergence?

Methodology

Study species and seed collection

Our study evaluated three rare species in Belgium occurring only in calcareous grasslands: *Pulsatilla vulgaris, Trifolium montanum* and *Veronica prostrata*. The species are highly threatened and listed in the regional Red Data Book (Saintenoy-Simon et al. 2006). Species descriptions were taken from Tutin et al. (1964-1980) and Saintenoy-Simon et al. (2006).

Pulsatilla vulgaris Mill. (Ranunculaceae) is a perennial species, flowering from March to May; seeds mature from May-June (J. Piqueray, pers. obs.). Flowers are borne singly on 3-12 cm stems. Each flower produces ca. 100-200 achenes bearing a ca. 3 cm appendage (J. Piqueray, pers. obs.). The species is distributed throughout a large part of Europe. However, *P. vulgaris* has been historically rare in Belgium and suffered a decline during the last century due to calcareous grassland fragmentation.

Trifolium montanum L. (Fabaceae) is a 15-60 cm high perennial species, flowering from May to August. Seed maturity occurs in July-September (J. Piqueray, pers. obs.). The plant produces 1-5 flowering shoots, each bearing 1-6 flower heads with ca. 150 flowers (Schleuning et al. 2009). The calyx forms a propagule and a one-seeded fruit (J. Piqueray, pers. obs.). The species distribution has been substantially reduced in Belgium, and currently occupies less than 25% of its historical range.

Veronica prostrata L. (Scrophulariaceae) is a prostrate perennial species up to 25(-40) cm high, flowering in May-July. Seed maturity occurs in June-July (J. Piqueray, pers. obs.). The flowers are borne in racemes usually 3-6 cm long. Fruits are capsules containing 10-20 seeds each (J. Piqueray, pers. obs.). Only *V. prostrata* subsp. *scheereri* occurs in Belgium.

Seeds of each species were hand-collected in 2006 for germination in Petri dishes and outdoor container experiments, and for in-situ experiments at maturity in 2007 (Table 1). For *P. vulgaris* and *T. montanum*, the seeds of 10 randomly selected flower heads were collected in each population, each year. For *V. prostrata*, the seeds of 15 randomly selected racemes were collected in each population, each year.

Germination in Petri dishes

We studied germination in Petri dishes each of the three species under three treatments as follows: (i) cold; (ii) heat; and (iii) smoke. Combinations of these treatments resulted in eight modalities (control (o), cold (c), heat (h), smoke (s), cold+heat (ch), cold+smoke (cs), smoke+heat (sh), and cold+heat+smoke (csh)). Seeds were subjected to the following treatments before germination: cold treatment for two months at 4°C; heat treatment of 20 min at 60°C in an oven, which corresponds to topsoil conditions during a fire (Auld & Bradstock 1996); and seeds exposed to a smoke treatment by burning *Brachypodium pinnatum* (Poaceae) hay for 20 minutes, with smoke produced using a bee smoker injected into airtight glass bottles containing seeds. Each of the eight modalities was repeated four times. Following seed treatments for each of the three study species, we established a total of 96 Petri dishes, containing 10 seeds each, placed on double filter paper moistened with distilled water. Petri dishes were randomly placed in a climatic chamber (18°C constant temperature, 16h photoperiod, 70-80% humidity). Germination was recorded two times a week until the end of germination (the absence of germination during a 2-week period).

Data were analyzed independently for each species. Final germination percentages in Petri dishes (when necessary, data was transformed to improve homoscedasticity) were compared among the eight modalities using a one-way ANOVA. In case of significant differences, pairwise comparisons were made using Tukey's tests.

Emergence and development in containers

In October 2006, an outdoor container experiment was established using a random design on an open homogenous area in Gembloux (Belgium; 50°33'N; 4°42'E; alt. 160 m). Container size was 0.6 m x 0.15 m x 0.15 m. Each container was filled with a bottom layer of calcareous stones (5 L, caliber 40 mm) followed by compost (pH = 7.5). Twenty seeds were sown in two rows in each container. Three treatments were set up: (i) no treatment = "control treatment"; (ii) seeds sown after heat and smoke treatments (see Petri dish experiment for modalities) = "fire treatment"; and (iii) containers covered with 2 cm *B. pinnatum* litter = "litter treatment". Each treatment was repeated four times for each species (total: 36 containers). In each container, emergence and survival rates were recorded every two weeks in winter (November to March) and every week during the vegetative season (April to October). In November 2007, seedling size for all three species (mean of the two largest perpendicular plant diameters in mm) and number of leaves (excluding cotyledons) of *P. vulgaris* was recorded. *B. pinnatum* litter was re-applied at this time. The final number of individuals was recorded at the end of the experiment in April 2008.

The percent final emergence, seedling size at the end of the first growing season and final number of individuals (when necessary, data was transformed to improve homoscedasticity) were compared among the three treatments using one-way ANOVAs, followed by Tukey's tests in the case of significant differences. Seedling fate (survival or mortality) at the end of the experiment was analyzed among the three treatments using a chi-square test.

In situ experiments

In situ experiments were conducted at five sites. Sites were selected to meet the following conditions: (i) the sites exhibited a large population (hundreds individuals) of one of the study species, called "target species"; (ii) a restored area occurred in close vicinity to the population; and (iii) the target species did not occur in the restored area. Each site was composed of two parcels, the "reference parcel" where the species occurred and the "restored parcel", where the species was absent. These sites were therefore suitable for studying the absence of re-colonization of restored sites by the target species, despite the close vicinity of a seed source. Under these very limited conditions, we were able to find only one site supporting *V. prostrata* and two sites for *T. montanum* and *P. vulgaris* (Table 1). The site "Montagne-aux-Buis" (target species: *V. prostrata*) is located in the Viroin Valley, the other sites are located in the Lesse Valley. Both valleys are situated in Calestienne, a narrow Devonian limestone strip running southwest to northeast. Both valleys support large areas of calcareous grasslands, and are considered as core areas for calcareous grassland conservation in Belgium (see Butaye et al. (2005b) and Piqueray et al. (2007) for vegetation descriptions). Restored parcels were formerly forest stands that were clear cut at different periods (Table 1). Since 2003, all sites have been managed by grazing using migrating sheep flocks.

Table 1: Study sites for the three study species. Time since the restoration of the restored parcels.

Name	Localisation	Target species	Time since restoration
Les Grignaux	50°07’N – 5°10’30”E	*Pulsatilla vulgaris*	3 years
Tienne des Vignes	50°06’N – 5°10’E	*Pulsatilla vulgaris*	10 years
Lorinchamps	50°06’N – 5°14’E	*Trifolium montanum*	4 years
Les Pairées	50°06’N – 5°11’E	*Trifolium montanum*	12 years
Montagne-aux-Buis	50°05’30”N – 4°34’E	*Veronica prostrata*	9 years

The experiment was settled at the five selected sites from May to August 2007, at the propagule release time for each species.

At four sites ("Tienne des Vignes, "Lorinchamps", "Les Pairées", "Montagne aux Buis"), vegetation data was extracted from the data set described by Piqueray et al. (2010). In order to have information for each parcel, a supplementary vegetation list was recorded at the fifth site ("Les Grignaux"). We computed a Detrended Correspondence Analysis (DCA) in order to analyze vegetation variability among sites and parcels.

Within each parcel (restored parcel and reference parcel) of each site we established four 2 m x 2 m experimental units. Each unit was divided into four 1 m x 1m subunits and two treatments leading to four modalities were randomly assigned to each subunit. The two treatments included the manual addition of seeds (25 seeds) and habitat disturbance (i.e. litter and above-ground vegetation were removed in 25 (five rows by five lines) 5 cm x 5 cm microsites). The modalities included (i) control (no treatment, O); (ii) seed addition (S); (iii) disturbance (D); and (iv) seed addition + disturbance (SD). At each site, added seeds were collected within the site in order to insure the genetic integrity of local populations. In May 2008, seedlings were counted in each subunit.

In-situ seedling emergence (when necessary, data was transformed to improve homoscedasticity) was analyzed using ANOVA. A preliminary analysis tested for site effects. In the case of significant site effects (single effect or interaction with modalities or parcel type), further analyses were computed independently for each site. Comparisons between modalities were computed using a two-way ANOVA testing the effects of modalities, parcel types (restored vs. reference) and their interactions. Pairwise comparisons were made using Tukey's tests.

Results

Germination initiated following 4, 11 and 18 days for *T. montanum*, *V. prostrata* and *P. vulgaris*, respectively. All three species germinated without treatment. The control treatment germination percentage for *T. montanum* was 37.5% (SE=8.54%) and was not significantly changed under the treatment conditions (Fig. 1). Control *V. prostrata* germination percentage was 57.5% (SE=4.79%). Germination percentage of this species significantly varied among modalities (P= 0.002). The highest germination percentages were globally obtained when cold treatment was applied (Fig.1). Germination in the absence of treatment was 80% (SE=8.16%) for *P. vulgaris*. ANOVA detected significant differences among the eight modalities (P=0.021). Furthermore, the difference between cold+smoke+heat (csh: 97.5 %) and smoke+heat (sh: 60 %) were significant (Fig. 1).

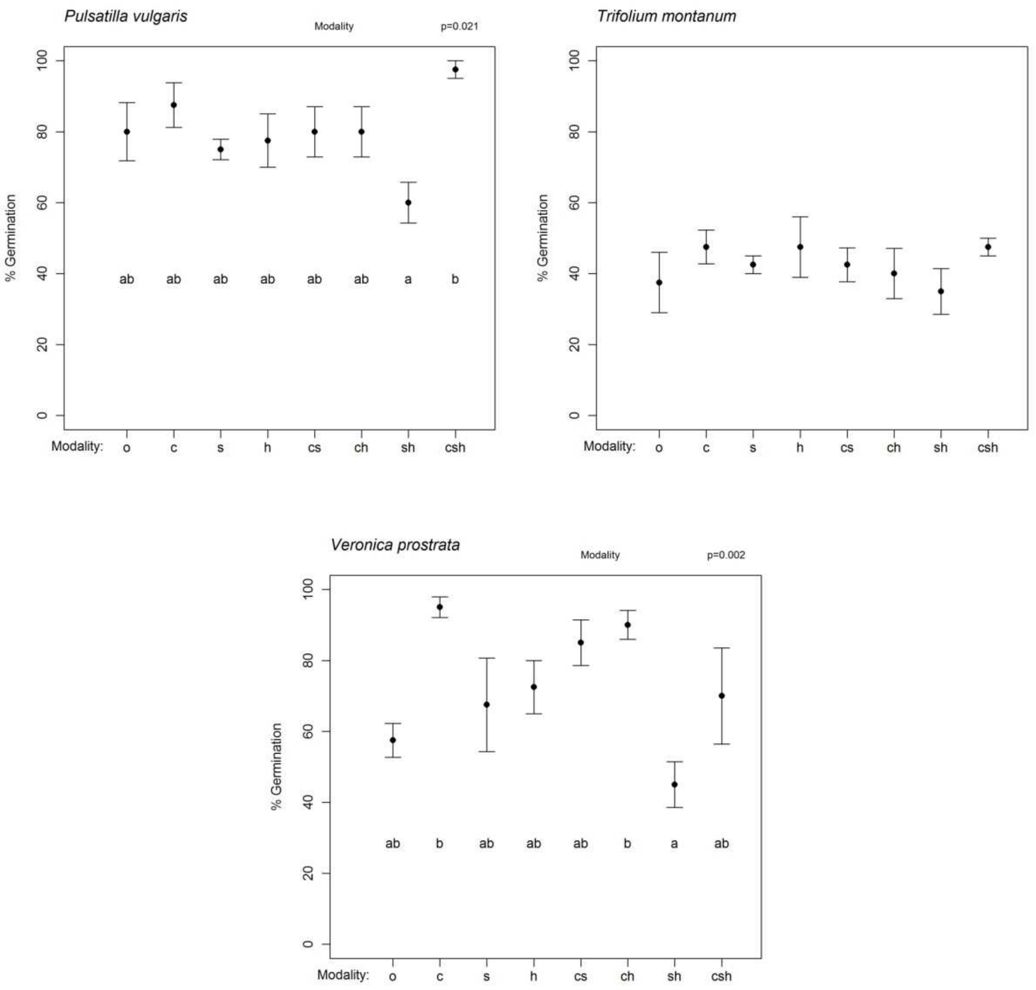

Figure 1: Germination in Petri dishes of the three study species under the eight treatment combinations. Error bars are 95% confidence intervals. The letters c, s and h respectively correspond to the cold, heat and smoke treatments. Letter combinations are treatment combinations (modalities).

Container growing experiments revealed different phenological patterns between species (Fig. 2). *T. montanum* and *V. prostrata* germinated both in autumn and spring, but in different proportions. Respectively, for *T. montanum* and *V. prostrata*, we recorded the following germination proportions: 32% and 11% in the first autumn (2006), 62% and 86% in the first spring (2007), and 6% and 3% in the second autumn (2007). Both species exhibited a germination peak in March and mortality occurred primarily in the first spring, with a peak in April. *P. vulgaris* germinated entirely in summer between mid-June and mid-August and the highest mortality occurred the following winter.

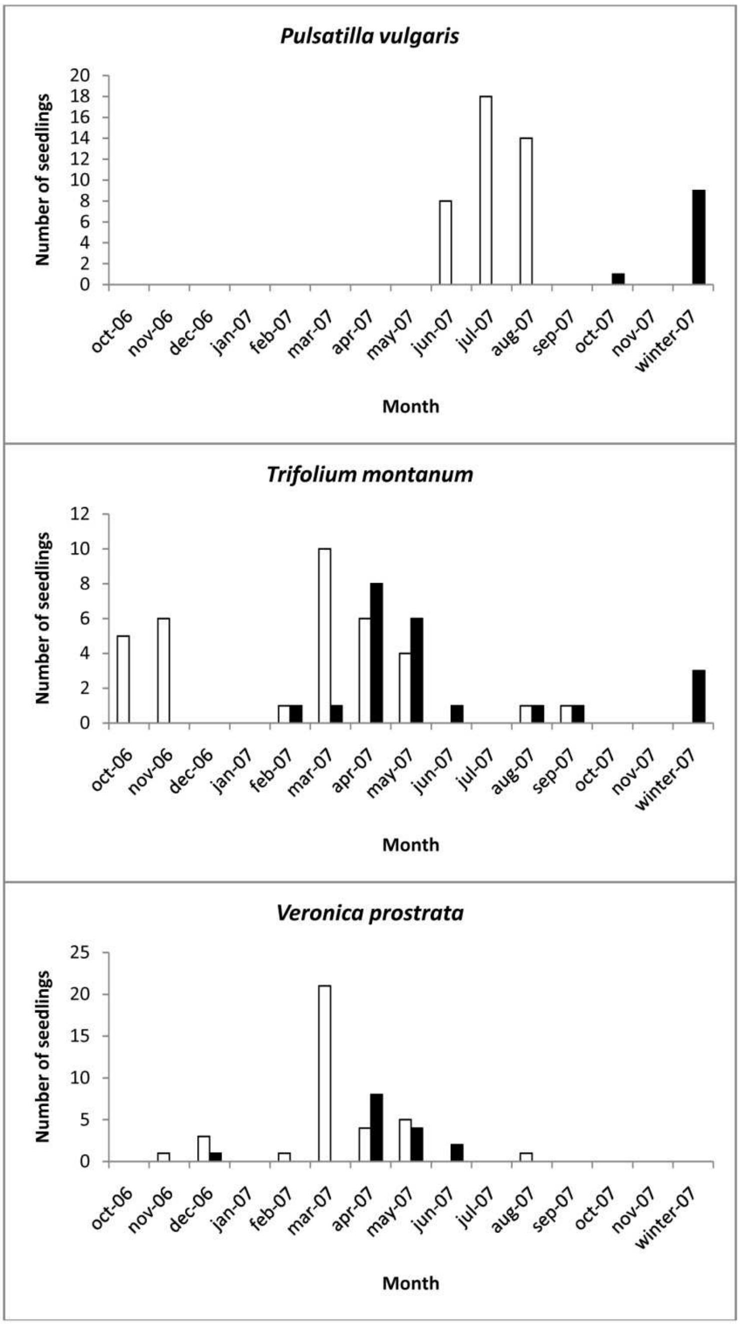

Figure 2: Emergence (white bars) and mortality (black bars) phenology in outdoor containers for the three study species.

V. prostrata germination and survival percentages were reduced by fire treatment (smoke + heat) relative to the control (0.06 vs. 0.31: P = 0.014, and 0 vs. 0.64, P = 0.012, respectively for germination rate and survival; Table 2). Moreover, *Brachypodium* litter reduced *V. prostrata* germination percentage (0.07 vs. 0.31, P = 0.014; Table 2). However, *Brachypodium* litter showed an increased in germination percentage (0.30 vs. 0.11, P = 0.014), final number of individuals (5.25 vs 1.25, P = 0.003) and survival (0.87 vs 0.44, P = 0.038) of *P. vulgaris* (Table 2). Finally, a significant positive effect of treatment was observed for *T. montanum* survival (0.12 vs. 0.57 and 0.60, respectively for control, fire and litter treatment, P = 0.016; Table 2). However the final number of individuals was not significantly increased despite an increase in germination percentage i.e. an inverse pattern was indicated.

Table 2: Response of the three study species in outdoor containers under three treatments. In Fire treatments, seed were submitted to heat (20 min at 60°C) and smoke (20min of smoke) before sawing. In litter treatment, brachypodium litter was set up on containers ground. P is the p-value of the performed test. When significant, pairwise comparison were computed; letter indicate significant differences.

	Treatment				
	Control	Fire	Brachypodium	test	*P*
Pulsatilla vulgaris					
Germination (/)	0.11 ^{ab}	0.09 ^{b}	0.30 ^{a}	$F_{[2;9]}$=5.84	**0.024**
Survivorship (/)	0.44	0.71	0.87	$\chi^2_{[2]}$=6.53	**0.038**
Final number of individuals	1.25 ^{b}	1.00 ^{b}	5.25 ^{a}	$F_{[2;9]}$=11.70	**0.003**
Seedling size (mm²)	270	215	577	$F_{[2;7]}$=2.88	0.122
Number of leaves	2.2	1.9	2.8	$F_{[2;7]}$=1.97	0.209
Trifolium montanum					
Germination (/)	0.21	0.09	0.12	$F_{[2;9]}$=0.30	0.750
Survivorship (/)	0.12	0.57	0.60	$\chi^2_{[2]}$=8.26	**0.016**
Final number of individuals	0.50	1.00	1.50	$F_{[2;9]}$=0.46	0.644
Seedling size (mm²)	49	2761	1732	$F_{[2;5]}$=1.24	0.366
Veronica prostrata					
Germination (/)	0.31 ^{b}	0.06 ^{a}	0.07 ^{a}	$F_{[2;9]}$=7.06	**0.014**
Survivorship (/)	0.64	0.00	0.83	$\chi^2_{[2]}$=8.87	**0.012**
Final number of individuals	4.00 ^{a}	0.00 ^{b}	1.25 ^{ab}	$F_{[2;9]}$=6.82	**0.016**
Seedling size (mm²)	14413	-	16768	$F_{[1;4]}$=0.10	0.771

DCA showed differentiation in species composition between reference and restoration parcels at each site (Fig. 3). It also detected differentiation among sites. "Les Grignaux" (target species: *P. vulgaris*) occupied more rocky microsites and included xerophilous species (e.g. *Carex humilis, Teucrium chamaedrys*). "Lorinchamps" (target species: *T. montanum*) was the most mesophilous site (Fig. 3). The three other sites (one for each target species) were more homogenous, although the three sites differentiated along axis 3 (results not shown).

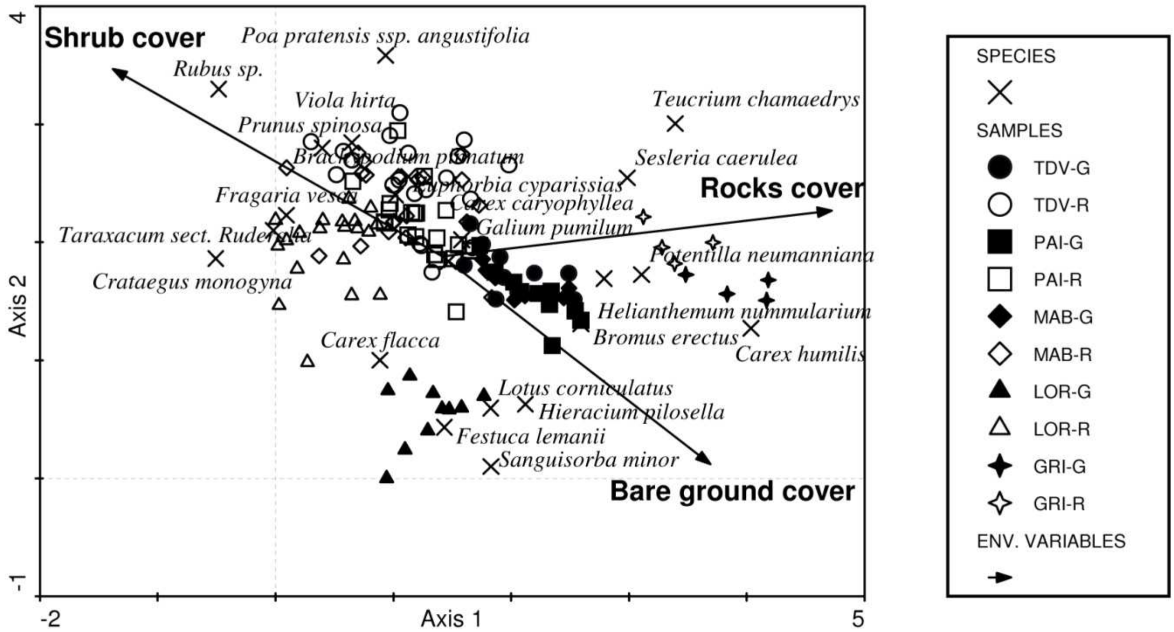

Figure 3: DCA scatter plot. Different shapes indicate different sites (TDV= "Tienne des Vignes"; PAI= « Les Pairées » ; MAB= « Montagne-aux-buis » ; LOR= « Lorinchamps » ; GRI= « Les grignaux »). Empty shapes are restored parcels (R), full shapes are reference grasslands (G). Shrubs, rocks and ground are respectively shrubs, rocks and bare ground covers.

In-situ experiments revealed highly contrasting patterns among species. Germination was not observed under any treatment conditions for *V. prostrata*. Twelve *T. montanum* seedlings were recorded. A preliminary ANOVA revealed several significant interactions for sites, notably for seed addition and disturbance (results not shown). As a consequence, we analyzed the effects of modalities and parcels in each site applying a two-way ANOVA (Fig. 4). The results revealed that germination did not occur in restored parcels at both sites (significant only at "Lorinchamps", P = 0.013). At "Les Pairées", only seed addition in undisturbed parcels lead to significant seedling emergence (2 seedlings, P = 0.05, Fig. 4). At "Lorinchamps", the combination of disturbance and seed addition increased seedling emergence in comparison to other modalities (7 seedlings vs. 0, 1 and 2 seedlings, respectively for the control, disturbance, and seed addition) but the differences were only marginally significant (P = 0.064). We observed a total of 105 *P. vulgaris* seedlings with similar patterns at "Lorinchamps" and "Les Pairées". Significantly lower seedling emergence was recorded in restored than in reference parcels (75 seedlings vs. 30 seedlings, P = 0.003; Fig. 4). Seedling emergence significantly increased with a combination of disturbance and seed addition, both in restored and reference parcels. The effect was more important when applied in reference grasslands (Fig. 4).

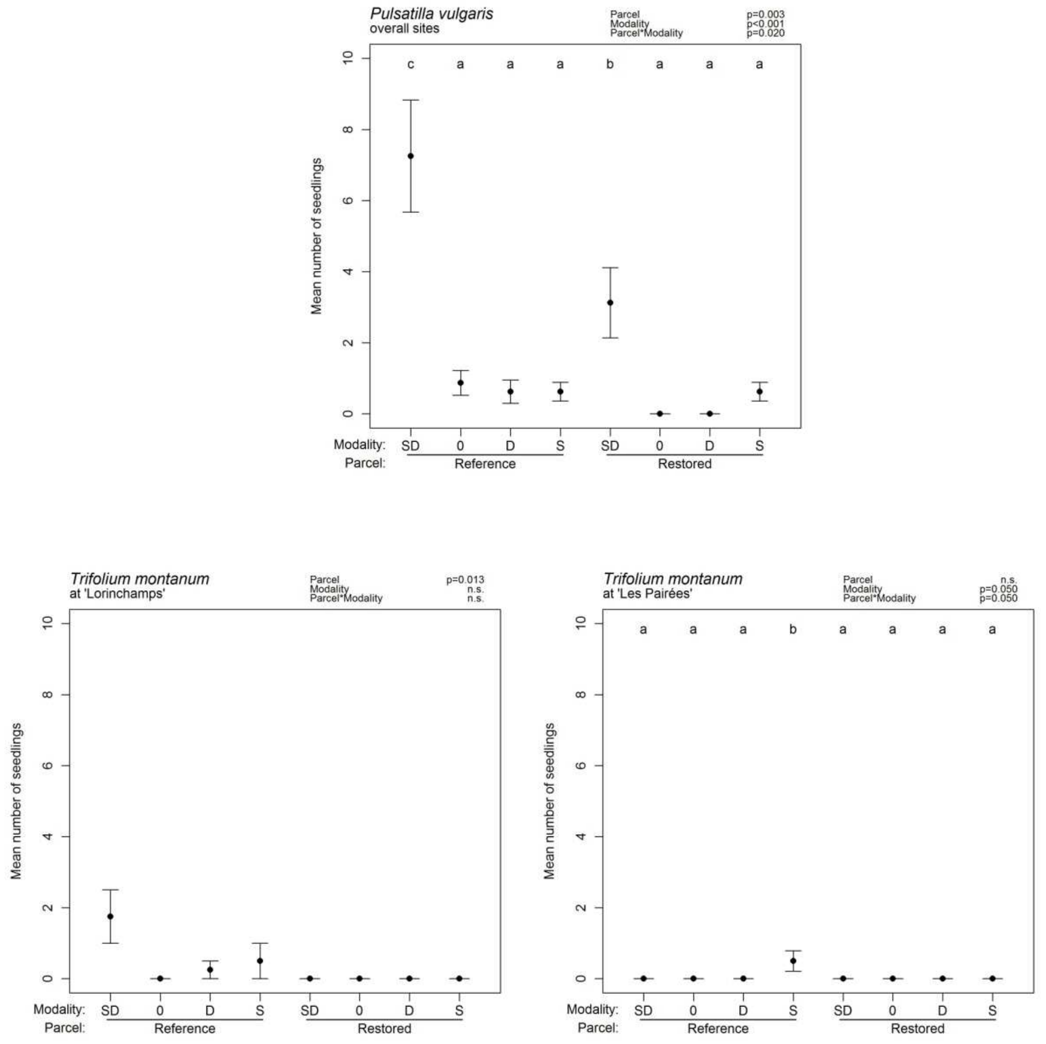

Figure 4: In-situ seedling emergence under the four treatments in the two parcel types for: (a) Pulsatilla vulgaris (2 sites pooled), (b) Trifolium montanum at the site "Lorinchamps", (c) Trifolium montanum at the site "Les Pairées". Error bars are 95% confidence intervals. The letters D and S were indicated respectively when disturbance and seed addition occurred.

Discussion

Germination and seedling development

The three species evaluated in this study generated viable seeds, with a germination percentage varying from 40% to 80% in Petri dishes in the absence of treatment. *T. montanum* germination without treatment was lowest. However, it reached approximately 45% over all treatments, which is consistent with the results of Schleuning and Matthies (2009). Dormancy breaking cues were species specific. Cold treatment increased the germination of *V. prostrata* and, to a lower extent, *P. vulgaris*. Cold treatment can be responsible for breaking physiological dormancy (Baskin & Baskin 1998). In outdoor containers, a small number of *V. prostrata* seeds germinated in autumn, the majority germinated in spring. This result could be related to a necessity for a cold period, which was demonstrated to enhance germination in Petri dishes. A similar phenological pattern was observed for *T. montanum*, although this species exhibited no significant increase in germinability after cold treatment. Mortality patterns in outdoor containers for *T. montanum* and *V. prostrata* revealed a mortality peak in spring and low mortality the following winter. *P. vulgaris* exhibited a different pattern, with all germinations occurring in summer and mortality observed the following winter. Spring germination allows a reduction in winter seedling mortality, while seedlings emerging in autumn have a competitive advantage if they survive, which is more likely during a mild winter. Bi-seasonality in seedling emergence can therefore be a bet-hedging strategy to reduce year-to-year variation in recruitment (Masuda & Washitani 1992). The *T. montanum* and *V. prostrata* mortality pattern likely reflects weather conditions than an intrinsic characteristic of the species. Indeed, the 2006-2007 winter was exceptionally mild in Belgium, with mean temperatures 3.5°C higher than usual over the period from December to February, with only a few days below 0°C. In other respects, an exceptional drought occurred in April 2007, with the total absence of rain for this month likely explaining the observed high mortality (MeteoBelgique 2010 for weather conditions).

None of the three species was favored by fire-related treatments (heat and smoke), regardless of the experimental conditions (Petri dishes or outdoor containers). *V. prostrata* exhibited a decreased germination percentage both in Petri dishes and outdoor containers following fire-related treatments. Fire-related cues, notably heat, can have a positive effect on germination by breaking dormancy. This has been shown in the seed bank for some specialist species of calcareous grasslands (Bossuyt & Honnay 2008). However, heat can also be responsible for the death of seeds (Baskin & Baskin 1998). Therefore, even in fire-prone habitats, response of germination to fire-related conditions remains species dependant (Crosti et al. 2006; Bossuyt & Honnay 2008).

Response to litter addition in outdoor containers was two-fold and species dependant. On the one hand, *T. montanum* and *V. prostrata* exhibited a decreased germination percentage in containers covered by *B. pinnatum* litter, although this effect was not significant for *T. montanum*. On the other hand, seedling survival was significantly higher for *T. montanum*. This dual effect of litter has already been described in fen grasslands (Rasran et al. 2007). Litter can compete with seedlings for light and mechanically prevent emergence (Xiong & Nilsson 1999). Alternatively, litter can protect seedlings against severe drought or chill. A similar effect was described for moss layer in calcareous grasslands (Jeschke & Kiehl 2008). As a whole, litter was favourable for *T. montanum* recruitment and exhibited a negative effect on *V. prostrata* evidenced by decreased germination. Finally, *P. vulgaris* exhibited the best performance in containers covered by litter. This result is congruent with Pfeifer et al. (2002), who found that artificial shadowing increased *P. vulgaris* performance under transplantation conditions.

Seed or microsite limitations?

A lack of seed can be limiting for both recruitment within populations or new site colonization in grassland habitats (Zobel et al. 2000; Donath et al. 2007; Edwards et al. 2007; Rasran et al. 2007). When seed addition enhances seedling emergence in restoration areas, this indicates dispersal limitations for new site colonization (Münzbergová & Herben 2005). We identified such a dispersal limitation for *P. vulgaris* that emerged in restored areas only with the addition of seeds.

V. prostrata and *T. montanum* did not emerge in restored areas. As a consequence, we cannot conclude that a positive or negative microsite or a dispersal limitation for colonization was operating in the restored site.

In reference grasslands, a positive effect i.e. an increase of seedling recruitment following seed addition was found for *T. montanum* and *P. vulgaris*. For *P. vulgaris,* it was only observed in combination with disturbance (a microsite limitation). This seed limitation result is not a consequence of a dispersal limitation but can be explained by a "source limitation" (sensu Clark et al. 2007), which implies that not enough seeds are produced to saturate the potential recruitment microsites. Moreover, seed addition can lead to predator satiation through a reduction in density-dependent seed mortality (Turnbull et al. 2000).

For *P. vulgaris*, a significant effect of seed addition and disturbance combination was shown in reference and restoration grasslands. In reference grasslands, each treatment alone did not result in more seedlings than the control treatment. Limitations to recruitment are therefore not a dichotomy of seeds vs. microsites, but rather a combination of both effects. Disturbance intensity can be responsible for the occurrence of different grassland species. Edwards et al. (2007) showed that the more intense the disturbance the greater the positive influence on seedling recruitment in restored chalk grasslands. It was already observed that disturbance increased the effectiveness of seed addition (Zobel et al. 2000; Hofmann & Isselstein 2004; Poschlod & Biewer 2005; Edwards et al. 2007). The same pattern was observed in *T. montanum* at the "Lorinchamps" site, suggesting microsite limitations at this locality. However, no significant test confirmed this trend. Microsite limitations alone seems to be species dependant (Kupferschmid et al. 2000; Zobel et al. 2000).

The results observed in outdoor containers are valuable to explain field results. Indeed, the better a species performed under *Brachypodium* litter in outdoor containers, the better it performed in the field. *P. vulgaris* that showed enhanced performance with litter exhibited the best emergence in the field in contrast to *V. prostrata* that did not emerge in the field. This pattern suggests that the 5 cm x 5 cm microsites used were too small for microsite dependant species, particularly *V. prostrata*. Therefore, it would be worth studying the effect of microsite size on *V. prostrata* recruitment and, to a lesser extent, *T. montanum.*

Finally, other factors not considered in this study could play a role in species recruitment. For example, *P. vulgaris* and *T. montanum* are known to host arbuscular mycorrhyzas (Harley & Harley 1987; Gucwa-Przepióra & Blaszkowski 2007). Mycorrhizal colonization level can be an important factor for seedling recruitment and colonization success of plant species (van der Heijden 2004; Eschen et al. 2009). Mycorrhizal fungi communities are known to differ sharply between grasslands and temperate forests (Öpik et al. 2006). To our knowledge, restoration of mycorrhizal fungal communities following grassland restoration by clear-cutting has never been studied.

Implications for conservation

One of the aims of ecological restoration is to provide new habitat opportunities for characteristic species in order to create new populations and increase population size and connectivity. In our study, while target species produce viable seed that survive under controlled conditions, the three target species failed at colonizing restored areas. Seedling emergence in restored areas could only be achieved after seed addition.

We observed between 1% and 5% in situ seedling emergence in *T. montanum* based on site. Although these percentages indicate low germination rates, they are slightly higher than those reported by Schleuning et al. (2009) for the same species and considered sufficient to maintain population size if management practices were suitable. A combination of sowing and disturbance facilitated the colonization of restored sites for *P. vulgaris*. However, fewer seedlings of *P. vulgaris* were observed in restored areas than in reference grasslands. Restored areas showed the absence of seedling emergence for *T. montanum* and *V. prostrata*. These results suggested that conditions in restored parcels are not suitable for species recruitment (seedling establishment and survival). DCA results indicated that habitats differed between restored and control/reference grasslands. Piqueray et al. (2010) reported that nutrient content did not differ between restored and reference parcels at these sites. However, it has been demonstrated that grassland forestation increased nutrient availability rather than nutrient content (Farley & Kelly 2004; Chen et al. 2008). Therefore, higher fertility and subsequent higher competition could persist in restored areas. Restored areas supported an increased frequency of the competitive grasses *B. pinnatum* and *Bromus erectus* (Piqueray et al. 2010).

Finally, our results revealed that both *T. montanum* and *V. prostrata* had lower seedling emergence under litter accumulation conditions and that the applied disturbance (in 5 cm x 5 cm microsites) was subject to a microsite limitations, particularly for these two species. Further studies are therefore necessary to determine if alternative management practices i.e. type, timing and intensity (different from the current grazing management) can improve microsite availability. Increased disturbance and gap creation is required to reduce litter accumulation, leading to better recruitment, essential in individual species population dynamics.

DISCUSSION GENERALE

Dynamique des paysages

La dynamique des paysages peut être appréhendée par la comparaison globale d'indicateurs de surface et de connectivité entre différentes périodes. Dans notre site d'étude du bassin de la Lesse, nous avons observé une forte diminution de la surface totale de pelouses calcicoles (448 ha en 1920 ; 59 ha en 2002), accompagnée d'une diminution de la taille moyenne de taches (4.67 ha en 1920 ; 0.31 ha en 2002) et de leur connectivité. Ce niveau de fragmentation (perte de 86% de la surface totale et diminution de 93% de la surface moyenne) est particulièrement élevé par rapport à ce qui a été observé dans d'autres régions d'Europe (Krauss et al. 2010). Par contre, le phénomène de fragmentation de grandes taches d'habitat en plus petites a eu pour conséquence d'augmenter le nombre de taches d'habitat (96 en 1920 ; 190 en 2002). Toutefois, cette augmentation ne s'est pas faite que par un morcèlement progressif des grandes taches d'habitat. Entre temps, de nombreuses taches d'habitat se sont formées et ont elles-mêmes subi un processus de fragmentation. Ce phénomène de création d'habitat n'est pas négligeable. Entre 1920 et 1965, 117 taches pour une surface totale de 152 ha se sont formées ; 57 autres (9 ha) sont apparues entre 1965 et 2002. Ces apparitions sont dues à différentes causes (création de routes, abandon de champs, création de layons de chasse en forêt, …). Une telle dynamique de coexistence de fragmentation et de création d'habitat a rarement été décrite dans la littérature (mais voir Jacquemyn et al. 2003b; Arroyo-Mora et al. 2004a; Cristofoli et al. 2009), la plupart des études décrivant un patron de fragmentation seul. Il est toutefois probable que l'apparition de nouvelles taches d'habitat au cours du temps ne soit pas l'apanage de notre site d'étude et de quelques autres, mais que cet aspect soit couramment négligé dans les études d'écologie du paysage. En réponse aux changements de structure de leur habitat, on s'attend à ce que les espèces répondent, mais pas forcément de manière immédiate. Il résulte que, durant une période, un état de déséquilibre subsiste où les taches d'habitat fragmentées conservent un surplus d'espèces hérité de leur structure paysagère passée et où les nouvelles taches d'habitat n'ont pas encore atteint leur richesse définitive (Hanski 2000). A la création involontaire d'habitats doit venir s'ajouter la restauration dirigée. Le projet LIFE-Nature mené dans la région a eu pour résultat la restauration d'environ 20 ha de pelouses calcicoles entre 2002 et 2005 (Piqueray et al. 2005). Toutefois, même en ajoutant ces 20 ha aux 59 ha présents en 2002, nous sommes encore loin des 448 ha de 1920, ou même des 353 ha de 1965. La fragmentation des pelouses calcicoles reste donc la tendance globale, malgré l'importante contribution relative des habitats récemment créés dans le paysage actuel.

Effets de la fragmentation

Dans notre site d'étude, les taches d'habitat ayant subi une forte fragmentation durant le siècle dernier arboraient, à l'heure actuelle, une richesse en espèces surestimée par rapport à ce qu'on pouvait attendre compte tenu de leur taille (Chapitre 4). Ces taches d'habitat présentent donc une dette d'extinction : un nombre d'espèces encore présentes actuellement, mais vouées à disparaître. Ce phénomène n'est pas rare dans les pelouses calcicoles. Il avait déjà été observé précédemment par Helm et al. (2006) et par Lindborg & Eriksson (2004a). Toutefois, il semble que ce ne soit pas un cas général, spécialement dans les paysages fortement fragmentés, tels que le nôtre (Adriaens et al. 2006; Cousins et al. 2007). Il faut toutefois noter que, comme Kuussaari et al. (2009) l'avaient déjà pressenti, nous avons mis en évidence que des différences dans les méthodes utilisées pour détecter la dette d'extinction pouvaient mener à des différences dans les résultats. Dès lors, notre étude dénote avec la tendance qui semblait se dégager que dans les paysages fortement fragmentés, les habitats étaient exempts de dette d'extinction, mais uniquement du fait de la prise en compte d'aspects méthodologiques. Nous avons montré que l'effet de la dynamique individuelle des taches d'habitat (pourcentage de perte) pouvait s'avérer plus important que l'état à un moment donné. Si ce paramètre n'avait pas été pris en compte dans notre étude, nous aurions conclu, tout comme Adriaens et al. (2006) et Cousins et al. (2007), que notre paysage ne présentait pas de dette d'extinction.

Au-delà de l'estimation de la dette d'extinction globale à l'échelle du paysage, il est important de considérer les effets de la fragmentation sur les espèces individuellement, cette information étant plus susceptible d'intéresser les gestionnaires. Par ailleurs, l'étude des traits fonctionnels rendant les espèces plus ou moins sensibles présente l'intérêt supplémentaire de la comparabilité à d'autres pools d'espèces. Comme cela avait déjà été mis en évidence par d'autres auteurs (Fischer & Stöcklin 1997; Poschlod et al. 2000), nous avons pu montrer que les espèces clonales étaient moins sensibles à la fragmentation. Du fait de leur long temps de vie, ces espèces sont moins susceptibles de s'éteindre. Par contre, la capacité à former une banque de graine persistante n'a pas été identifiée comme un avantage pour la survie dans un paysage fragmenté, bien que cela ait été décrit dans d'autres études (Stöcklin & Fischer 1999; Fréville et al. 2007). Nous avons par ailleurs montré que la capacité à former des populations denses, donc de grandes populations dans des habitats de taille réduite était un avantage. Il est toutefois difficile de distinguer cet effet de celui de la clonalité, les deux traits étant fortement liés.

La présence de cette dette d'extinction n'est pas sans conséquences en termes de restauration des habitats. D'une part, elle peut contribuer à l'établissement des priorités quant aux sites à restaurer. En effet, on peut espérer que la restauration des paramètres spatiaux des sites ayant subi une forte fragmentation pourra permettre d'éviter une partie des extinctions. D'autre part, cette dette doit absolument être prise en compte lors de l'évaluation de la restauration. On ne peut en effet pas attendre que les habitats restaurés atteignent l'état des pelouses actuelles si celui-ci n'est que le reflet d'une situation passée. Une situation similaire peut être observée lorsque la composition floristique d'une pelouse est en partie le résultat de son utilisation ancienne (Dutoit et al. 2004; Karlík & Poschlod 2009).

Réponse à la création d'habitats

Filtre à la dispersion

Les études théoriques et expérimentales sur les capacités de dispersion des espèces typiques des pelouses calcicoles ont abouti à la conclusion que ces espèces possédaient des capacités de dispersion relativement limitées, de l'ordre de quelques mètres (Verkaar et al. 1983). Stampfli & Zeiter (1999) ont même affirmé que la plupart des espèces des pelouses calcicoles auraient des capacités de dispersion inférieures à 25 m. De même, la plupart de ces espèces sont connues pour leur incapacité à former une banque de graines viable (Kalamees & Zobel 1997; Thompson et al. 1997; Bisteau & Mahy 2005b) et peu d'entre elles sont capables de persister dans les habitats qui découlent de leur abandon (Poschlod et al. 1998). Dès lors, on pourrait s'attendre à ce que le filtre à la dispersion soit une contrainte importante pour la reconstitution du cortège floristique typique lorsqu'une nouvelle opportunité est créée dans le paysage. Toutefois, l'étude de zones restaurées il y a moins de 15 ans a permis de montrer que de nombreuses espèces typiques des pelouses calcicoles étaient capables de coloniser ces sites dans ce laps de temps. D'autres études similaires étaient arrivées aux mêmes conclusions. Dans un premier temps, les espèces rudérales dominent, mais après une dizaine d'années, ce sont principalement des espèces typiques des pelouses qui sont présentes (von Blanckenhagen & Poschlod 2005; Schrautzer et al. 2009). De même, la richesse spécifique de ces anciennes restaurations est le plus souvent proche de celle des pelouses de référence (Zobel et al. 1996; Dzwonko & Loster 1998; Lindborg & Eriksson 2004b). Le succès observé a le plus souvent été attribué à la réintroduction du pâturage ovin suite à la restauration, tel que ce fut le cas dans notre site d'étude. Il a en effet été montré que les moutons transportaient de nombreuses graines dans leur toison et leurs excréments (Hellström et al. 2003; Couvreur et al. 2004b) et pouvaient donc jouer un rôle important dans la dispersion des espèces des pelouses calcicoles (Fischer et al. 1996; Adriaens et al. 2007). Cependant, il découle des résultats de cette thèse que le pâturage, bien qu'il joue probablement un rôle important, ne peut être considéré comme seul responsable du succès de la colonisation dans les pelouses calcicoles. En effet, l'étude des taches d'habitat nouvellement créées dans le paysage (< 40 ans), et qui n'avaient jamais été pâturées au moment des relevés de terrain se sont révélées être aussi riches en espèces et de composition floristique très proche des anciennes pelouses. Ce résultat est en contradiction avec les études qui ont démontré que les espèces des pelouses calcicoles possédaient de faibles capacités de dispersion (voir références dans Hutchings & Stewart 2002). Cependant, ces études ont principalement analysé la pluie de graines sur des surfaces et des laps de temps limités. Ces méthodes laissent peu de chance d'observer des évènements de dispersion à longue distance qui sont rares, mais qui peuvent suffire à la colonisation de sites distants (Nathan et al. 2002). Par ailleurs, certaines espèces peuvent survivre dans des habitats marginaux (bords de routes, talus, lisières et clairières en forêt, …), ce qui peut réduire les distances sur lesquelles les espèces doivent se disperser (Kiviniemi & Eriksson 1999). Il faut enfin noter que certains de ces nouveaux sites ont pu bénéficier, par le passé, d'un paysage nettement moins fragmenté que celui observé actuellement.

Cependant, que ce soit dans les parcelles restaurées ou dans les taches d'habitat nouvellement créées, nous avons pu observer que certaines espèces présentaient des difficultés à coloniser les sites. Dans les deux cas, certaines espèces restaient mieux représentées dans les zones de référence. Dans le cas des parcelles restaurées, nous avons même pu identifier des espèces totalement absentes, malgré leur présence dans les parcelles de référence adjacentes. Un cas similaire avait déjà été mis en évidence par Kiefer & Poschlod (1996). Il a été montré qu'une faible disponibilité en graines pouvaient limiter la colonisation de nouveaux sites (Zobel et al. 2000; Foster 2001; Hofmann & Isselstein 2004; Donath et al. 2007; Edwards et al. 2007). Dans le cas de *Pulsatilla vulgaris*, nous avons pu montrer que le transfert de graines vers les sites restaurés aboutissait à l'installation de l'espèce, ce qui nous permet de conclure à une limitation à la dispersion (Münzbergová & Herben 2005). Dès lors, il serait intéressant de tester l'effet de semis dirigés de graines dans les zones restaurées en compléments des actes de gestion actuellement pratiqués dans le site d'étude.

Filtres abiotique/biotique

Les conditions abiotiques, en particulier les conditions physico-chimiques du sol peuvent être un facteur limitant la restauration des habitats (Marrs 1993; Walker et al. 2004). Les pelouses calcicoles étant, par nature, des milieux pauvres en éléments nutritifs et de faible productivité (Grime 1979; Critchley et al. 2002a), leur restauration requiert de retrouver des conditions de sol adéquates. Toutefois, les études sur la restauration qui ont abouti à la conclusion que les conditions physico-chimiques du sol étaient un facteur limitant la restauration des pelouses calcicoles ont étudié la restauration à partir d'anciennes terres cultivées, et donc bien souvent amendées. Dans ce cas, la réduction du niveau de fertilité s'avérait effectivement, et de manière assez générale, comme une des contraintes principales (Walker et al. 2004).

En ce sens, le cas de la restauration à partir de parcelles forestières, pour lequel l'étude des conditions physico-chimiques du sol n'avait jamais été abordée, s'est avéré drastiquement différent. Nous n'avons pu mettre en évidence aucun enrichissement en éléments nutritifs majeurs (N, P et K), tel que cela a été montré dans le cas des restaurations à partir de terres cultivées (Walker et al. 2004; Fagan et al. 2008). Cependant, suite à l'afforestation, nous avons observé un ralentissement de la minéralisation (augmentation du rapport C:N) persistant dans les jeunes restaurations. Toutefois, plus aucune différence n'a pu être observée entre les anciennes restaurations et les pelouses de référence, ce qui laisse entendre que les conditions physico-chimiques du sol, telles que nous les avons estimées, ne sont pas une contrainte majeure pour la restauration de pelouses calcicoles à partir de sites forestiers. Cependant, d'autres caractéristiques du sol que nous n'avons pas mesurées pourraient avoir été modifiées, notamment les formes et donc la disponibilité de l'azote ou du phosphore (Farley & Kelly 2004; Chen et al. 2008).

Dans le cas des pelouses calcicoles, les filtres abiotiques ne sont pas toujours évidents à distinguer des filtres biotiques. En effet, la richesse en éléments nutritifs n'est probablement pas le facteur limitant directement les espèces des pelouses calcicoles, comme en témoigne l'aisance avec laquelle nous avons pu cultiver dans un terreau commercial trois espèces strictement inféodées à ces pelouses: *Pulsatilla vulgaris*, *Trifolium montanum* et *Veronica prostrata*. En réalité, l'enrichissement en éléments nutritifs mène à la dominance de quelques espèces compétitives au détriment des espèces qui le sont moins (Bakker 1987; Pywell et al. 2002). Notamment, l'enrichissement en azote tend à favoriser la dominance de *Brachypodium pinnatum* dans les pelouses calcicoles (Bobbink et al. 1998). Bien que les causes n'aient pu être établies, nous avons observé dans les pelouses restaurées une dominance croissante des graminées sociales (*Brachypodium pinnatum* et *Bromus erectus*), qui sont des espèces très compétitives. Dès lors, le niveau de compétition plus important qui en résulte pourrait agir comme un filtre biotique à la colonisation des sites restaurés.

Aspects méthodologiques

Au-delà des causes «biologiques» de l'absence de certaines espèces dans les pelouses restaurées, d'autres éléments sont à prendre en compte, notamment les aspects méthodologiques. Premièrement, les hasards de l'échantillonnage peuvent être responsables de l'absence observée de certaines espèces. Toutefois, il reste que certaines espèces étaient, sans équivoque, effectivement absentes des zones restaurées. Deuxièmement, le choix des anciennes pelouses comme sites de référence peut introduire un biais dans les conclusions. Il a été montré que la structure ancienne du paysage avait encore actuellement une influence sur la richesse des pelouses et sur l'occurrence de certaines espèces, créant une dette d'extinction dans le paysage. Lindborg & Eriksson (2004a) ont en outre montré que la structure du paysage pouvait influencer la densité en espèces (nombre d'espèces par unité de surface). Par ailleurs, l'utilisation ancienne des pelouses, notamment pour l'agriculture[1] peut aussi influencer la composition floristique actuelle (Dutoit et al. 2004; Karlík & Poschlod 2009). Il résulte de cela que l'état actuel des pelouses de référence est le fruit de son histoire récente et ancienne, et

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[1] Plusieurs pelouses qui ont été choisies comme références montrent des traces anciennes d'agriculture, notamment des pierriers et la présence d'espèces messicoles.

que ces milieux sont appelés à subir des changements dans le futur. On peut dès lors se poser la question de la pertinence de choisir ces milieux comme référence et du réalisme de vouloir les restaurer à l'identique. Cette affirmation doit toutefois être balancée par le fait qu'aucune action de restauration ne devrait être réalisée sans objectif défini (Perrow & Davy 2002). Atteindre l'état des pelouses calcicoles anciennes est l'objectif évident d'un programme de restauration de ces milieux.

Espèces invasives

L'étude sur la naturalisation de *Cotoneaster horizontalis* dans les pelouses calcicoles a permis de montrer, qu'à l'heure actuelle, l'espèce n'est présente que de manière relativement limitée dans ces habitats. Il semble que seules les pelouses du *Xerobromion* soient touchées. En Grande-Bretagne, il a été rapporté que les pelouses des falaises côtières étaient aussi touchées (Crofts & Jefferson 1999). Etant donnés les impacts importants occasionnés dans les zones envahies, il convient de surveiller cette espèce afin de limiter son expansion. En effet, il a été montré dans d'autres milieux ouverts d'intérêt que les invasions biologiques pouvaient se déclarer très rapidement. Dans les milieux dunaires, où l'on retrouve entre autres des pelouses sèches, *Rosa rugosa* a envahi de nombreux habitats à travers toute l'Europe durant les dernières décennies (Bruun 2006; Kollman et al. 2007). Cette espèce est, comme *C.horizontalis*, une Rosaceae originaire d'Asie, introduite pour l'ornement, clonale et dispersée par les oiseaux. Depuis quarante ans, l'espèce s'est largement répandue, notamment aux Pays-Bas, au Danemark et en Allemagne (Bruun 2005), occasionnant des pertes de diversité, notamment de richesse spécifique (Grootjans et al. 2002; Isermann 2007; Kiehl & Isermann 2007). Ce cas montre que les invasions biologiques peuvent se déclencher rapidement et qu'il est nécessaire d'agir aux premiers stades de l'invasion. Dans l'état actuel des choses, étant donné le nombre limité d'individus, il est probable que la gestion des populations établies soit encore possible. Toutefois, cette gestion ne sera totalement efficace que si elle est accompagnée d'actions de sensibilisation ou de législation visant à limiter le commerce de l'espèce.

Conclusions

Apports de la thèse à l'étude de la conservation des pelouses calcicoles et perspectives

Cette thèse a permis d'identifier deux menaces pesant sur les pelouses calcicoles de Belgique. D'une part, la réponse différée des espèces à la fragmentation a entraîné la formation d'une dette d'extinction; un nombre d'espèces persiste dans certaines taches d'habitat malgré une structure spatiale qui leur est défavorable. D'autre part, il a été montré que *Cotoneaster horizontalis*, une espèce horticole originaire d'Asie, était capable de s'implanter, de croître et de fructifier dans les pelouses calcicoles, principalement dans les faciès les plus secs. Ces deux menaces présentent un point commun : ce sont des menaces latentes contre lesquelles des solutions potentielles existent.

L'état actuel des populations de *C.horizontalis* est tel qu'une gestion ciblée de cette espèce est probablement réalisable. Il est toutefois nécessaire que des tests soient réalisés afin d'identifier les techniques de gestion les plus efficaces. Par ailleurs, la prise de conscience progressive des pouvoirs politiques et du secteur horticole permet d'espérer que le commerce d'espèces à caractère invasif se réduise dans les années à venir.

Par ailleurs, les effets de la fragmentation, y compris la dette d'extinction pourraient être réduits par la restauration d'un réseau fonctionnel d'habitats. Les résultats de cette thèse ont montré que la restauration était efficace pour un grand nombre d'espèces typiques qui sont capables de coloniser les sites restaurés. Pour les autres espèces, on peut espérer que le temps fera une partie du travail. Dans le cas contraire, il sera peut-être utile d'envisager de réintroduire les espèces. Notre étude sur les limites à la colonisation de trois espèces typiques des pelouses calcicoles a permis de montrer que la disponibilité en graines et en microsites de germination pouvaient être responsable de l'échec de certaines espèces à coloniser les sites restaurés. Toutefois, cette étude a surtout abouti à de nouvelles questions. D'une part, pour une des espèces (*Veronica prostrata*), l'ensemencement n'a permis d'obtenir aucune plantule, même dans la pelouse ancienne où l'espèce était présente. L'étude comparative de différents modes de gestion permettrait de déterminer les modalités les plus profitables à cette espèce. D'autre part, *Trifolium montanum* et *Pulsatilla vulgaris* ont montré des taux de recrutement plus faibles en zones restaurées que dans les anciennes pelouses, ce qui suggère que la qualité de l'habitat serait moins bonne dans ces zones restaurées. Ceci rejoint la conclusion de l'étude floristique de ces zones restaurées qui a montré que les communautés végétales étaient différentes. Les analyses de sols n'ont pu révéler de différences entre les pelouses anciennes et restaurées. D'autres indicateurs pourraient permettre de déterminer les causes des différences observées telles que la productivité des milieux (biomasse produite annuellement), les formes de l'azote dans le sol ou encore les taux de mycorhyzation.

Il serait utopique de vouloir restaurer le réseau d'habitat de 1920 ou de 1965, bien qu'il s'agisse probablement du moyen le plus efficace d'éviter le « paiement » de la dette d'extinction. On peut raisonnablement penser qu'une partie importante des sites anciennement occupés par des pelouses calcicoles en Lesse et Lomme et dans le Viroin (voir Adriaens et al. 2006), notamment les sites qui ont été aforestés, sont potentiellement restaurables. La question est de savoir si notre société veut se donner les moyens de conserver la biodiversité exceptionnelle de notre pays.

Implications en termes de stratégie de conservation

La présence de *Cotoneaster horizontalis* dans les pelouses calcicoles de Haute-Meuse devrait être surveillée. La présence de l'espèce est encore assez limitée actuellement. Il en résulte qu'une gestion ciblée de l'espèce est encore envisageable. Des essais sont nécessaires afin de définir les modalités de gestion. L'interdiction du commerce de cette espèce s'avère toutefois indispensable pour limiter son expansion.

La présence d'une dette d'extinction dans les pelouses calcicoles de la région de Han-sur-Lesse – Rochefort implique que la menace qui pèse sur ces milieux a été sous-estimée. La stratégie qui s'impose afin d'éviter le « paiement » de cette dette est de continuer l'effort de restauration initié dès la fin des années 1990. La présence d'une dette d'extinction apporte aussi un argument quant au choix des sites à restaurer. Les sites dont la surface a été fortement réduite durant le siècle dernier sont les plus susceptibles de présenter une dette d'extinction et sont donc à restaurer en priorité. Il en va de même pour les sites abritant des populations d'espèces dépendantes de grandes surfaces de pelouses (voir chap. 5)

Les études sur la colonisation de nouvelles taches d'habitats (chap. 6) et d'habitats restaurés (chap. 4) ont montré que les pelouses calcicoles se reformaient assez rapidement lorsque de nouvelles opportunités se présentaient. Cet argument appuie l'idée de maintenir l'effort de restauration, étant donné que celui-ci ne se fait pas en vain. Toutefois, il a été montré que certaines espèces présentaient des difficultés à coloniser les sites nouvellement créés. Dans certains cas, l'introduction par semis suffit pour que ces espèces s'établissent dans les sites restaurés. Cette technique peut être améliorée par la création de microsites pour la germination.

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