

THÈSE DE DOCTORAT D'AVIGNON UNIVERSITÉ

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'Sciences et Agrosciences '

Spécialité / Discipline de doctorat : Biologie

Aix Marseille Univ. Avignon Université, CNRS, IRD, IMBE, Avignon, France
ECO-MED, Marseille, France

Présentée par

Aure Durbecq-Bassoul

**Ecological restoration and early plant community
assembly of degraded mountain grasslands
in the Southern French Alps**

**Restauration écologique et assemblage précoce des communautés
végétales de prairies montagnardes dégradées dans les Alpes du
sud de la France**

Soutenance publique prévue le 31/05/2021 devant le jury composé de :

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Dr Vicky Temperton, Professeure, Université de Lüneburg, Allemagne
Dr Marie-Lise Benot, Maître de conférences, BIOGECO, Université de Bordeaux
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Mr. Alexandre Cluchier, Directeur R&D et Directeur International, ECO-MED
Dr. Truman Young, Professor Emeritus, University of Davis, California, USA

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Résumé

La dégradation écologique à grande échelle des prairies et pelouses augmente dans le monde entier, principalement en raison des changements d'utilisation des terres et de la destruction des habitats. La construction d'une ligne électrique à haute tension dans la zone d'étude, la vallée de la Haute-Durance dans les Hautes-Alpes, a entraîné une dégradation des sols et des communautés végétales le long de voies d'accès transitoires et de plates-formes de construction. Dans les écosystèmes perturbés de la zone d'étude, la restauration est limitée par un manque de propagules disponibles et un faible recrutement des graines sur sols peu profonds et caillouteux caractérisés par une sécheresse estivale marquée. Mon étude se concentre sur la restauration de prairies sèches à mésophiles riches en espèces couvrant une grande partie de la zone de construction, situées entre 1000 et 1400 mètres d'altitude. La plupart de ces prairies sont des habitats prioritaires Natura 2000 tels que N6210 'Prairies sèches semi-naturelles de Festuco-Brometalia et faciès de garrigue sur substrats calcaires'(directive UE habitat 92/43 / CEE).

Ma thèse vise à mieux comprendre les facteurs limitant la restauration des prairies de montagne après une perturbation du sol, et à identifier des techniques de restauration pour compenser la dégradation. Le chapitre 1 se concentre sur l'identification d'une référence appropriée qui est une étape cruciale pour la restauration écologique. Afin de comprendre les mécanismes favorisant l'établissement des espèces cibles, cinq pelouses semi-naturelles dégradées ont été utilisées pour mettre en place trois expériences différentes, en utilisant une approche basée sur les filtres d'assemblage des communautés pour analyser les résultats de la restauration. Dans la première expérimentation (Chapitre 2), j'ai examiné si la préparation du sol était nécessaire pour fournir des conditions de sol plus appropriées et réduire la compétition avec la végétation préexistante, et pour voir si le pâturage extensif traditionnel devrait être exclu lors des premières étapes de restauration. Dans la deuxième expérimentation (Chapitre 3), je me suis concentrée sur différentes techniques de transfert de propagules de plus en plus utilisées dans les prairies de montagne (propagules récoltées à la brosseuse et transfert de foin), et j'ai analysé l'effet potentiellement facilitateur d'un couvert végétal mort (foin) et vivant (blé) sur le recrutement des propagules transférées et sur l'érosion du sol. Enfin, comme l'assemblage des communautés est également fortement influencé par la dispersion, qui elle-même peut conduire à des effets de priorité, j'ai testé le semis séquentiel de différentes combinaisons d'espèces végétales (dominantes vs. subordonnées) dans une troisième expérimentation et j'ai analysé comment il affecte l'assemblage des communautés (Chapitre 4).

Les résultats ont montré que l'analyse des interactions plante-environnement fournit un outil important pour identifier les communautés de référence ou les sites sources pour la récolte de propagules, d'autant plus si ces sources ne sont pas disponibles à proximité des habitats dégradés.

Les techniques de restauration appliquées ont indiqué que la réduction de la compétition par la préparation du sol avant l'ajout de graines issues de la brosseuse avait un effet positif sur le recrutement des espèces transférées, et peut donc être clairement recommandée dans mon système d'étude. De plus, j'ai constaté que le faible effet négatif du pâturage ne justifiait pas toujours les coûts des exclos. Le paillis de foin a favorisé le recrutement des semis d'espèces cibles, mais le transfert de propagules sans couche de paillis devait être compensé par un couvert végétal temporaire pour être efficace, ce qui suggère que la restauration des prairies de montagne avec des sols peu profonds et caillouteux bénéficie clairement d'un effet facilitateur de couvert végétal mort (foin) ou vivant (blé). Enfin, le chapitre 4 a fourni des preuves que l'assemblage de la communauté végétale était influencé par l'ordre d'arrivée des espèces, mais a aussi mis en évidence de fortes variations de réponse entre les espèces, suggérant des effets de priorités spécifiques à chaque espèce. La force des effets de priorité varie en fonction des caractéristiques de la niche qui diffèrent entre les espèces et peuvent influencer (négativement ou positivement) l'établissement des espèces arrivant ultérieurement.

Mots clés : assemblage des communautés végétales, écologie de la restauration, écosystème de référence, effets de priorité, dégradation du sol, pelouse de montagne, prairie de montagne, succession végétale, restauration écologique, transfert de propagule.

Abstract

Large-scale ecological degradation of grasslands is increasing worldwide mainly due to land use changes and habitat destruction. The construction of high-voltage transmission lines in my study area, the Haute-Durance valley in the Southern French Alps, involves a degradation of grassland soil and plant communities along transitory access tracks and construction platforms. In these degraded ecosystems of the montane altitudinal belt, restoration is limited by a lack of available propagules and low seedling recruitment on stony and shallow soils characterised by summer drought. I focus on the restoration of species-rich dry to mesophilic grasslands covering a large part of the construction zone between 1000 and 1400 m. Most of these grasslands are Natura 2000 priority habitats, such as N6210 “Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates” (EU directive habitat 92/43/EEC).

My thesis aims to understand better the factors limiting the restoration of mountain grasslands after soil disturbance, and to identify successful restoration techniques to compensate the degradation. Chapter 1 is focused on the identification of appropriate reference communities, which is a crucial step in ecological restoration. In order to understand the mechanisms favouring target species establishment, five degraded semi-natural grasslands were used to set up two different experiments, using a filter-based community assembly approach of community dynamics to analyse the restoration outcome. In the first one (Chapter 2), I examined whether soil preparation is required to improve soil conditions and to reduce competition of pre-existing vegetation, and whether traditional extensive grazing should be excluded in initial stages of grassland restoration. In the second experiment (Chapter 3), I focused on different techniques of propagule transfer increasingly used in mountain grasslands (brush-harvested propagules and hay transfer), and I analysed the potentially facilitative effect of dead (hay) and living (wheat) plant cover on the recruitment of transferred brush-harvested propagules, and on soil erosion. Finally, as community assembly is also highly influenced by dispersal that may lead to priority effects, I tested sequential sowing of different combinations of plant species (dominants vs. subordinates) (Chapter 4) and I analysed how they affect community assembly.

The results demonstrated that the analysis of plant-environment interactions provides a useful tool to identify reference communities or donor sites for seed transfer, particularly if donor sites are not available adjacent to degraded habitats. The restoration techniques applied indicated that the reduction of competition by soil preparation before seed addition of brush material had a positive effect on the transferred species recruitment and can thus clearly be recommended in my study system. Furthermore, I found that the weak negative effect of grazing may not always justify fencing costs.

Hay mulch favoured seedling recruitment of target species, but propagule transfer without mulch layer needed to be compensated by additional temporary plant cover, suggesting that restoration of mountain grasslands with shallow and stony soils clearly benefits from a facilitative effect of dead (hay) or living (wheat) vegetation cover. The chapter 4 provided evidence that plant community assembly is influenced by the order of arrival but highlighted strong response variations between species suggesting high species-specific priority effects. The strength of priority effects probably varies according to the niche characteristics of different species and may influence (negatively or positively) the late-arriving species establishment.

Keywords: ecological restoration, mountain grassland, plant community assembly, plant succession, priority effects, propagule transfer, reference ecosystem, restoration ecology, soil disturbance.

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Chaque chose de la nature contient une part de merveilleux

Aristote



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Ecological restoration and early plant community assembly of degraded mountain grasslands in the Southern French Alps

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General introduction

I. Ecological theory and restoration ecology

I.1. Ecological restoration and restoration ecology

Ecological restoration and restoration ecology are closely related (Palmer et al. 2006). The Society for Ecological Restoration (SER), a worldwide organisation founded in 1987 to guide restoration science and policy, defines restoration ecology as the scientific process that develops theories guiding ecological restoration, and ecological restoration as “the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed” (hereafter collectively referred to as ‘disturbance’; Gann et al. 2019). Disturbance is an event that disrupts ecosystem, community, or population structure, thus changing the physical environment and its resources (Pickett and White 1985). Such ecological disturbance may result in a partial or total loss of plant biomass (Grime 1979; Sousa 1984; Rykiel 1985; White & Jentsch 2004). Therefore, the aim of most restoration ecologists is to recover the ecosystem with its original structure, composition, functions, and dynamics, by assisting habitat repair and recolonisation of local animal and plant species that were present before the disturbance.

Although ecosystems are continuously facing natural disturbances (White & Jentsch 2004), human activities are nowadays the most important source of disturbance (UN Environment 2019). Since the beginning of the industrial revolution, humanity has been responsible for global environmental changes transforming ecosystems at an unprecedented rate causing significant damage to systems on which living beings depend (Hobbs and Harris 2001; Steffen et al. 2006). Human disturbances are responsible for massive natural habitat losses, for the extension of urban and agricultural areas (Donald 2004; Pansu 2014) and for an unprecedented global species extinction, often described as the irreversible sixth extinction crisis (Ceballos et al. 2010; Chapin III et al. 2000; Lenzen et al. 2012). Land use transformation facilitated biological invasions and overexploitation of natural resources, pollution and climate change are continuous key processes contributing to biodiversity loss (Vitousek et al. 1997). The growing need to ‘repair ecosystems’ in the face of global change is now taken into account in political decisions. The United Nations have even declared the years 2021-2030 as the “Decade of Ecosystem Restoration”. Hence, there is an increasing need to improve knowledge on *how to restore degraded habitats?* Since the end of 20th century, many standards and practical guides have been published including the *SER International Primer on Ecological Restoration* (SER 2004), *Ecological Restoration for Protected Areas: Principles, Guidelines and Best Practices* (Day et al. 2019), the *National Standards for the Practice of Ecological Restoration in Australia* (McDonald et al. 2016), and

the *International principles and standards for the practice of ecological restoration* (Gann et al. 2019). These guides highlight the key stages of ecological restoration projects. However, the application of these principles must be adapted to the local context, such as habitat type and specific disturbance effects. Ecological restoration is therefore a context-based approach and restoration ecologists need to be familiar with general ecological theory that is the baseline of all restoration projects (Gornish et al. 2019; Lindenmayer 2020; Palmer et al. 2006; Palmer et al. 2016; Temperton et al. 2004; Young et al. 2005).

I.2. A synthesis of theory and practice

Today, it is commonly accepted by restoration ecologists that ecological theory and ecological restoration are closely connected. Since the beginning of restoration ecology as a discipline, many authors have emphasized this interdependence of theory and practice, which has spurred a particular enthusiasm resulting in several book publications (Bradshaw 1987; Hobbs et al. 2009; Palmer et al. 2006, 2016; Temperton et al. 2004; Walker et al. 2007). However, ecological theory is still not well integrated in restoration practice and recently, several authors advocated a stronger consideration of theory (Audino et al. 2017; Corline et al. 2021; Halassy et al. 2019; Lindenmayer 2020). The objectives of restoration ecology and ecological sciences are different since the former intends to guide the repair of ecosystems that have been degraded, while the latter aims to ultimately understand and explain how organisms interact with their environment. However, the restoration of degraded ecosystems requires a good understanding of ecosystem structure, mechanism, dynamics and natural variability (Menninger & Palmer 2006; Suding & Gross 2006). In fact, the theoretical models of succession and assembly of communities are at the heart of restoration practice (Young et al. 2005). Ecological theory provides a framework that helps identifying the underlying mechanisms and ecological processes behind the observed patterns, and guidelines may be designed to improve predictions for the outcome of restoration actions (Temperton et al. 2004; Palmer et al. 2016; Gornish et al. 2019; Lindenmayer 2020). Thus, many authors agree that restoration projects must be 'science-based restoration projects' by systematically integrating scientific methods and theoretical predictions as guiding concepts (Menninger & Palmer 2006; Palmer et al. 2016 2018; Temperton et al. 2004; Young et al. 2005).

Furthermore, the relationship between theory and practice is mutually beneficial (Falk et al. 2013; Palmer et al. 2006; Suding & Gross 2006). Ecological theory and scientific knowledge also benefit from applied restoration research since experiments offer the chance to test and examine the theories (Bradshaw 1987; Palmer et al. 1997, 2006; Young et al. 2001). Restoration projects provide *in situ* 'playgrounds' to analyse biological processes complementary to *ex situ* experiments, by manipulating community responses under different environmental conditions (Menninger & Palmer 2006; Young et al. 2001). Testing

theories under controlled and repeatable conditions of restoration projects provide opportunities to analyse successional trajectories of restored communities which contributes to an improvement of ecological theory (Menninger and Palmer 2006; Hobbs and Harris 2001; Palmer et al. 2006; Laughlin 2014; Falk et al. 2013).

I.3. Restoration ecology and plant community assembly models

Among the fundamental theories and concepts of restoration ecology (lists of concepts are detailed in: Lindenmayer 2020; Palmer et al. 2006; Perring et al. 2015; Young et al. 2005), my thesis focuses on early plant community assembly and succession, and the associated filter-based models (Chang & HilleRisLambers 2016; Halassy et al. 2016; Hulvey & Aigner 2014; Temperton et al. 2004). Plant succession and assembly models aim at identifying the processes driving the establishment of species from local species pools. The identification of key processes allows predictions of plant community trajectories in restoration projects (Young et al. 2001). A hierarchical framework with three main filters has been suggested to classify environmental constraints of restoration: i) the dispersal filter - the first barrier limiting species dispersal (Öster et al. 2009), (ii) the abiotic filter - the environmental conditions that favour or limit recruitment (Hobbs & Norton 2004), and iii) the biotic filter - the interactions between species (e.g. competition, facilitation; Menninger and Palmer 2006). The filters are interdependent, and feedbacks between environmental and biotic filters continuously modify the ecosystem, drive niche modification and therefore, the processes of community assembly (Fukami 2015; Koffel et al. 2018). This filter-based 'integrated community concept' framework was synthesized by Lortie et al. (2004) and is nowadays largely applied in ecological restoration (Buisson et al. 2021; Halassy et al. 2016; Hulvey and Aigner 2014; Török et al. 2018).

In my thesis, I particularly focus on the processes affecting community assembly in early successional stages after soil disturbance. The dispersal filter was overcome by adding propagules combined with different techniques to manipulate both the biotic and abiotic filters, in order to enhance restoration success. Niche modification, niche pre-emption (Fukami 2015; Vannette & Fukami 2014), competition and facilitation between plant species (Callaway & Walker 1997; Koffel et al. 2018) are the main theoretical concepts that are discussed. Despite increasing knowledge on successional dynamics, associated filters and community assembly processes (Chang & HilleRisLambers 2016; Halassy et al. 2016 2019; Hulvey & Aigner 2014), the occurrence of stochastic processes may compromise predictions and may result in alternative stable states (Suding et al. 2004; Suding & Gross 2006; Temperton & Hobbs 2013; Young et al. 2005). The potential existence of alternative stable states needs to be considered in the identification of the pre-disturbance community that may also have oscillated within a range of states (**Fig. I.1**). Similarly, such alternative

states may characterise the endpoints of succession and restoration outcome (Suding et al. 2004; Suding & Gross 2006; Temperton & Hobbs 2013; Young et al. 2005). Therefore, the reference community may not be unique, and choosing a range of reference communities that encompass the range of community states before the disturbance may be more representative of the natural variability of community responses to environmental conditions (Gann et al. 2019; Ruiz-Jaen & Aide 2005; Suganuma & Durigan 2015).

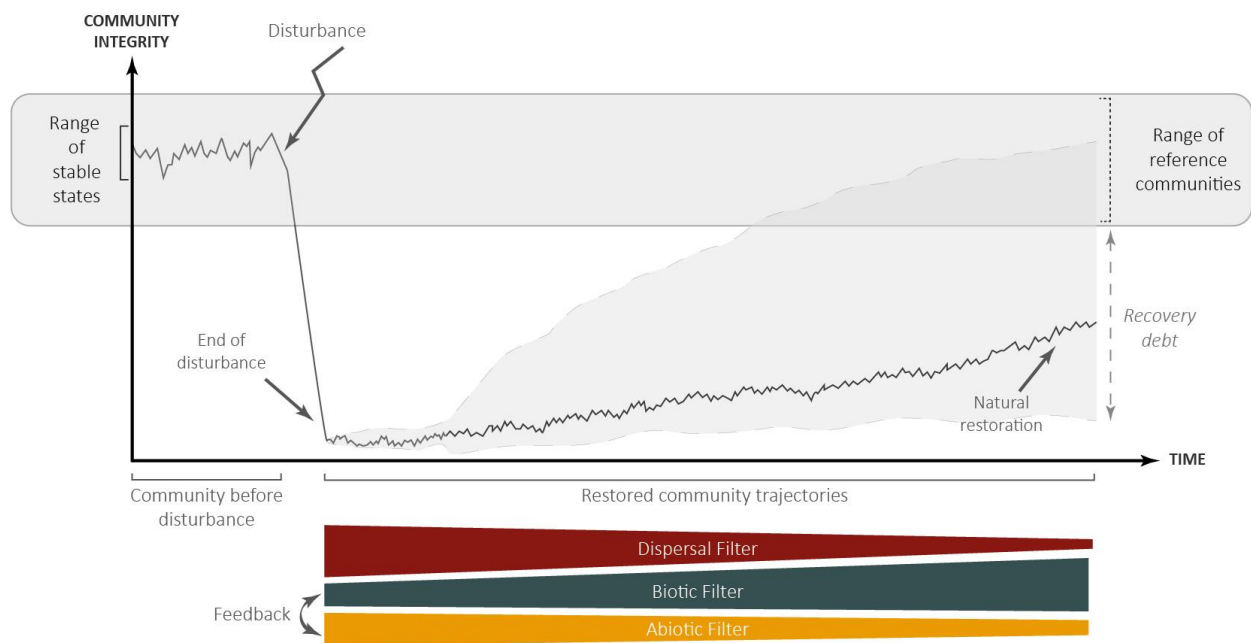


Fig. I.1. Integrated framework of ecological restoration and community assembly filters after a disturbance. The natural variability of the community before disturbance is characterised by the range of stable states. A range of reference communities encompass the range of stable states to consider the potential restored community trajectories under both deterministic and stochastic processes. At the end of the disturbance, non-linear community trajectories are driven by three main assembly filters (different filter sizes indicate their hypothesized relative strength over time and double arrow indicates biotic and abiotic filters feedbacks). The *recovery debt* represents the gap between the community under restoration and the references (Moreno-Mateos et al. 2017; Strobl et al. 2019). Modified from Hobbs and Norton (1996), including elements of Chang and HilleRisLambers (2016).

II. The study system

II.1. Habitat type - Semi-natural mountain grasslands

Grasslands are open habitats, with few shrubs or trees, dominated by grasses and forbs. They represent between 25% and 40% (including shrublands and tundra) of Earth's terrestrial biomes (Blair et al. 2014; Wilsey 2018). In Europe, natural grasslands are limited to continental steppes or extreme habitats such as high elevation, unstable chalk scree and cliff grasslands. All other grasslands are 'semi-natural' depending on extensive grazing and/or cutting (Kuneš et al. 2015) or 'intensive' including sowing of forage grasses and fertilization (Hejcman et al. 2013). European semi-natural grasslands developed with livestock farming during the Neolithic Age (Gibson 2009; Pärtel et al. 2005). Since this period (3000 BC – AD 1000), human occupancy was closely related to livestock keeping and grazing, which explains the absence of shrub encroachment and the open character of these occupied areas (Kuneš et al. 2015).

Semi-natural grasslands are often species-rich with specialized species not occurring in other habitats (Dengler et al. 2014; Habel et al. 2013; Pärtel et al. 2005; WallisDeVries et al. 2002; Wilson et al. 2012). They provide numerous ecosystem services such as regulation of carbon storage and nutrient cycling, reduction of water run-off and soil erosion, nutrition of grazing livestock and open habitats for plants and animals (e.g. pollinators) (Amiaud & Carrère 2012; Byrne & del Barco Trillo 2019; Peyraud et al. 2012; Yan et al. 2019). Due to land use intensification and land abandonment, semi-natural grasslands, and their related biodiversity and ecosystem services, are severely endangered and show a dramatic decline (-12% land cover from 1975 to 1998 in the EU) (Amiaud & Carrère 2012; Stoate et al. 2009; Török et al. 2018; Valkó et al. 2016). Additionally, direct habitat destruction is still contributing to semi-natural grassland losses (Poschlod & WallisDeVries 2002; Veen et al. 2009). However, since 1979, the conservation of species-rich grasslands has become a major concern in Europe: their conservation value was recognized by environmental policy, and an inventory of these habitats was requested to preserve their endangered vegetation and to develop adequate management (Wolking et al. 1981; EEA 2004). Following the creation of the Habitats Directive, many sites comprising semi-natural grasslands were integrated in the Natura 2000 network, in order to prevent habitat degradation, to improve the guidelines and practices of ecological restoration, and to increase grassland species diversity (Stoate et al. 2009; WallisDeVries et al. 2002; Wilsey 2020).

II.2. Localisation - The 'Haute-Durance' valley in the French Alps

The upper Durance valley ('Haute-Durance') is located in the southern France Alps, in the Hautes-Alpes (05) department, Provence-Alpes-Côte d'Azur region (Fig. 2). The construction of a high-voltage transmission line led to a local destruction of semi-natural grasslands along a 100 km strip from L'Argentière-la-Bessée (44°78'78"N, 6°59'41'E) via Embrun (44°60'04"N; 6°54'24'E) to La-Bâtie-Neuve (44°57'93"N, 6°20'77'E). These semi-natural grasslands are located between 1,000 and 1,300 meters above sea level (asl), at the upper montane altitudinal belt. The climate is temperate to sub-Mediterranean depending on elevation and exposition. The Durance valley is characterized by 209 hours of sunshine per month (based on data from 1973 to 2010, meteorological station of Embrun), and 740 mm of annual precipitation with considerable intra-valley variation. The rainfall is thus lower than in other regions of the Alps as the valley is protected by the Pelvoux massif in the west and the Mont Viso in the east. At the Embrun meteorological station (800 m asl), average July and January temperatures are 20 °C, and 2°C, respectively, with an annual mean of 10.7 °C. Due to the higher altitude, temperatures of the study sites are 1.5 to 3 °C lower. At the study sites, snow and frost occur from October to April with a usually continuous snow cover in January and February. The geology of the region is complex. The bedrock is predominantly calcareous, including black marlstone, dolomite, gypsum and siliceous glacial deposits occur at the surface.

The 'Haute-Durance' grasslands are traditionally managed and maintained open by grazing by cattle or sheep and to a lesser degree by mowing. Mesobromion plant communities are dominating, with transitions to Arrhenateretum at more humid sites, and to Xerobromion at dryer sites. The main environmental factors driving plant communities are elevation, slope, aspect, and dominant bedrock. The 'Haute-Durance' grasslands belong to the Natura 2000 priority habitat type "Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates" (N6210, EU directive habitat 92/43/EEC; Calaciura and Spinelli 2008). Grasslands of this habitat type are often nutrient-poor and particularly species-rich, with up to 80 plant species/m² (WallisDeVries et al. 2002) including rare or endangered plant and animal species such as *Astragalus alopecurus* Pall. and the butterfly *Maculinea arion* L.. The two main threats to these grasslands are i) decreasing livestock densities and subsequent land abandonment that led to shrub encroachment (Ostermann 1998), and ii) soil destruction by land use transformation.



Fig. I.2. Location of the study area with the five degraded sites selected to set up restoration experiments (in light green) along the construction zone of the electricity line (in orange).

II.3. Restoration context - **Environmental degradation and ecological compensation**

The renovation of the high-voltage transmission lines of the 'Haute-Durance' valley including the construction of transitory access tracks and platforms involved different measures to compensate for the ecological impact. This thesis project is part of the ecological compensation action 'Experimental restoration of degraded open habitats', set up by the environmental consultancy ECO-MED and two laboratories IMBE (Mediterranean Institute of Biodiversity and Ecology – Avignon University, CNRS, IRD) and LESSEM (Mountain Ecosystems and Societies Lab – INRAE Grenoble) in the framework of a four-party agreement with the construction company RTE.

The compensation takes place according to the sequence “avoid, reduce, and compensate” (called ‘séquence ERC’ in France). Article R. 122-14 of the Environment Code defines the compensation measures as follows:

The purpose of the compensatory measures is to provide compensation for the direct and indirect negative effects of the project that cannot be avoided or sufficiently reduced. They are primarily implemented on the damaged site in order to recover and then guarantee its long-term ecological functionality. The whole sequence should preserve and, if possible, improve the environmental quality of the concerned habitat.

If a project leads to the degradation of environmental quality and if significant residual negative effects persist despite avoidance and reduction measures, the contracting authority needs to set up sustainable and effective compensation measures.

In our study, the compensation action includes (1) an experimental approach comparing the effectiveness of restoration techniques and aiming at a better understanding of plant-plant and plant-environment interactions during the restoration process (this thesis), and (2) a large-scale direct propagule transfer using brush harvesting to restore all degraded open habitats of the study area (10 ha). The construction work ended between May and July 2018 and the experiments of part (1) were set up on five construction sites in autumn 2018 (Fig. I.2).

III. Thesis aims

III.1. Grassland restoration: the identification of reference communities, limiting factors and potential solutions

Identifying the ecosystem that has been degraded and represents the state of the undisturbed system is the first step of any ecological restoration project. The definition of this hypothetical state is therefore a starting point that provides a framework to guide the planning and the implementation of the project towards a reference system to reach (James Aronson et al. 2016; Giardina et al. 2007; Hobbs & Norton 1996). The reference concept emerged in the 1990s to identify the desirable state for restoration (Aronson et al. 1993). The reference has been particularly used in forest and aquatic restoration projects but was less well developed in terrestrial restoration processes until the end of the last century. Only since the 21st century, the concept has been adopted by the scientific community initiated by the SER Primer (2004), that highlights the importance of references to define restoration goals and to evaluate the success of the project. However, the identification of references is still not sufficiently taken into account in restoration projects (Wortley et al. 2013), and the specific selection of references often lacks clearly defined criteria. Furthermore, as the reference may already be degraded prior to the disturbance, and/or the information on the historical community might not be available, identifying references based on robust environmental indicators of the restoration site may be a straightforward solution. It also allows taking into account alternative stable states, as well as the natural variability and dynamics of the study system (Gann et al. 2019; Ruiz-Jaen & Aide 2005; Suganuma & Durigan 2015).

In my study area, plant communities are shaped by traditional mowing and grazing, and as in several grassland restoration projects, restoration ecologists must negotiate with farmers to fence the areas under restoration and protect sown seedlings from herbivory and trampling. As extensive agricultural management shaped semi-natural mountain grasslands by mowing and grazing for millennia (Gibson 2009; Kuneš et al. 2015), the maintenance, or even reintroduction of traditional extensive management is essential (Hejcman et al. 2013). Many plant species and ecosystem services of semi-natural grasslands are totally dependent on the maintenance of these practices (Petit et al. 2004; Schermer et al. 2016), which deserves further consideration for both efficient conservation and restoration. Intensive grazing may result in the loss of biodiversity while extensive grazing is essential to preserve these ecosystems (Petit et al. 2004; Hejcman et al. 2013; Janicka and Pawluśkiewicz 2018). The viability of seeds in the soil contributed to the preservation of semi-natural grasslands but also seed dispersal, in particular by grazing and mowing (Janisová 2018). However, long-term extensive grazing has favoured clonal reproduction whilst decreasing sexual reproduction of grasslands species (Halassy et al. 2016; Piqueray & Mahy 2010; Römermann

et al. 2005).

'Passive' or 'Natural grassland restoration' (*sensu* Atkinson and Bonser 2020) may be hampered by the poor density of grassland species in the soil seed bank (Buisson et al. 2018; Grman et al. 2015; Thompson & Grime 1979; Turnbull et al. 2000), by their low dispersal capacity and by the fragmentation of grassland habitats limiting the seed rain (Bischoff et al. 2009; Muller et al. 2014; Münzbergová & Herben 2005). Therefore, overcoming dispersion limitation is an important issue and seed addition is often a successful method to restore species-rich grasslands (Öster et al. 2009; Török et al. 2012) or, at least, to accelerate restoration (Halassy et al. 2019; Kövendi-Jakó et al. 2019). The use of local and native seeds is recommended to assure adaptation to local environmental conditions and to maintain genetic structure (Barrel et al. 2015; Bucharova et al. 2019; Vander Mijnsbrugge et al. 2010). However, native species of local origin are still not widely available, and the seed mixtures used for revegetation in Europe are often dominated by species of non-local origin. Consequently, transferring local seeds from nearby non-degraded communities is increasingly used in grassland restoration (Kiehl et al. 2010; Scotton 2019; Scotton et al. 2012). A multitude of techniques are currently being used and studied, such as dry hay transfer vs. sowing of seed mixtures (Auestad et al. 2015; Kövendi-Jakó et al. 2019), brush-harvesting material sowing vs. green hay transfer (Albert et al. 2019) and seed sowing combined with various additional treatments (Havrilla et al. 2020; Pawluśkiewicz et al. 2019).

Seed addition experiments demonstrated that limited recruitment due to unfavourable site conditions is the next major constraint influencing plant establishment (Bissels et al. 2006; Pywell et al. 2002; Tilman 1997; Turnbull et al. 2000). Although soil disturbance may negatively affect soil structure and components (Di & Cameron 2002; Holland 2004), soil preparation, such as harrowing or topsoil removal, may promote the recruitment of plant species (Hölzel et al. 2003; Klaus et al. 2017; Long et al. 2014; Myers & Harms 2009) by limiting the competition for resources by an already established vegetation (Bischoff et al. 2018; Kupferschmid et al. 2000; Poschlod & Biewer 2005; Stevenson & Smale 2005). Soil preparation may also improve abiotic conditions and increase seed trapping and adhesion (Chambers 2000; Isselin-Nondedeu et al. 2006; Kiehl et al. 2010).

Seedling recruitment and early plant community assembly are influenced by abiotic factors as well as by species strategy (Grime 1979) influencing biotic interactions (i.e. plant-plant and plant-soil interactions) (Bever 2003; Fukami & Nakajima 2013; García-Girón et al. 2020). Biotic interactions can be either negative (e.g. competition, predation, amensalism, parasitism) (Callaway & Walker 1997; Grime 1973) or positive (e.g. facilitation, commensalism, mutualism, symbiosis) (M. Bertness & Leonard 1997; Brooker et al. 2008; Bruno et al. 2003; Choler et al. 2001). All types of interactions are important in ecological restoration, because plant-soil feedback and spontaneous colonisation continuously reshape the habitat since early successional stages. Therefore, the use of biotic drivers, such as ecosystem engineers (Jones et al. 1996) or nurse species, may favour ecosystem

restoration (cf. also keystone or nexus species, Temperton & Hobbs 2013). For example, in active restoration by seed addition, the use of mulch may facilitate seedling recruitment by protecting seedlings against high solar radiation and drought (Eckstein & Donath 2005; Havrilla et al. 2020; Mollard et al. 2014; Wright et al. 2014). Sowing of annual ('nurse') plant species together with target species may compensate for the absence of a protecting mulch layer. Such annuals provide temporary living cover that may facilitate seedling recruitment and establishment (Padilla & Pugnaire 2006; Wright et al. 2014). Furthermore, plant cover may reduce soil erosion (Graiss & Krautzer 2011; Gu et al. 2020; Liu et al. 2018; Peratoner 2003), an important criterion for the success of restoration measures. In particular in mountain grasslands, soil stabilisation and erosion control are important because steep slopes and low vegetation cover favour soil erosion (Huc et al. 2018; Löbmann et al. 2020; Pohl et al. 2009; Scotton 2019). However, a living cover may also increase root and light competition (Donath et al. 2006). Biotic and abiotic filters need to be taken into account in restoration actions, since both filters continuously interact with each other through feedback loops (**Fig. 1.1**).

After degradation, plant community assembly may be modified by natural succession or by management actions leading to different trajectories (Clewett & Aronson 2013). These different trajectories may result in alternative states that are more or less similar to the reference (Suding et al. 2004; Temperton & Hobbs 2013). In ecological restoration, it is important to determine relevant management strategies to drive succession towards the desired states. Stochastic dispersal events, biotic and abiotic interactions influencing community assembly induce priority effects. Priority effects occur when one species arrives first and affects its neighbourhood and/or its environment and the establishment of later species (Temperton et al. 2013; Gillhaussen et al. 2014; Weidlich et al. 2018). They have been identified as a core concept in restoration ecology since they influence early community succession (Van der Putten et al. 2000; Vaughn & Young 2015) and can have mid- to long-term consequences on plant community assembly (Fry et al. 2017; Fukami 2004; Garcia-Giron et al. 2021; Mergeay et al. 2011; Švamberková et al. 2019; Werner et al. 2016). Therefore, modifying the sequence of the arrival of plant species, and thus modifying priority effects is one possible way to influence the structuring of communities (HilleRisLambers et al. 2012; Vannette & Fukami 2014; Gillhaussen et al. 2014). The first arriving species may modify the micro-local environmental conditions and consequently the establishment of later arriving species by inhibiting (Cole 1983) or facilitating (Bertness & Shumway 1993) their seedling recruitment (Donath et al. 2007; Fukami 2015). Many other mechanisms may also drive priority effects, such as niche pre-emption or niche modification (Fukami 2015; Helsen et al. 2016), plant-soil feedbacks and soil legacies (Bever 2003; Fukami & Nakajima 2013; Grman & Suding 2010; van der Putten et al. 2013) or even allelopathic effects (Levine et al. 2003). Finally, priority effects do not only influence the early community assembly but also shape the potential future community trajectories, including their composition and associated functions (Grman & Suding 2010; Stuble & Young 2020; Gillhaussen et al. 2014; Werner et al. 2016).

III.2. Research questions

The thesis project aims to better understand the factors limiting the restoration of mountain grasslands after soil disturbance, and to identify successful restoration techniques compensating the degradation. I addressed the following scientific questions:

In restoration ecology, the reference is a key concept that is well defined in theory. In practice, however, the specific selection of references often lacks clear methodology. Thus, my major questions are: **(1.a)** How should we determine the references? **(1.b)** Which factors influencing species composition are appropriate for the choice of references? **Chapter 1**

Taking into account the main filters limiting plant recruitment, I asked: **(2.a)** Does soil preparation by harrowing before sowing improve recruitment and survival of the transferred species? **(2.b)** What is the effect of early extensive grazing on seedling recruitment and survival of the transferred species? **Chapter 2**

In order to understand the mechanisms favouring target species seedling establishment, I asked: **(3.a)** Which method of plant material transfer is most successful in establishing species transferred from the donor community? **(3.b)** Does the sowing of a nurse plant (wheat) facilitate seedling establishment from brush material? **(3.c)** Do transfer techniques and wheat sowing increase the similarity between restored and reference communities? **(3.d)** Does propagule addition limit soil erosion? **Chapter 3**

And finally, I manipulated priority effects to restore plant community composition, asking: **(4.a)** Does the order of species arrival manipulated by sequential sowing influence community establishment? **(4.b)** More specifically, does sowing dominant species first results in competitive exclusion of subordinate species? And **(4.c)** Does sowing subordinates first provide a sufficient establishment of both subordinates and dominants? **Chapter 4**

III.3. Thesis chapters

Chapter 1 focused on the identification of an appropriate reference community, which is a crucial step in ecological restoration as it allows defining restoration targets and evaluating restoration success (Gann et al. 2019; Giardina et al. 2007; Hobbs & Norton 1996). The community prior to its degradation is considered as the reference (**Fig. 3**; Aronson et al. 1995; SER 2004). However, information on the reference may not be available and communities may have already been degraded before the latest anthropogenic disturbance. As in our project, this situation is very common in European grassland restoration (Baasch et al. 2016; Bischoff 2002). Furthermore, the use of several positive references is

increasingly recommended to include alternative stable states and natural variability of the study system (Gann et al. 2019; Ruiz-Jaen & Aide 2005; Suganuma & Durigan 2015). However, selecting references in practice often lacks clear criteria. In this chapter, I suggest a framework based on ecological theory, and more precisely on relationships between vegetation and environmental drivers, to facilitate the identification of a range of reference communities.

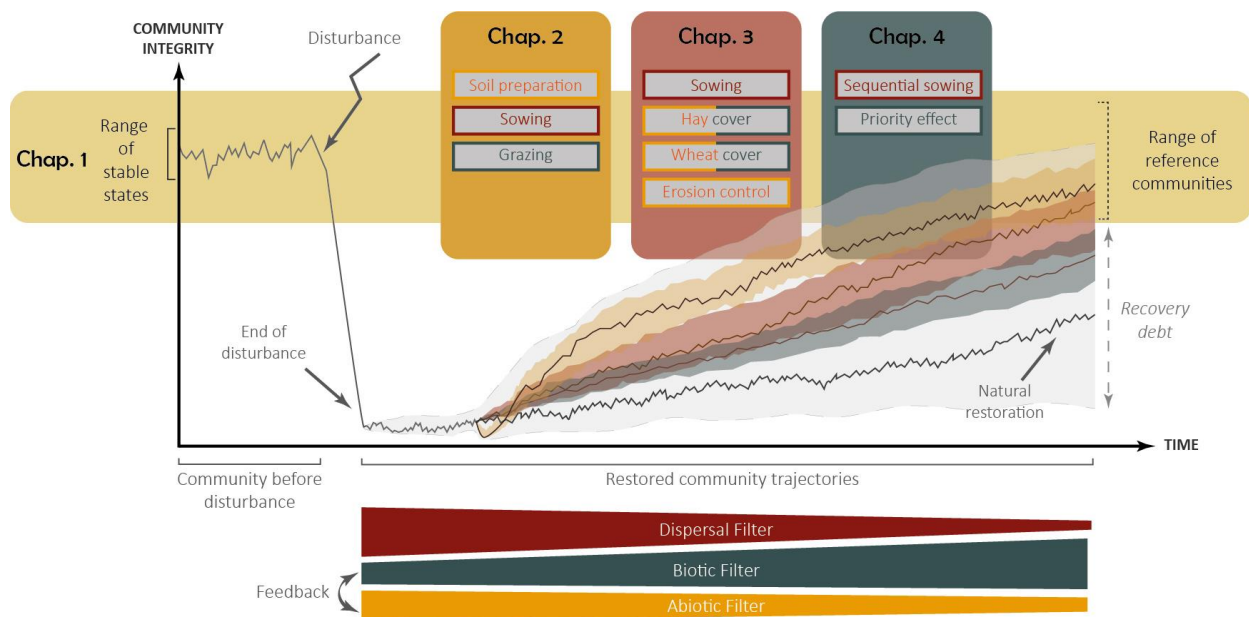


Fig. I.3. Illustration of the thesis structure (see Fig. I.1. for further explanations).

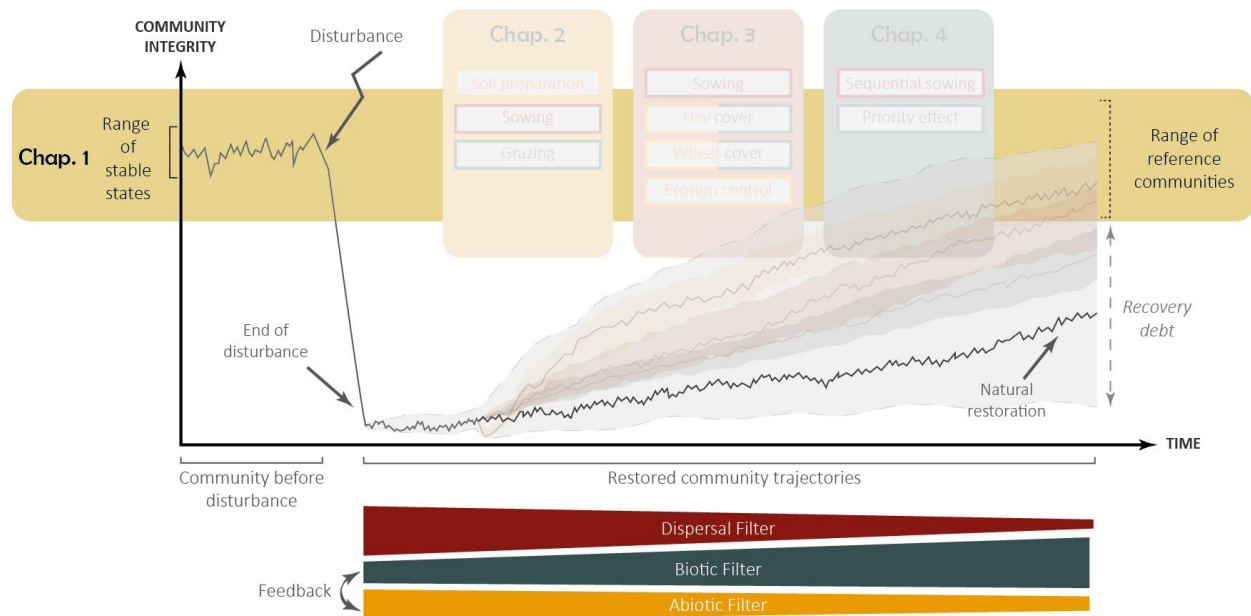
In the next three chapters of my thesis, I suggest a filter-based community assembly approach of community dynamics by simultaneously manipulating dispersal, abiotic and biotic filters to analyse restoration outcomes (Chang & HilleRisLambers 2016; Halassy et al. 2016; Hulvey & Aigner 2014; Temperton et al. 2013). In order to answer **questions (2) and (3)**, five degraded semi-natural grasslands were used to set up two different experiments (**Fig. I.1**) corresponding to the **chapters 2** and **3**, respectively. Another experiment manipulating the arrival of dominant and subordinate species to test priority effects (**question (4) and chapter 4**) was set up on one of the five degraded sites with ten spatially randomized replicates. Different types of propagule transfer techniques were carried out in the three experiments to overcome the dispersal filter (**Fig. I.3, Table 1**).

In the first experiment, I manipulated both abiotic and biotic assembly filters by examining whether soil preparation is required to provide more suitable soil conditions and to reduce competition of pre-existing vegetation, and whether traditional extensive grazing should be excluded in initial stages of grassland restoration to protect seedlings. This would justify soil tillage and fencing costs in grassland restoration projects (**chapter 2**). In the second experiment, I focused on different techniques of propagule transfer increasingly used in mountain grasslands to understand the mechanisms favouring seedling establishment of transferred species. I analysed both biotic and abiotic filters by testing the effects of brush-harvested propagules and hay transfer, as well as the potentially facilitative effect of dead and living plant cover on the recruitment of transferred brush-harvested propagules (biotic filter), and on soil erosion (abiotic filter; **chapter 3**). Finally, as community assembly is also highly influenced by dispersal that may lead to priority effects, I altered the biotic filter by sowing different combinations of plant species (dominant vs. subordinate) in a third experiment and I analysed how sequential sowing affects community assembly (**chapter 4**).

Table I.1. Filter-based restoration actions carried out during the thesis

Thesis chapter	Restoration actions	Associated assembly filter
1	Reference identification	Environmental drivers of plant community assembly
2	Soil preparation	Abiotic
	Transfer of brush-harvested material	Dispersal
	Livestock grazing management	Biotic (herbivory) & Abiotic (trampling)
3	Transfer of brush-harvested material	Dispersal
	Hay transfer (dead plant cover)	Dispersal, Biotic (plant-plant interactions) & Abiotic (micro-climate)
	Wheat addition (living plant cover)	Biotic (plant-plant interactions) & Abiotic (micro-climate)
	Erosion measurement	Abiotic
4	Sequential seeding	Dispersal
	Priority effect	Biotic (plant-plant interactions) & Abiotic (micro-climate change)

Identifying reference communities in ecological restoration: the use of environmental conditions driving vegetation composition



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Identifying reference communities in ecological restoration: the use of environmental conditions driving vegetation composition

Abstract

In restoration ecology, the reference ecosystem represents a key concept, which is well defined from a theoretical point of view. In practice, however, selecting reference systems, such as reference plant communities, often lacks clear methodology. In order to facilitate this selection, we provide a framework based on ecological theory and more precisely on relationships between vegetation and environmental factors to identify reference plant communities. The four major steps are: 1) the delimitation of a geographical zone in which habitat types similar to restoration sites occur; 2) the identification of environmental factors structuring non-degraded plant communities within this geographical zone; 3) the comparison of the environmental factors between non-degraded and degraded sites; 4) the selection of the non-degraded sites most similar to restoration sites in terms of environmental factors to use them as references. We concept-proved our approach by identifying reference communities using environmental factor combinations for five mountain grassland sites degraded by the construction of a high-voltage line. In a multivariate analysis of eighteen non-degraded sites, we identified six major environmental factors explaining plant species compositions. A second multivariate analysis including degraded sites provided environmental distances of the eighteen non-degraded to each of the degraded sites. The results demonstrated that the environmentally most similar sites were not necessarily the geographically closest ones. In conclusion, the analysis of regional plant-environment interactions provides an important tool to identify reference communities or donor sites for seed transfer if not available adjacent to degraded habitats.

Keywords: abiotic factors, grassland restoration, plant succession, degradation, target reference

1.1 Introduction

The definition of reference systems is a crucial step in ecological restoration as it allows defining restoration targets and evaluating restoration success (Hobbs & Norton 1996; Giardina et al. 2007; McDonald et al. 2016). The reference paradigm represents a cornerstone concept, which is well defined from a theoretical point of view (Aronson et al. 1993 2016; SER Primer 2004). However, in practice, the specific selection of reference ecosystems or communities often lacks clear scientific criteria, or this step is not sufficiently taken into account in restoration planning. A review of 301 articles focusing on terrestrial ecological restoration projects showed that less than 75% of the studies used a reference, 62% used “negative reference(s)” representing the pre-restoration degraded state, and only 60% used “positive target reference(s)” defined by the pre-degradation system, with only 22% presenting both. Both negative and positive references may be used together to evaluate restoration success: i) a negative one to evaluate the success of an active restoration compared with the degraded system, and ii) a positive one to assess success by comparing the restored and the reference systems (Benayas et al. 2009; Wortley et al. 2013). In many studies, the ecosystem prior to its degradation is considered as a (positive) reference (Aronson et al. 1995; SER Primer 2004). However, ecosystems may already be degraded prior to the latest disturbance that is considered as starting point of restoration, and/or the information on the state of the ecosystem before degradation might not be available. This is a very common situation in European grassland restoration where ecosystems suffer from land abandonment or intensification (Bischoff 2002; Baasch et al. 2016).

An important objective of restoration is to accelerate or jumpstart succession by facilitating late-successional species (Palmer et al. 1997). Late-successional stages or even endpoints of succession are determined by abiotic conditions although historical contingency may influence final community composition (Fukami et al. 2005; Young et al. 2005). While it is useful to consider endpoints (attractors) of succession, earlier successional stages and potential variation of endpoints also need to be taken into account (Prach et al. 2016). The succession towards natural vegetation representing such endpoints is often prevented by human land management. A constant and/or predictable disturbance regime, such as grazing by livestock of semi-natural grasslands, may result in stable intermediate states of plant succession (or “alternate steady states” or metastable states; Clewell & Aronson 2013). These intermediate states can thus be considered as quasi-stable representing an equilibrium with environmental conditions and land management (Young et al. 2001; Bouzillé 2007).

The use of several positive references may be a solution to take into account such intermediate states and is increasingly common in ecological restoration (Ruiz-Jaen & Aide 2005; Suganuma & Durigan 2015; McDonald et al. 2016). The definition of various reference

systems resulting from different land management types would provide a range of restored ecosystem trajectories (Le Floch & Aronson 1995; Cortina et al. 2006).. Furthermore, considering the potential variation in successional endpoints allows integrating ecosystem dynamics and natural variability (Asbjornsen et al. 2005; Sluis et al. 2018; Erskine et al. 2019). In cases of high spatial heterogeneity such as varying slope and aspect, small-scale differences may compromise the preference of adjacent plant communities and populations usually recommended for active ecological restoration (Kiehl et al. 2010, Vander Mijnsbrugge et al 2010; Scotton et al. 2011). Additionally, restoration sites may sometimes be surrounded by other more or less degraded ecosystems. In such cases, references need to be found at higher distances requiring a clear concept and methodology to identify the most appropriate sites.

In our study, we provide a framework to define positive reference communities by using environmental factors that drive plant species composition. This method is particularly useful in the following cases: i) information on plant species composition prior to latest degradation is not available; ii) the sites were already (at least partially) degraded before the latest disturbance for which restoration is required and several environmental factors structuring plant communities remain within the natural range after a particular ecosystem degradation; iii) if due to economic or legal constraints, only one propagule donor site can be selected for several restoration sites.

We first present a framework to select reference communities by using environmental factors that determine plant species composition. To concept-prove our framework, we applied the approach to a mountain grassland project involving the restoration of temporary access tracks required to build a new high-voltage electricity line. Habitat conditions at the sites change at relatively small scales thus complicating the search for appropriate reference communities. Such extensively grazed and/or mowed grassland ecosystems represent a particular case of succession in which regular management truncates succession by preventing the development of woody vegetation (Young et al. 2001). Most European grasslands are thus considered as such alternative quasi-stable states since they are maintained by regular grazing and mowing. Their species composition is not only dependent on climate and soil conditions but also on management type (Isselstein et al. 2005; Härdtle et al. 2006). However, management regimes are similar in our study region allowing an analysis of environmental factors driving plant community composition. Due to the scope of the construction work, the different non-degraded grasslands selected as potential references should thus make it possible to obtain a wide range of quasi-stable states representative of the different restoration sites.

1.2 Framework to identify reference communities by combinations of environmental factors

We suggest a 4-step methodological framework to select references by analyzing the species composition of non-degraded systems and measuring the environmental factors structuring them (**Fig. 1.1**): 1) delimit a geographical zone in which habitat types similar to restoration sites occur; 2) identify environmental factors structuring non-degraded plant communities within this geographical zone and measure them in non-degraded and degraded sites; 3) evaluate environmental distances between degraded sites and non-degraded sites; 4) identify the non-degraded sites most similar to restoration sites in terms of environmental factors.

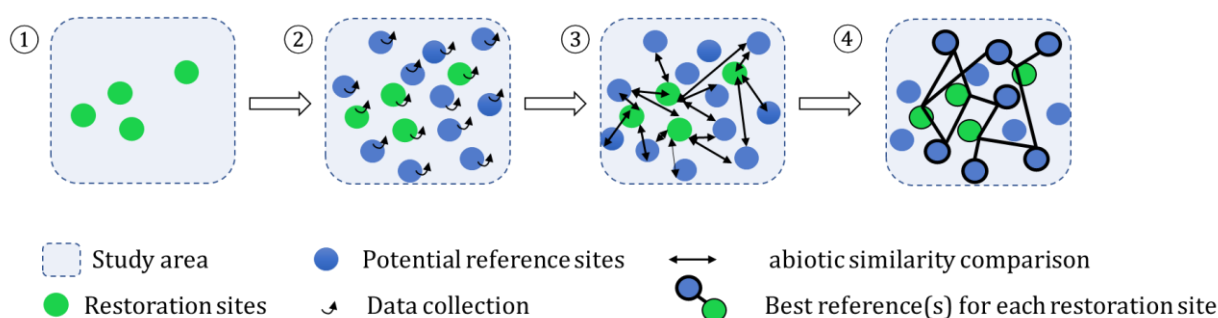


Fig. 1.1. The four major steps of the proposed method to select references. ① Delimit an extend study area around restoration sites; ② Identify environmental factors structuring plant communities (data collection and driver analysis); ③ Evaluate environmental distances between restoration and potential reference sites (dissimilarity coefficient); ④ Select the most similar references to restoration sites (dissimilarity coefficient).

First, a rough estimation of habitat type based on existing habitat or biogeographical maps is required to delimit the geographical zone in which the reference communities may be found (Alard 2002). Existing pedoclimatic or historical vegetation data may increase the precision of habitat type information. Secondly, within the delimited geographical zone, major environmental factors that typically drive species composition in the study area are measured on non-degraded sites. Factors that are significantly modified by the habitat degradation cannot be taken into account. At the same time, the relationship between these environmental factors and plant species composition is analysed. The number of analysed sites needs to be representative of the variation of the geographical zone and sufficient to identify the best subset of environmental factors using multivariate statistics. Once identified, the major environmental drivers of non-degraded sites need to be measured on sites to be restored. The non-degraded sites presenting the most similar values compared

with restoration sites can therefore be used as reference sites. This method allows identifying the best corresponding plant communities for each restoration site within the study zone. For each restoration site, one or more reference sites that correspond best in terms of structuring environmental factors and thus of potential plant community composition will be chosen.

1.3 Proof of concept

We tested our approach to identify reference communities using combinations of environmental factors in mountain grasslands degraded by the construction of a high-voltage electricity line. The construction work included the creation of access tracks and working platforms on 13 ha of mountain grasslands. The grasslands were separated in hundreds of patches separated by woodlands. Due to small-scale changes in abiotic conditions and degradation of adjacent sites by land abandonment, arable use or afforestation, the closest sites were often inappropriate references. The identification of reference communities was thus a priority action to define restoration targets but also to find appropriate grassland sources for hay transfer used as a restoration technique (Kiehl et al. 2010). Following the four suggested steps we asked: (1) What are the environmental factors explaining best the species composition of non-degraded mountain grassland sites? (2) What are the non-degraded sites most similar to the degraded sites in terms of these environmental factor combinations? (3) Are the environmentally most similar sites also the geographically closest ones?

1.3.1 Material and methods

1.3.1.1 Restoration sites and delimitation of geographical zone

The study area is the upper Durance valley (“Haute-Durance”) in the Southern French Alps from L'Argentière-la-Bessée to La-Batie-Neuve (44°78'78"N, 6°59'41'E; 44°57'93"N, 6°20'77'E, **Fig.2**). The study sites are located 100 to 600 m above the valley floor on slopes at an elevation of 1000 to 1400 m (upper montane altitudinal belt). The climate is temperate to sub-Mediterranean depending on elevation and exposition. The Pelvoux and Mont Viso mountains provide a rain shadow, reducing precipitation to 740 mm per year, with a mean annual temperature of 10.7°C (based on data from 1991 to 2010, meteorological station of Embrun). The bedrock is predominantly calcareous, but many sites are covered by quaternary glacial deposits. The degraded areas were characterized by dry to mesophilic grasslands. Most of these grasslands are Natura 2000 priority habitats: “Semi-natural dry

grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates” (N6210, EU directive habitat 92/43/EEC). The construction work affected soil structure and soil organic matter composition by stripping soil, compacting and decompacting. A total of 18 species-rich non-degraded grassland sites as well as five restoration sites were selected as study sites in the Haute-Durance valley (Fig. 1.2). Selection criteria were traditional management by grazing (sheep, cattle) and mowing, geographical representation of the study area and representative microclimatic conditions (semi-dry to mesophilic) in avoiding driest and most humid sites. We further excluded restoration sites with particularly strong soil modification by gravel addition.

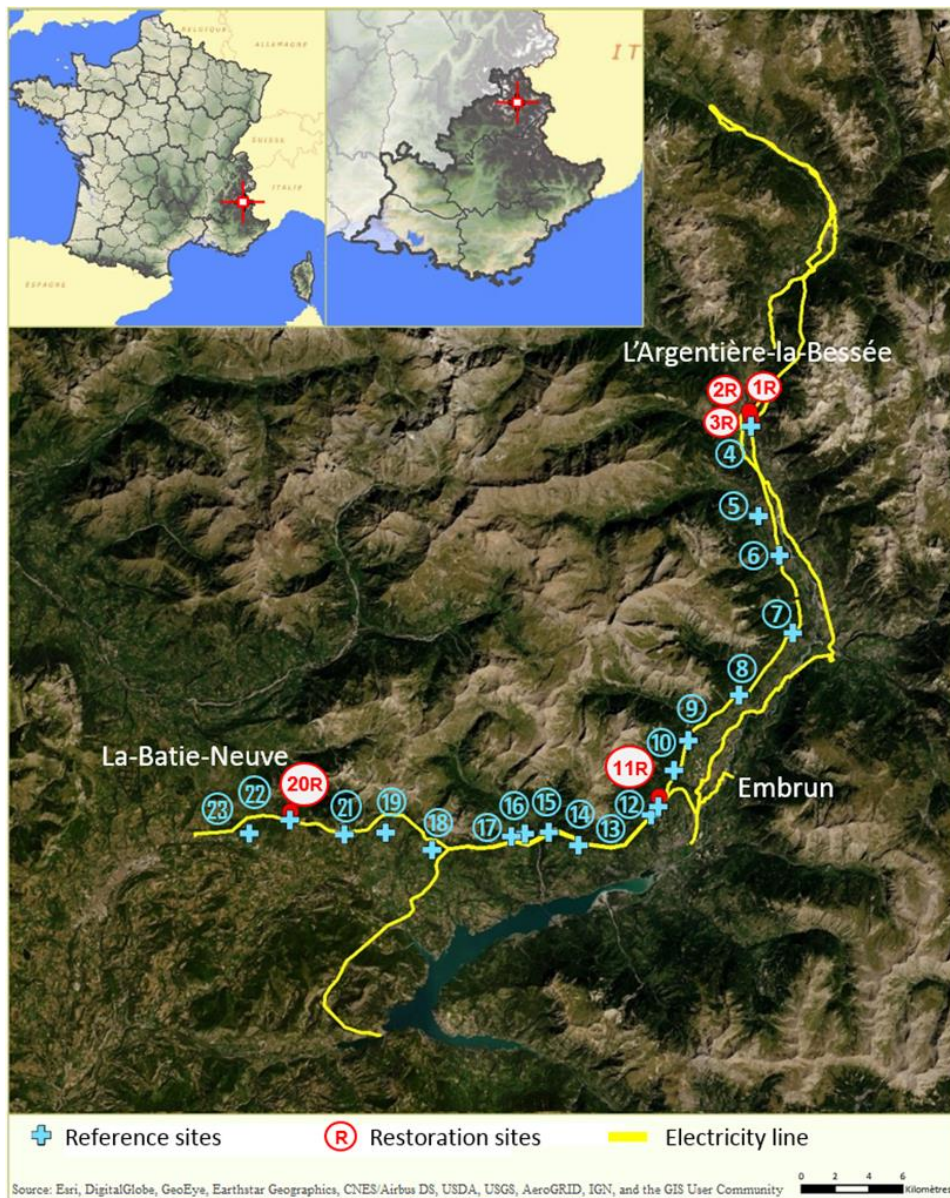


Fig. 1.2. Location of the study area with restoration sites (1R, 2R, 3R, 11R and 20R, in red) and reference sites (numbered from north to south, without R, in blue) along the construction of a new electricity line.

1.3.1.2 Data collection

To link species composition to environmental conditions, vegetation cover of all vascular plant species was evaluated between 21 June and 5 July 2018 in 3 randomly placed quadrats (2m x 2m) within each of the 18 non-degraded grassland sites resulting in a total of 54 quadrats. We measured abiotic factors that are usually not or little influenced by soil disturbance involved in access track or working platform creation. These factors included soil variables such as phosphorus, potassium and carbonate content, pH value and moisture, as well as geomorphological variables influencing climatic conditions such as slope, aspect and elevation. Three soil samples per site were thus taken to a depth of 20 cm (diameter 5 cm). They were dried and sieved using a 2 mm mesh size. Plant available phosphorus (P_2O_5) was determined using the Olsen Sterling method (1954), exchangeable potassium ions (K_2O) was determined according to Thomas (1982) and Ciesielski et al. (1997). Soil moisture was measured in the field using Theta Probe ML3 sensors. The soil pH was measured in a 1:5 water solution. The carbonate content was estimated using a 3-level scale of effervescence with 1 M HCl (non-effervescent; slightly effervescent; strongly effervescent). In mountain ecosystems, plant species composition and richness are strongly influenced by slope and aspect driving solar radiation (Pykälä et al. 2005). We estimated the annual direct incident solar radiation (ASR) using the method of McCune & Keon (2002). The ASR takes into account that southwest facing slopes show a higher radiation than southeast-facing slopes in folding the aspect (**Supplement S1.1**). This folded aspect is combined with slope and latitude to calculate the ASR.

1.3.1.3 Statistical analysis

Factor selection: test of variance inflation to avoid collinearity and correlation matrix

In order to reduce collinearity within the measured environmental variables we tested Variance Inflation Factors (VIF; Zuur et al. 2010). Highly correlated variables ($VIF > 2$) were sequentially removed (R package “vegan”, Oksanen et al. 2019). We used the pairwise correlation analysis (Pearson correlation coefficient) to crosscheck VIF removal procedure. Since slope and aspect are included in the ASR, they were not considered. Carbonate content (correlated to pH) was removed according to VIF analysis whereas elevation, ASR, soil moisture, soil pH, P_2O_5 and K_2O were kept and used in subsequent analyses in order to test the response of plant species composition to environmental factors of non-degraded sites.

Analysis of factors driving species composition

We analysed the plant species composition of the non-degraded sites using redundancy analysis (RDA) to model the relationship between species composition and environmental variables (R package “vegan”, Oksanen et al. 2019). Species cover was first Hellinger-

transformed since this transformation provides a better resolution in linear ordination techniques than chi-square distance (Legendre & Gallagher 2001). We removed rare species (occurring in less than eight plots) from the analysis in order to improve the detection of relationships between community composition and abiotic factors. Removing rare species reduces bias due to stochastic sampling effects since rare species occurrence is often a poor predictor of environmental conditions (McCune and Grace 2002). The significance of the six selected variables (ASR, elevation, soil moisture, soil pH, P₂O₅, K₂O) was tested using the “envfit” function with N = 999 permutations (R package “vegan”; Oksanen et al. 2019) to determine relationships with plant community composition.

Environmental distances between non-degraded and restoration sites

The environmental distance (= dissimilarity) between each restoration and non-degraded sites was assessed by calculating the Euclidean distance using the “vegdist” function (R package “vegan”; Oksanen et al. 2019). Non-metric multidimensional scaling (NMDS) based on Euclidean distances of environmental factors was used to illustrate the most similar reference for each restoration site (R code: <https://github.com/RenaudJau/ChooseRef>). In order to compare the distribution of plant communities according to environmental factors values and to geographical zone, a second NMDS was performed on plant species composition of non-degraded sites. Sites were grouped by each environmental factor (high, medium, low factor values) and major geographical zones (south; central; north) in different ordination plots. All analyses were performed using R 3.5.3 (R Core Team 2019).

1.3.2 Results

1.3.2.1 Environmental drivers of plant species composition

One-hundred and eighty plant species belonging to 40 families were identified across the 18 study grasslands. The RDA model relating plant species composition and environmental variables at non-degraded sites explained 44.8% of the total variance. The first two constrained axes accounted for 20.2% and 11.4% of total variance and were mainly determined by P₂O₅, soil pH, soil moisture and solar radiation (**Fig. 1.3**). The environmental variables retained by the VIF analysis did not show strong correlations confirming that applying it efficiently removed correlated environmental variables (**Table S1.1**). Four of the six selected variables (ASR: $p = 0.004$, P₂O₅: $p = 0.025$, soil moisture: $p = 0.023$, pH: $p = 0.026$) had a significant influence on plant species composition whereas the influence of elevation ($p = 0.201$) and K₂O ($p = 0.830$) was not significant (**Table S1.2**). Sites located on the right side of Fig. 3 were associated with low ASR, relatively low (neutral) pH and high soil moisture, as well as high P₂O₅. They were well represented by mesophilic species, such as *Dactylis glomerata*, *Galium verum*, *Poa pratensis*, *Plantago lanceolata* and *Lathyrus pratensis*.

In contrast, plants associated with higher ASR and pH included *Bromopsis erecta*, *Brachypodium rupestre*, *Pilosella officinarum*, *Lotus corniculatus* and *Teucrium chamaedrys*. Sites with higher abundance of *Astragalus danicus*, *Pilosella officinarum*, *Lotus corniculatus* and *Teucrium chamaedrys* were negatively correlated with elevation and soil moisture, whereas sites with higher abundance of *Salvia pratensis* and *Dactylis glomerata* were positively correlated with both environmental variables.

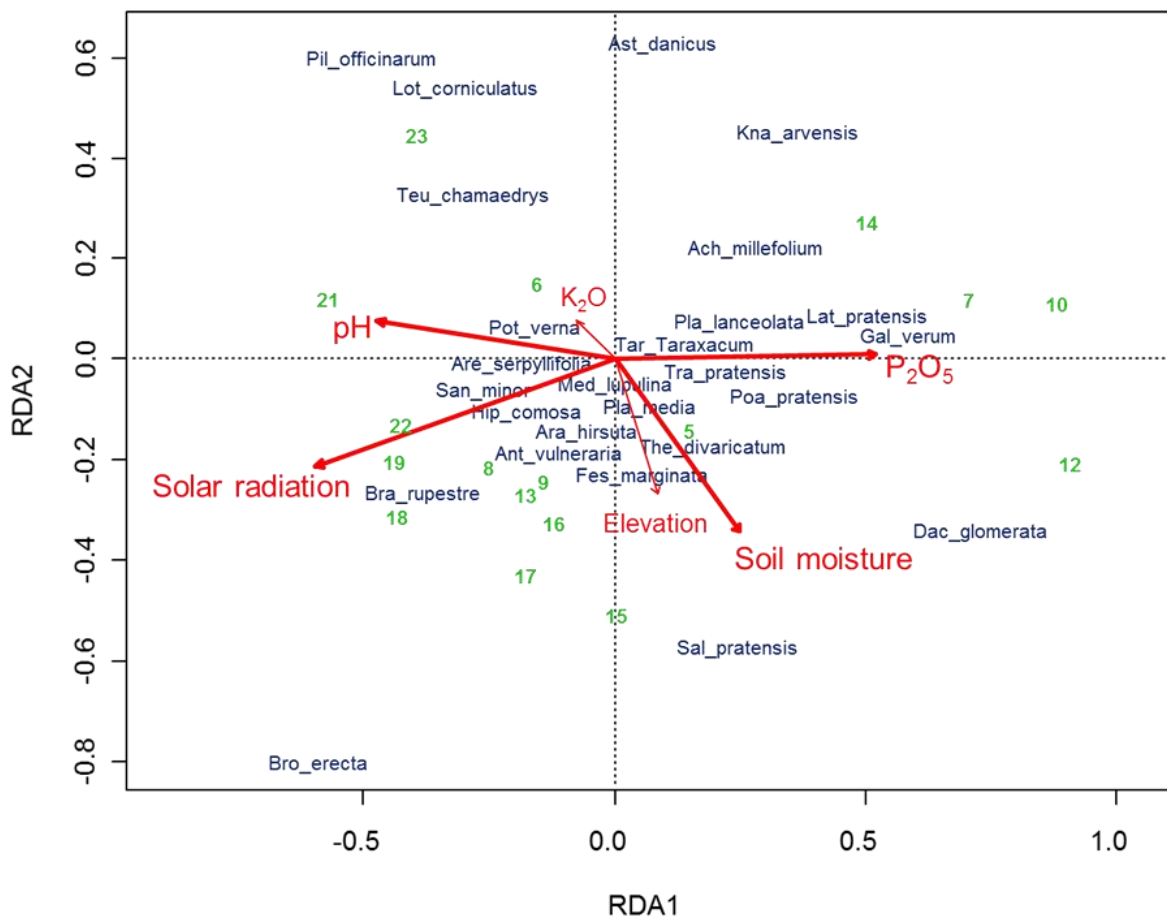


Fig. 1.3. RDA biplot using Hellinger-transformed plant species cover (species names in blue) and reference sites (green) constrained by environmental variables (red arrows). Environmental variables: ASR = Annual Solar Radiation; P₂O₅ = plant available phosphorous; soil moisture; soil pH; elevation and K₂O = plant available potassium. Full species names: Table S1.3.

1.3.2.2 Identification of reference sites by comparing similarity in environmental factors

The five restoration sites revealed different combinations of environmental factors resulting in different environmental distances with non-degraded sites (**Fig. 1.4**). Restoration sites 1R and 3R showed a low environmental distance to the non-degraded site 21 indicating high similarity in measured abiotic factors. However, the second environmentally closest non-degraded sites were different (1R: site 22, 3R: site 18). The three sites closest to 2R in terms of environmental variables were 6, followed by 4 and 5. The most similar sites to 11R were 12, 10 and 16, and the most similar to 20R were 9, 17 and 14 (**Fig. 1.4.a, Table 1.1**). If technical and/or logistic constraints require the choice of one reference community as a donor site for plant material for all five degraded sites, the non-degraded site 9 would represent the best reference (**Table 1.1**), which has the lowest mean distance to five restored sites (**Fig. 1.4.b**).

Table 1.1. Similarities between restoration sites (1R, 2R, 3R, 11R, 20R) and the non-degraded sites (from site 4 to 24, without R) of the study zone (similarity decreasing from left to right) using the Euclidean distance for environmental variables shown in **Fig. 1.3**. Here the colors show examples for choosing only one reference for **(a)** one site (example of restoration site 2R: 6); **(b)** 2 sites (example of 1R and 3R: 16); **(c)** all the restoration sites (1R, 2R, 3R, 11R and 20R: 9).

Restoration sites	Reference sites																	
	Higher similarity \longrightarrow Lower similarity																	
1R	16	22	9	23	15	19	4	17	18	13	6	5	8	16	10	7	12	14
2R	6	4	5	15	9	16	16	8	18	7	19	13	23	10	22	12	17	14
3R	16	18	15	6	19	9	4	22	8	23	5	13	16	17	7	10	12	14
11R	12	10	16	13	9	14	7	5	8	15	17	19	18	22	4	6	16	23
20R	9	17	14	19	10	22	16	12	7	13	16	18	4	15	8	6	5	23

The geographical zonation of plant species composition hardly corresponded to environmental factor values. NMDS according to geographical position separated the southernmost zone from the northernmost zone whereas the central zone was not well separated from the latter one (Fig. S1.1). The Northern zone was characterized by low levels of P_2O_5 and soil moisture, low solar radiation (ASR) and neutral pH. The Central zone showed highest soil moisture and P_2O_5 , medium ASR and the most acidic pH. The Southern zone was represented by high solar radiation and a basic pH. However, there were many exceptions to these general trends and environmental zonation was very different from geographical zonation in P_2O_5 and soil moisture. The lack of correspondence between geographical and environmental zonation explains that the environmentally most similar non-degraded site was not necessarily the geographically closest one.

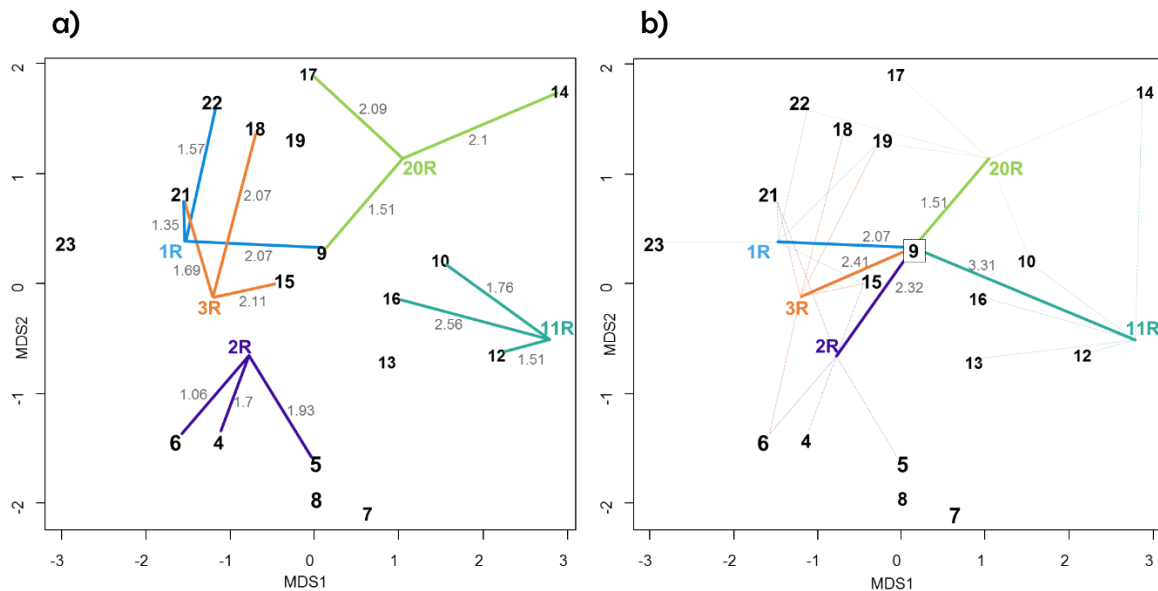


Fig. 1.4. NMDS based on environmental variables showing **a)** the three most similar non-degraded sites (without R) for each restoration site (with R) and **b)** the non-degraded site that presents the lowest average environmental distance with all the restoration sites. Different restoration sites are represented by different colours. The grey numbers represent the environmental distance (dissimilarity coefficient). See **Table 1** for more details.

1.3.3 Discussion

Our results indicate that among the seven tested environmental factors, ASR, soil moisture, P_2O_5 and soil pH best explain the distribution of plant communities in our 18 species-rich mountain grasslands and can thus be used as indicators for reference communities in this particular case.

The simultaneous analysis of environmental factors such as pedoclimatic variables, and vegetation, allows identifying major ecological processes that govern the studied system, since they are considered as major drivers of plant community composition (Box 1981). Elevation known has an important driver of mountain grassland composition did not significantly influence plant species composition since limited to a relatively small elevational gradient of 400 m. ASR included slope and aspect that are known to determine plant species composition in mountain grasslands (Srinivasan et al. 2005). As many other plant communities, grasslands are controlled by edaphic factors, such as water content, organic matter and nutrient concentration (Sebastiá 2004; Klimek et al. 2007). Several studies have emphasized the relation between pH and phosphorus. Phosphorus availability decreases with increase in pH because at alkaline pH, phosphate ions react with calcium or magnesium (Cerozi & Fitzsimmons 2016; Li et al. 2019). This is consistent with our results that show a negative correlation between soil pH and exchangeable phosphorus (P_2O_5).

The lack of methods for reference community choice has often been cited as a shortcoming in restoration ecology (Halle & Fattorini 2004) although some recent studies have developed a more explicit methodology. Suganuma & Durigan (2015) selected nine references divided into three categories of riparian forest according to their ecological integrity (3 references in old growth forest, 3 in degraded forest and 3 in secondary forest). Erskine et al. (2019) combined vegetation surveys and mapping data to select five reference sites for the ecological restoration of uranium mines, while McManamay et al. (2018) suggest a spatial framework in six steps to identify reference systems for stream restoration. All these studies used multiple references that were selected according to relationships between environmental factors and vegetation patterns. They included floristic surveys (contemporary data) and the search of historical data. These studies suggest extending the surveys beyond the close vicinity of restoration sites and using statistical tools to identify and to rank structuring variables in order to select appropriate reference communities. In our framework, we filled this gap in providing a method to identify reference plant communities based on statistical analyses of specific relationships between environmental factors and plant community composition.

Since soil conditions are among the most important factors driving plant species composition, a careful variable choice is needed to exclude factors strongly influenced by previous ecosystem degradation. Soil disturbance, such as tillage or soil removal, may lead

to a drastic reduction in organic matter and total nitrogen storage, and can strongly affect their distribution in the soil profile (Hölzel & Otte 2003; Dolan et al. 2006; Du et al. 2010). Intermediate arable use, as the most common degradation of European grasslands, increases soil nutrient content, in particular plant available potassium and phosphorous (Bischoff et al. 2009). In such a case of eutrophication, the depletion of fertilizer residues is crucial to obtain abiotic conditions that are appropriate for the establishment of plant species adapted to the pre-degradation state (Tallowin et al. 1998; Bischoff et al. 2009). Thus, our method of reference community identification is difficult to apply to ecological restoration of fertilized soils. However, if habitat degradation is limited to mechanical soil disturbance, many soil variables typically driving plant species composition remain unchanged. In our approach, we avoided the use of the soil parameters cited above (total and mineral nitrogen, organic matter) that are known to be influenced by mechanical soil disturbance. Thus, we are convinced that our selected abiotic factors are appropriate for the search of best matching reference sites. Such data collected in situ (contemporary data) may be complemented by historical data (wildfire records, land use changes) that may still affect plant species composition (Asbjornsen et al. 2005; Urgenson et al. 2018).

Our method is applicable to ecological restoration beyond grasslands if the specific environmental factors structuring the plant communities are correctly identified. For example, in ecological restoration of lagoon ecosystems corresponding to ancient salt marshes (EU habitat code 1150), environmental factors structuring plant communities are water level, duration of flooding and degree of salinity that may also be used to identify reference communities (Bouzillé 2007).

In restoration practice, adjacent sites of very similar environmental conditions are not always available. Either the degradation includes the whole habitat with completely different conditions outside the degraded zone (e.g. degraded grasslands within woodlands), adjacent sites are not accessible (or not available as donor sites for seed transfer) or abiotic conditions change at very small scales. In such cases, our approach allows the identification of geographically more distant reference or donor sites that match best with environmental conditions of restoration sites. In this case, the selection of multiple potential references is therefore necessary to select the one(s) that will be closest to the restored sites in terms of environmental variables driving plant community composition. The concept of multiple references is a helpful tool in restoration practice allowing a more flexible choice depending on availability (identification of donor sites) or on different priorities for environmental factor match. Additionally, even if the restoration sites are close, the best potential reference sites are not necessarily the same, depending on the environmental factors that can vary on a relatively small scale. Finally, in grassland restoration practice, the identification of multiple references allows to combine different donor sites if non-degraded sites do not comprise the entire species pool (area not sufficient, environmental conditions do not fully correspond). Although not directly tested in our proof-of-concept, the method also allows the integration of land management variation resulting in different quasi-stable states of plant succession.

To conclude, our approach to use the close relationships between environmental conditions and plant community composition is a straightforward method to identify reference communities when environmental structuring factors are not affected by degradation. It can be easily applied to restoration practice but requires a good knowledge of plant ecology, in particular on plant-environment interactions, environmental filters and local species pools (Lortie et al. 2004; Cristofoli & Mahy 2010). A better understanding of plant-environment interactions and of potential effects of degradations on physical and chemical soil conditions would be required to improve the method.

1.4 Supporting Information

Supplement S1.1. Estimation of annual direct incident solar radiation (ASR) using the method of McCune and Keon (2002):

- a) “To approximate heat load, the equation can be shifted from a maximum on south slopes to a maximum on southwest slopes and a minimum on northeast slopes. This is accomplished by ‘folding’ the aspect about the NE - SW line, such that NE becomes zero degrees and SW becomes 180°”: $Folded\ aspect = |180 - |aspect - 225||$
- b) $f(latitude, slope, aspect) = 0.339 + 0.808 * \cos(latitude) * \cos(slope) - 0.196 * \sin(latitude) * \sin(slope) - 0.482 * \cos(aspect) * \sin(slope)$ [Eq. 3 in McCune et Keon 2002]
- c) f returns $\ln(\text{Rad, MJ.cm}^{-2}\text{.yr}^{-1})$. It is therefore returned to an arithmetic scale with the $\text{EXP}(x)$ function. We obtain Annual solar radiation: $\text{ASR} = \text{EXP}(f)$

Table S1.1. Correlations between environmental variables.

	Elevation	ASR	Soil moisture	Soil pH	P205	K20
Elevation	1.000	0.081	0.035	-0.017	0.207	0.247
ASR	0.081	1.000	0.071	0.442	-0.367	0.175
Soil moisture	0.035	0.071	1.000	0.073	0.450	0.104
Soil pH	-0.017	0.442	0.073	1.000	-0.330	0.398
P205	0.207	-0.367	0.450	-0.330	1.000	0.118
K20	0.247	0.175	0.104	0.398	0.118	1.000

Table S1.2. Relationships between grassland plant composition (as represented in Fig. 1.2) and six environmental gradients using “envfit” with $N = 999$ permutations (R package “vegan”). The significant variables are in bold.

	R ²	P
Elevation	0.19	0.201
ASR	0.59	0.004
Soil moisture	0.39	0.023
Soil pH	0.36	0.026
P205	0.40	0.025
K20	0.02	0.830

Table S1.3. Complete names of plant species represented in the RDA biplot (**Fig. 1.3**)

RDA names	Full names	RDA names	Full names
Ach_millefolium	<i>Achillea millefolium</i>	Lot_corniculatus	<i>Lotus corniculatus</i>
Ant_vulneraria	<i>Anthyllis vulneraria</i>	Med_lupulina	<i>Medicago lupulina</i>
Ara_hirsuta	<i>Arabis hirsuta</i>	Pil_offcinarum	<i>Pilosella officinarum</i>
Are_serpyllifolia	<i>Arenaria serpyllifolia</i>	Pla_lanceolata	<i>Plantago lanceolata</i>
Ast_danicus	<i>Astragalus danicus</i>	Pla_media	<i>Plantago media</i>
Bra_rupestre	<i>Brachypodium rupestre</i>	Poa_pratensis	<i>Poa pratensis</i>
Bro_erecta	<i>Bromopsis erecta</i>	Pot_verna	<i>Potentilla verna</i>
Dac_glomerata	<i>Dactylis glomerata</i>	Sal_pratensis	<i>Salvia pratensis</i>
Fes_marginata	<i>Festuca marginata</i>	San_minor	<i>Sanguisorba minor</i>
Gal_verum	<i>Galium verum</i>	Tar_Taraxacum	<i>Taraxacum officinale agg.</i>
Hip_comosa	<i>Hippocrepis comosa</i>	Teu_chamaedrys	<i>Teucrium chamaedrys</i>
Kna_arvensis	<i>Knautia arvensis</i>	The_divaricatum	<i>Thesium divaricatum</i>
Lat_pratensis	<i>Lathyrus pratensis</i>	Tra_pratensis	<i>Tragopogon pratensis</i>

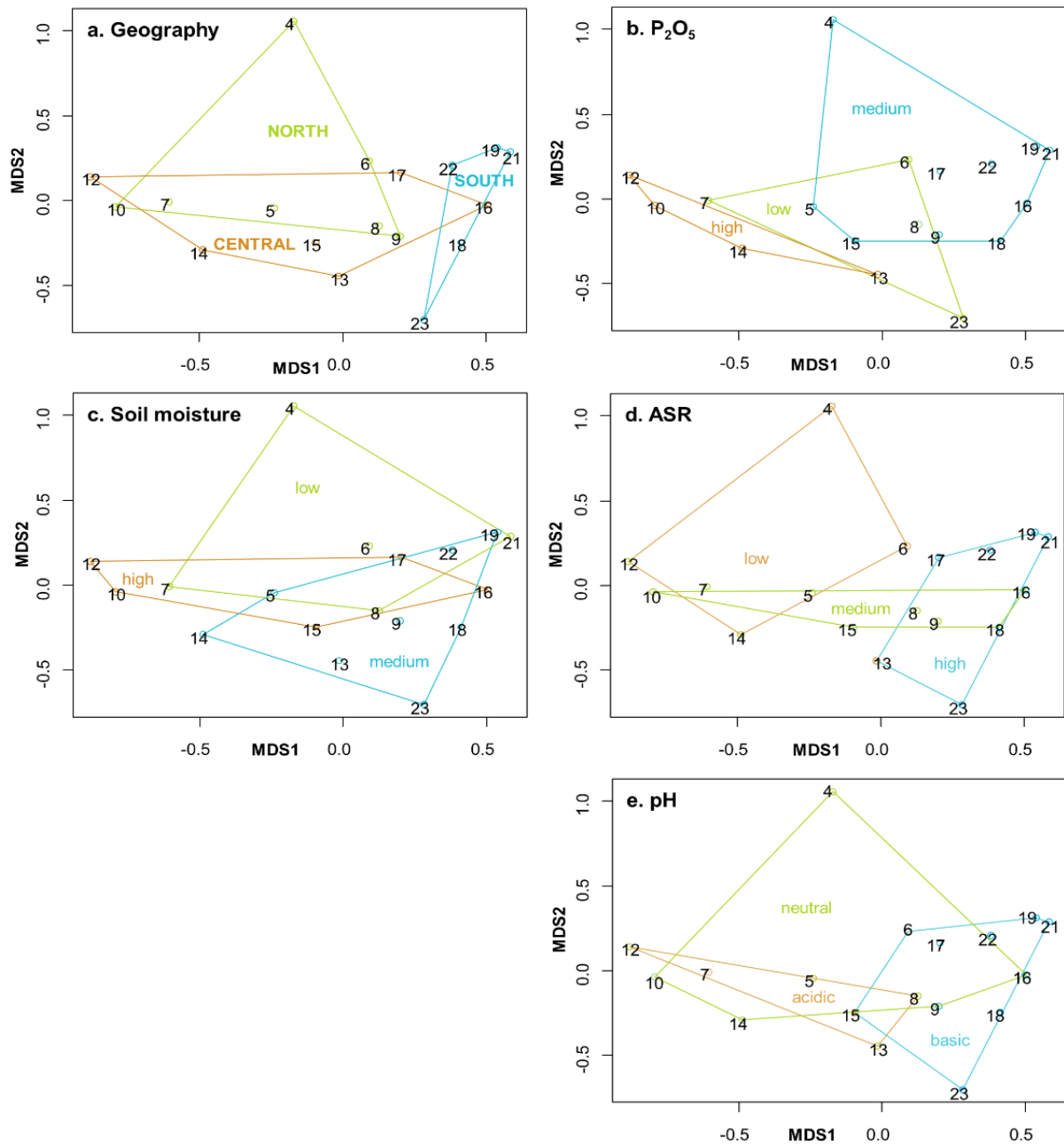


Fig. S1.1. NMDS based on plant species composition showing groups of the reference sites according to **a.** geographical position (south; central; north) in comparison with groups according to significant environmental variables: **b.** P₂O₅; **c.** soil moisture; **d.** annual direct incident solar radiation (ASR) and **e.** soil pH.

Transition to Chapter 2

(1.a) How should we determine the references?

In **chapter 1**, I suggest a framework based on relationships between vegetation and environmental factors to identify the best reference communities. The evaluation of similarities between environmental factors in degraded sites and in non-degraded sites highlighted that the environmentally most similar sites were not necessarily the geographically closest ones. Therefore, the analysis of plant-environment interactions provides an useful tool to identify reference communities or donor sites for seed harvest if not available adjacent to degraded sites.

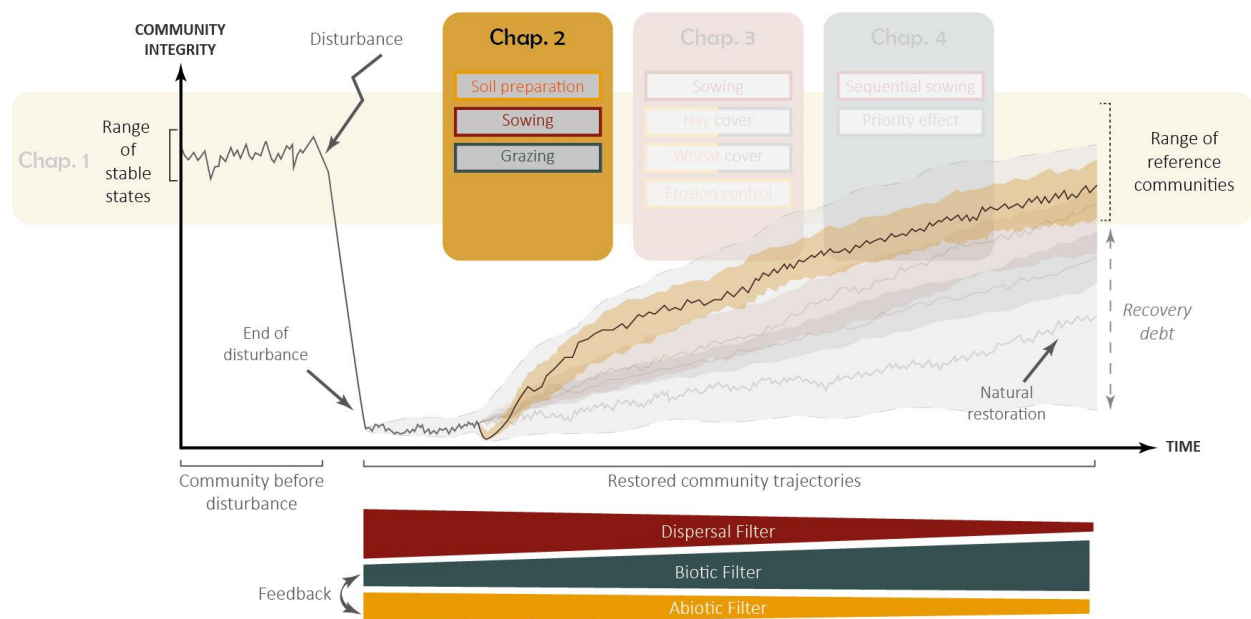
(1.b) Which factors influencing species composition are appropriate for the choice of references?

Slope and aspect driving direct incident solar radiation were identified as major factors influencing species richness and composition in the mountain grasslands of this study. Edaphic factors such as soil moisture, soil pH and exchangeable phosphorus were also important parameters structuring plant communities of the 'Haute-Durance' valley.

Chapter 2 focuses on drivers limiting seedling recruitment in mountain grassland restoration. Seeds were harvested using a brush harvester in one of the 18 references determined in chapter 1 and transferred to five degraded sites. I set up a combined soil preparation and grazing experiment to evaluate the effect of both factors on seedling recruitment.



Seedling recruitment in mountain grassland restoration: effects of soil preparation and grazing



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Seedling recruitment in mountain grassland restoration: effects of soil preparation and grazing.

Abstract

Questions: Seedling recruitment is a key step in any seed-based ecological restoration project. There is a controversial discussion i) whether soil preparation is required to reduce competition of pre-existing vegetation, or whether vegetation cover facilitate seedling recruitment, and ii) whether grazing should be excluded in initial stages of grassland restoration to protect seedlings, because grazing exclusion may also favour competitive ruderal species. We set up a combined soil preparation and grazing experiment to evaluate the effect of both factors on seedling recruitment of seeds transferred from a species-rich donor site.

Location: Upper Durance valley, Hautes-Alpes, France.

Methods: The experiment was set up using a full factorial split-plot design with five replicate sites. The treatments included soil preparation (harrowed or not) and grazing (excluded or not). Seeds were transferred using plant material brushed in a non-degraded reference grassland. The individuals of all occurring species were counted on the restoration sites, and the survival and reproduction of two focal species, *Bromopsis erecta* and *Rhinanthus alectorolophus*, were recorded for three months.

Results: Soil preparation by harrowing reduced the density of spontaneously emerging species and increased the seedling density of species transferred from the donor site. Grazing had only a weak negative effect on the recruitment of transferred species. The main effect of both treatments on seedling survival was not significant, but a significant interaction indicated that the grazing effect depended on soil preparation, with a negative effect of grazing only in non-harrowed plots.

Conclusions: The reduction of competition by soil preparation before seed addition of brush material had a positive effect on the seedling recruitment of transferred species and can thus clearly be recommended in our study system. The weak negative effect of grazing may not always justify fencing costs.

Keywords: *Bromopsis erecta*, brush harvesting, community ecology, ecological engineering, grassland restoration, grazing, harrowing, montane grasslands, *Rhinanthus alectorolophus*, seedbed preparation, seedling establishment, survival.

2.1 Introduction

Seedling recruitment is an important process driving the composition and diversity of plant communities (Grubb 1977; Zeiter et al. 2006). Understanding the factors constraining seedling recruitment is thus crucial to predict and improve restoration success. Dispersal (Münzbergová and Herben 2005; Frances et al. 2010) and microsite limitation (Myers and Harms 2009; Long et al. 2014) are known to be such constraining factors in re-colonisation processes. In grassland restoration, the small soil seed bank of typical late-successional species may result in insufficient seedling recruitment (Grman et al. 2015; Buisson et al. 2018). Poor seed dispersal, as well as the lack of connectivity between source populations due to landscape fragmentation, often limit the seed rain (Münzbergová and Herben 2005; Bischoff et al. 2009; Muller et al. 2014) and successful restoration may require seed addition to overcome seed limitation (Öster et al. 2009; Török et al. 2012; Valkó et al. 2016). Thus, seed addition methods such as sowing local seed mixtures, hay transfer or soil transfer, are increasingly used in grassland restoration (Kiehl et al. 2010; Scotton et al. 2012; Kiss et al. 2020).

Seed-addition experiments demonstrated that limited recruitment is the next major constraint influencing plant establishment (Tilman 1997; Turnbull et al. 2000; Pywell et al. 2002). Unfavourable microsite conditions may hamper germination and reduce seedling survival often resulting in microsite limitation (Bissels 2006). Soil disturbance, such as topsoil removal or soil tillage, increases recruitment opportunities (Hölzel and Otte 2003; Myers and Harms 2009; Long et al. 2014; Klaus et al. 2017). Soil preparation (also called “seedbed preparation”; Shaw et al. 2020) may promote the recruitment of plant species by (i) limiting the competition of an already established vegetation, and by (ii) improving abiotic conditions of seedling recruitment (Kiehl et al. 2010). A low pre-existing vegetation cover may have a facilitating effect on recruitment by protecting seedlings from high solar radiation and drought stress (Callaway and Walker 1997). However, if pre-existing vegetation is dense, the competitive effect on introduced species may prevail because already established species limit available space and resources (Kupferschmid et al. 2000; Poschlod and Biewer 2005). Preparing the soil may favour seedling recruitment of new species by opening the vegetation and destroying the root systems of competitive perennial species (Bischoff et al. 2018). Soil preparation further creates micro-reliefs improving seed and water retention as well as seed adhesion (Chambers 2000). However, soil disturbance increases nitrogen mineralisation increasing plant available nitrogen in the soil (eutrophication) and may negatively affect the soil fauna and soil structure potentially favouring erosion (Di and Cameron 2002; Holland 2004).

Human land management by grazing and mowing is a key factor regulating the functioning and structure of semi-natural grasslands (Hejcman et al. 2013). Several studies confirmed the essential role of grazing in maintaining plant species richness of grassland

ecosystems (Dupré and Diekmann 2001; Pykälä 2003, Saatkamp et al. 2018). However, in the initial stages of grassland restoration, grazing may hamper seedling recruitment since seedlings are particularly vulnerable to trampling and biomass removal by herbivory (Bakker 2003; Buisson et al. 2015). Trampling may prevent species from germinating by crushing and damaging seeds or coleoptiles (Rother et al. 2013). Moreover, trampling on slopes may lead to soil destabilization (Tasser et al. 2003), increases soil erosion (Farrell and Fehmi 2018) and soil compaction (Allington and Valone 2011). However, in mountain ecosystems, in which the particular pedo-climatic conditions (soil erosion, shallow and stony soils) reduce seedling recruitment, micro-reliefs created by hoof prints may increase seed trapping (Isselin-Nondedeu et al. 2006) and improve seed adhesion to the soil (Chambers 2000). Accordingly, Eichberg and Donath (2018) found an increased seedling recruitment in a trampling simulation experiment on sandy soils also suffering from low stability and high run-off. Biomass removal by herbivory damages seedlings and often reduces initial seedling survival (Buisson et al. 2015; Vidaller et al. 2019b). However, Farrell and Fehmi (2018) only found a combined negative effect of trampling and herbivory whereas herbivory alone did not affect seedling establishment. Furthermore, Kladivová and Münzbergová (2016) even revealed a positive effect of grazing on both seedling recruitment and establishment but the grazing effect depended on habitat conditions at the specific microsite.

In our study, we aim to test the effect of soil preparation and grazing on seedling recruitment (combining germination and early survival) in a seed-addition experiment conducted in semi-natural mountain grasslands. Using brush material transferred from a species-rich donor grassland to restore degraded sites, we addressed the following questions: (1) Does soil preparation by harrowing before sowing improve seedling recruitment and survival of the transferred species? (2) What is the effect of early grazing on seedling recruitment and survival of the transferred species?

2.2 Material and methods

2.2.1 Study area

The study area is the upper Durance valley (“Haute-Durance”) in the Southern French Alps. The construction of a high-voltage transmission line led to a local destruction of species-rich grassland communities within a 1 km wide and 100 km long strip from L'Argentière-la-Bessée to La-Batie-Neuve (44°78'78"N, 6°59'41"E; 44°57'93"N, 6°20'77"E). The experimental sites representing a subsample of these grasslands are located between 1100 and 1300 meters above the sea level (upper montane altitudinal belt). The climate is temperate to sub-Mediterranean depending on slope, exposition and elevation. At the closest meteorological station (Embrun), the annual precipitation is about 740 mm and the

mean annual temperature 10.7°C. Considering the lower elevation of the station (880 m) the temperature is roughly 2°C lower resulting in an annual mean of 8.7°C at our experimental sites. The bedrock is predominantly calcareous with some quaternary glacial deposits. Non-degraded grasslands of the study area belong to Natura 2000 priority habitats: “Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates” (N6210*, EU directive habitat 92/43/EEC; Calaciura and Spinelli 2008) that are traditionally managed either by mowing, cattle and sheep grazing. On transitory access tracks and construction platforms, the vegetation was removed, and the soil compacted to facilitate the transport of heavy equipment. To recreate former soil conditions after electricity line construction the soil was de-compacted by deep tillage between May and July 2018.

2.2.2 Donor site and seed harvest

The donor site to collect brush material is located within the study area at Freissinières at an elevation of 1100 m (44°73'61"N; 6°56'72"E). The soil and climatic conditions of the donor site corresponded to the average of the five experimental sites. The seed material was harvested in July 2018 using a brush harvester mounted on a quad and equipped with an integrated vacuum system. On a surface of 4000 m², we obtained 2.6 kg of brush material. The brush material comprised seeds (60% of total mass) and vegetative parts (40%). Seed counts in ten subsamples of 0.5 g revealed an average density of 887 seeds/g of brush material. A single harvest was considered as sufficient since the short summer season in the study area has resulted in a concentration of the seed production period for most species.

2.2.3 Experimental set-up and design

The experiment was set up in October 2018 using a full factorial split-plot design replicated on five sites (blocks) to test the effect of previous soil preparation, grazing and its interaction on seedling recruitment (see Durbecq et al. 2020, **chapter 1**, for the position of experimental sites). Each of the five sites comprised eight plots of 16 m² each (4 m × 4 m) resulting in a total of 40 plots. Four plots representing a half-block were exposed to extensive grazing and the remaining four were fenced preventing grazing (whole-plot factor, **Fig. 2.1**). The sowing and soil preparation treatments (split-plot factors) were randomly assigned within half-blocks. The distance between plots was 1 m except for the Embrun site where this distance was reduced to 50 cm due to limited space.



Fig. 2.1. Restoration site before (left) and after (right) experimental set-up involving enclosure by fencing and soil preparation by harrowing.

The full design also comprised unsown control plots randomly assigned to half-blocks. The unsown plots were used to evaluate which plant species of the donor grassland already occurred at the restoration sites before the brush material was transferred (soil seed bank, seed rain). Average vegetation cover of these unsown controls was 28% in the first year compared with 90% at the donor site. Species occurring in the control plots of a given site were considered as “spontaneously emerging” for this site. Only plant species of the donor site not occurring in the control plots were considered as “transferred” (**Table and Fig. S2.1**). The method may underestimate the density of transferred species since species occurring in unsown control plots may still have been transferred to the sown plots.

Sites already covered with spontaneous vegetation were mown prior to experimental set-up. Harrowing was selected as a soil preparation method using a rotary harrow adjusted to a working depth of 8 cm. In October 2018, just after harrowing, the brushed material was spread at a density of 104 g/plot corresponding to a seed density of 3.75 g/m² and approximately 5765 seeds/m². The chosen seed density was based on recommendations of a previous research project in the French Alps (Koch et al. 2015). In agreement with the traditional local grazing regime, the unfenced experimental plots were grazed twice a year: in late October just after experimental set up, and in early June. Two sites were grazed by cattle, three sites by sheep, corresponding to the average proportion of both grazing types in the study region. The sites were neither fertilised nor irrigated.

2.2.4 Data collection

2.2.4.1 Vegetation relevés at the donor site and germination tests in the greenhouse

Prior to seed harvest, the cover of all occurring vascular plant species was estimated in five representative 3 m × 3 m plots as the vertical projection cover of above-ground vegetation in late June 2018. We used Tison et al. (2014) for plant species identification and as reference for plant species names. At the harvest in July 2018, the average phenological stage of all species was recorded in order to evaluate seed availability. Eighty percent of the species recorded in June were found to have seeds in July.

In order to obtain information on the germination potential of seeds contained in the brush material and to evaluate germination in the field compared with germination potential, we set up a germination test in November 2018. Subsamples of the brush material were transferred to five trays (0.03 m²) filled with standard peat substrate and placed in an unheated greenhouse. Each tray received 0.54 g of brush material corresponding to 18 g/m² and a density of approximately 18 000 seeds per m². The trays were regularly watered, and germination was recorded until April 2019. Greenhouse germination of the harvested brush material was recorded once a week for four months and seedlings were removed after identification.

2.2.4.2 Seedling survey and vegetation relevés at restoration sites

To quantify seedling recruitment of both transferred species and spontaneously emerging species, plant numbers were counted in three quadrats of 40 cm × 40 cm placed along the diagonal of each plot. Since we were not able to distinguish seedlings and plants re-sprouting from below-ground organs, the seedling counts may include young ramets of clonally growing plants. In order to identify spontaneously emerging species, we conducted at the same time vegetation relevés on the unsown control plots (9 m²).

2.2.4.3 Seedling survival of focal species

Seedlings of two typical species of the donor site, *Bromopsis erecta* (Huds.) Fourr. (former name: *Bromus erectus*) and *Rhinanthus alectorolophus* (Scop.) Pollich were monitored over 11 weeks in order to assess seedling survival. *B. erecta* is the characteristic species of semi-dry grasslands (Mesobromion type *sensu* Ellenberg 1996) occupying a large range in Europe. It is further one of the typical species of the habitat type (N6210*, EU directive habitat 92/43/EEC; Calaciura and Spinelli 2008). *B. erecta* is a perennial grass growing on calcareous and often nutrient-poor soils. *R. alectorolophus* is a summer annual, hemi-parasitic species growing in semi-dry to mesophilic calcareous grasslands. As *B. erecta*, it is among the characteristic species of the N6210*-habitat type. *R. alectorolophus* has a wide range of hosts that includes grasses and legumes (Sandner and Matthies 2018).

The species is used as a tool in restoration of species-rich grasslands as it reduces competition by grasses and may thus have a positive effect on plant diversity (Bullock and Pywell 2005; Heer et al. 2018). In all sown plots (four plots per site), ten randomly chosen seedlings of both species were tagged in May 2019 using coloured rings and poles (**Fig. S2.2 S2.2**). A total of 400 seedlings were tagged over the five sites. The position of each individual was mapped, and each seedling was numbered in order to facilitate localization and recognition. Seedling survival and reproduction of these two focal species were recorded in late July 2019.

2.2.4.4 Data analysis

We analysed (i) seedling numbers and species richness of the total plant community (“all species” = spontaneously emerging species + transferred species), and of transferred species only, as well as (ii) the survival of the two focal species, *Bromopsis erecta* and *Rhinanthus alectorolophus*.

Generalized linear mixed models were applied to assess the effect of soil preparation (harrowing: yes/no) and of grazing (yes/no) on seedling recruitment and the survival of *B. erecta* and *R. alectorolophus*. The full model included the two factors and the grazing-by-soil preparation interaction as fixed effects and site as a random effect. In order to include the split-plot design of the experiment, a grazing-by-site interaction (random) was fitted to the model to test the whole-plot factor (grazing). Harrowing (split-plot factor) and the site-by-harrowing interaction were tested against the model residuals. Abundance data were right-skewed but models with Poisson error distribution and log-link function showed overdispersion. Thus, negative binomial error distribution and log-link function were finally used to test total abundance and abundance of transferred species. Species richness was tested using Gaussian error distribution and identity link. Both models additionally included quadrat within plots as a random factor. Survival was analysed at plot level and did not include quadrat. A binomial error distribution was fitted using a logit-link function.

Non-Metric Multidimensional Scaling (NMDS) based on Bray–Curtis similarity was applied to compare plant species composition in different treatments (Borcard et al. 2011). A permutation multivariate analysis of variance (PERMANOVA, Anderson 2001) with 9999 permutations was used to analyse whether the community composition was significantly different between the grazing and soil preparation treatments (R package “vegan”, Oksanen et al. 2019). All analyses were performed using R 3.5.3 (R Core Team 2019).

2.3 Results

2.3.1 Seedling emergence

A total of 21 plant species germinated in the greenhouse corresponding to 37.5% of the total species number observed at the donor site. At the restoration sites, 29 donor site species (51.8%) were found. Taking into account the difference in the amount of seed material used in the greenhouse test and at the restoration sites, the final germination was 40 times higher in the greenhouse (7.31 seeds/g) than in the field (0.17 seeds/g; **Table and Fig. S2.1**). In particular, *Plantago media* L. *Festuca marginata* (Hack.) K.Richt. and *Schedonorus pratensis* (Huds.) P.Beauv. showed a much higher greenhouse germination whereas the difference between greenhouse and field was much smaller in *B. erecta* (4 times higher in the greenhouse). *R. alectorolophus* did not germinate in the greenhouse.

2.3.2 Seedling recruitment

Soil preparation by harrowing reduced overall seedling recruitment including that of spontaneously emerging species. Grazing had a marginally significant negative effect on seedling recruitment (**Table 2.1, Fig. 2.2a**). The opposite effect of harrowing was observed for transferred plant species. Twice as many seedlings of transferred species were found in the harrowed than in the non-harrowed plots (**Fig. 2.2b**). Grazing resulted in a 50% reduction of the seedling density of transferred species, but due to the high between-site variation the grazing effect was only marginally significant. The grazing-by-soil preparation interaction was not significant for both response variables.

Table 2.1. Effect of harrowing and grazing on number of seedlings per m², species richness and the survival of two focal species. Results on seedling number and richness are presented separately for the whole plant community and the subsample of transferred species. H x G = grazing-by-harrowing interaction. Results of GLMM with Chi-Square (χ^2) and significance levels: . P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001, NS not significant.

	df	Seedling number		Species richness		Survival	
		All species	Transferred species	All species	Transferred species	<i>Bromopsis erecta</i>	<i>Rhinanthus alectorolophus</i>
Harrowing	1	7.097 **	6.435 *	3.476 .	6.308 *	2.484 NS	0.741 NS
Grazing	1	3.726 .	3.440 .	2.661 NS	2.804 .	1.799 NS	2.585 NS
H x G	1	0.560 NS	0.011 NS	0.014 NS	1.095 NS	5.089 *	4.851 *

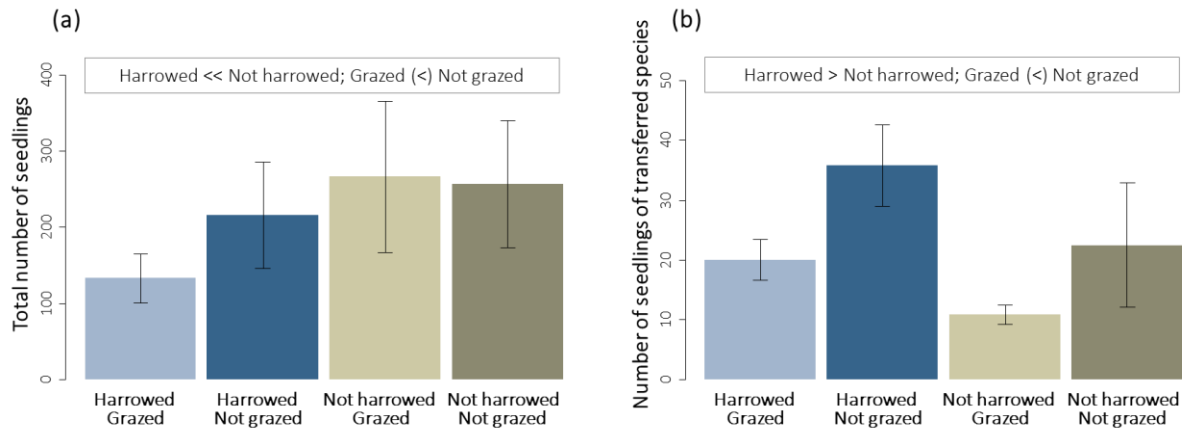


Fig. 2.2. Number of seedlings per m² of **(a)** all species, and of **(b)** transferred species only, in the four restoration treatments. The direction of main effects is indicated using “<<” (P<0.01), “<” (P<0.05), “(<)” (P<0.1; marginally significant).

2.3.3 Plant species richness

The treatment effects on overall species richness were not (grazing) or marginally (increase when harrowed) significant (Table 2.1, Fig. 2.3a). The species richness of transferred species was 30% higher in the harrowed compared to the non-harrowed plots (Fig. 2.3b). Similarly to the abundance of transferred species, the richness of transferred species was reduced by grazing but the effect was only marginally significant. No significant grazing-by-soil preparation interaction was observed.

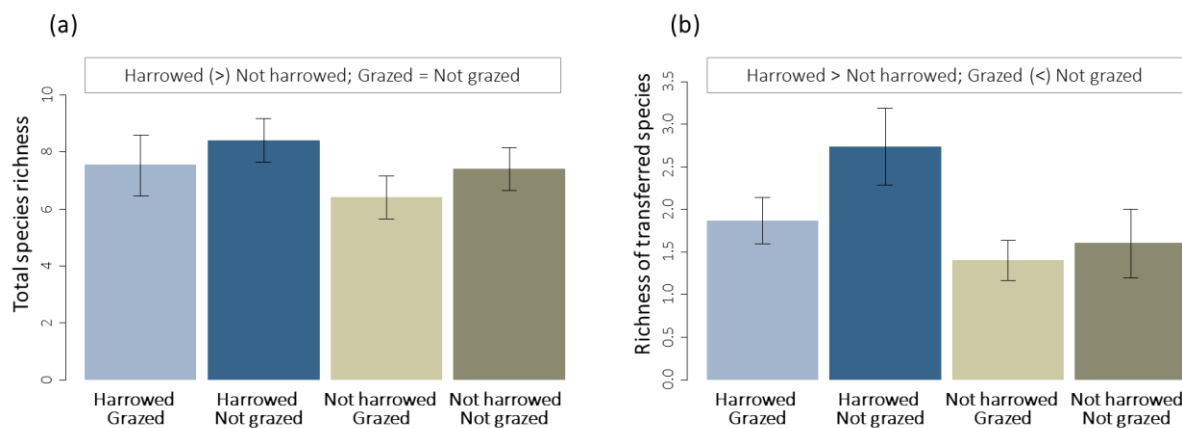


Fig. 2.3. **(a)** Species richness of seedlings of all species and of **(b)** transferred species in the four restoration treatments. The direction of main effects is indicated “<” (P<0.05), “(<)” (P<0.1; marginally significant) and “=” (not significantly different).

2.3.4 Plant species composition

The species composition was not clearly separated between treatments resulting in a large overlap of NMDS polygons (**Fig. 2.4**). Neither harrowing ($P = 0.198$), nor grazing ($P = 0.392$) nor the grazing-by-soil preparation interaction ($P = 0.461$) were significant. The plots without soil preparation nor grazing showed higher variation in species composition resulting in a larger polygon. Independent of treatment, semi-dry grassland species such as *B. erecta*, *Saponaria ocymoides* L. and *Eryngium campestre* L. occurred in the upper part of the ordination plot, and ruderal species such as *Capsella bursa-pastoris* (L.) Medik. *Atriplex patula* L. *Anisantha sterilis* (L.) Nevski (former name: *Bromus sterilis*), *Veronica polita* Fr. and *Polygonum aviculare* L. in the lower right part.

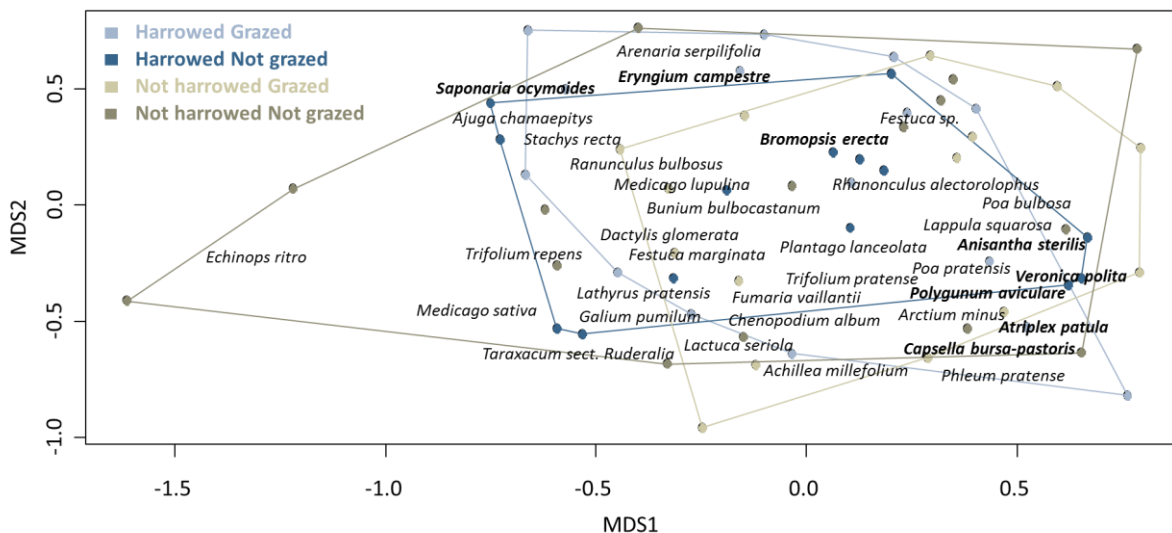


Fig. 2.4. Plant species composition in the four restoration treatments using NMDS. Polygons indicate the position of the outmost plots in each treatment (NMDS stress: 0.190).

2.3.5 Seedling survival of two focal species

Seedling survival of *B. erecta* was high in all treatments ($\geq 75\%$, **Fig. 2.5a**). The main effects of grazing and soil preparation were not significant, but a significant grazing-by-soil preparation interaction indicated that the grazing effect depended on soil preparation (**Table 2.1**). Grazing reduced seedling survival only in non-harrowed plots. With few exceptions, *B. erecta* did not reach the reproductive stage in the first year. The survival rate of *R. alectorolophus* was low ($<45\%$) compared to that of *B. erecta* (**Fig. 2.5b**). Similar to *B.*

erecta, no significant main effect was found for *R. alectorolophus* but a significant grazing-by-soil preparation interaction explained by a negative grazing effect in non-harrowed plots (Fig. 2.5b). Most surviving *R. alectorolophus* individuals of the un-grazed, non-harrowed plots were flowering in July, with a significant negative effect of grazing ($\chi^2 = 7.348$, $df = 1$, $P = 0.007$; Fig. S2.3). Few individuals reached the reproductive stage in the three other treatment types. Similarly to survival, the significant grazing-by-soil preparation interaction ($\chi^2 = 4.100$, $df = 1$, $P = 0.043$) was explained by a negative grazing effect in non-harrowed plots.

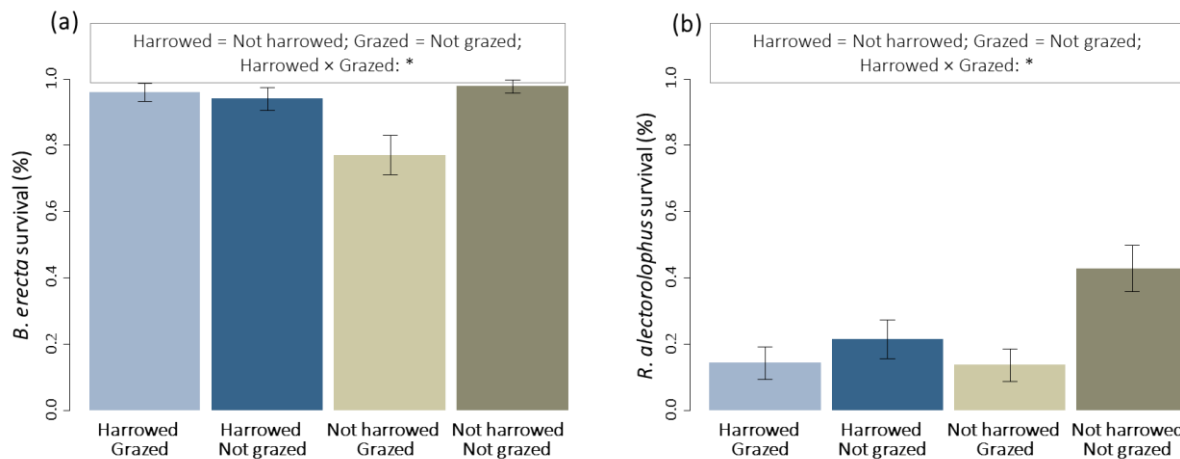


Fig. 2.5. Final seedling survival of (a) *Bromopsis erecta* and (b) *Rhinanthus alectorolophus*. The direction of main effects is indicated using “<” ($P < 0.05$) and “=” (not significantly different). Significant grazing-by-soil preparation interactions are presented using “*” ($P < 0.05$).

2.4 Discussion

The low field emergence compared with greenhouse germination showed that seedling recruitment may be a bottleneck in ecological restoration of grasslands (Öster et al. 2009). In our study on mountain grasslands, soil preparation largely increased the recruitment of transferred species, whereas competition by spontaneously emerging ruderal species was reduced. Grazing had only a weak negative effect on seedling density. While neither harrowing nor grazing had an effect on focal species survival when applied alone, a significant interaction effect indicated that the grazing effect depended on soil preparation or vice versa. The negative grazing effect was generally stronger in non-harrowed plots. Despite significant grazing and harrowing effects on seedling recruitment and early survival,

none of the treatments influenced the first-year plant community composition.

Several studies have demonstrated that soil preparation prior to seed addition increases germination success and emergence of added seeds because it limits competition of pre-existing vegetation (Edwards et al. 2007; Bischoff et al. 2018, Harvolk-Schöning et al. 2020). However, pre-existing vegetation or a mulch layer may also facilitate seedling recruitment (Donath et al. 2007; Scotton et al. 2012). In particular in dry and open habitats, the absence of vegetation cover increases drought stress reducing germination and seedling survival (Callaway and Walker 1997; Eckstein and Donath 2005). Our restoration sites in the Upper Durance valley clearly benefitted from previous harrowing indicating that the reduction of competition and improved conditions for germination (Chambers 2000) prevailed over the reduction of facilitation effects. Similarly, Schmiede et al. (2012) and Harvolk-Schöning et al. (2020) found that the cover of target species was higher following soil disturbance and seed introduction in a floodplain grassland. To our knowledge, our study is the first showing a simultaneous negative effect of soil preparation on spontaneously emerging species and a positive effect on transferred species. This effect was observed shortly after construction work leading to vegetation degradation and may be even stronger at restoration sites with higher spontaneous vegetation cover (here only 28%). The reduced competition by already established species created favourable microsite conditions for the recruitment of transferred species. Soil tillage particularly reduced the abundance of ruderal, competitive species (sensu Grime 1988), such as *Anisantha sterilis* (L.) Nevski, *Capsella bursa-pastoris* (L.) Medik. *Atriplex patula* L. and *Polygonum aviculare* L. Without soil disturbance, such competitive early successional species hamper the establishment of the transferred late-successional species (Donath et al. 2007; Jaunatre et al. 2014). Regardless of competition, harrowing has also been shown to create favourable micro-reliefs improving the adhesion of the transferred seeds to the soil and thus favouring recruitment and establishment (Isselin-Nondedeu et al. 2006). Chambers et al. (2000) showed that seed movements and seedling establishment clearly depended on soil surface structure with holes limiting seed removal and increasing germination. The quantity of removed seeds also depends on the number of transferred seeds and their morphology (size, weight, shape). A better understanding of interactions between soil preparation, seed adhesion to the soil and seed morphology may help to increase the recruitment of transferred species and thus improve the efficiency of restoration measures (Jakobsson and Eriksson 2000).

Contrary to the soil treatment, the effect of grazing was not selective between transferred species representing the target species of restoration, and non-target species. Grazing had a small but generally negative effect on seedling recruitment. The negative grazing effect was smaller than in other studies of the same geographical region but under Mediterranean climate (Buisson et al. 2015; Vidaller et al. 2019b) in which the transferred species clearly established better when grazing was excluded. Grazing animals negatively affect seedling recruitment by trampling and later on by biomass removal. Trampling by livestock does not only damage seedlings but also strongly affects the soil by erosion (Farrell and Fehmi 2018) or destabilization (Tasser et al. 2003), particularly in mountain grassland

slopes. On the other hand, trampling may have a positive effect on seedling recruitment of transferred species, since pre-existing vegetation is damaged by bruising, crushing, plant displacement or burial in mud (Bilotta et al. 2007), thus limiting competition with non-target species. Similar to soil preparation, trampling may also create holes reducing seed removal of transferred species (Isselin-Nondedeu et al. 2006). Soil preparation and grazing may thus have a common positive effect: the creation of microsites favourable to recruitment by limiting competition, increasing seed adhesion and water retention (Isselin-Nondedeu et al. 2006; Chambers 2000). The outcome of the trade-off between microsite creation and seedling damage depends on the productivity of the sites and the timing of grazing. Biomass removal by grazing or mowing is particularly required on highly productive sites where competitive grasses often hamper seedling recruitment and establishment (Schmiede et al. 2012; Kladivova and Münzbergova 2016). Nutrient-poor sites rather need protection of seedlings against grazing than biomass removal since competition is much lower (Kirmer et al. 2012; Scotton et al. 2012; Vidaller et al. 2019a 2019b). The marginally significant negative grazing effect in our study may thus be explained by the intermediate productivity level of our restoration sites. Post-pasture sowing has been cited as a method to optimise timing, because it allows seedlings to develop before the next grazing, reducing trampling and herbivory at the most vulnerable stage (Eichberg and Donath 2018). Our study sites were first grazed before seedling emergence, 2-3 weeks after brush material transfer, and the following grazing period occurred seven months later. Thus, the first grazing probably had a positive effect in reducing competition by pre-existing vegetation, but the second grazing period may have negatively affected young seedlings that predominantly emerged in spring, finally resulting in a slightly negative effect of grazing. The vegetation period usually starts after snow melt in March resulting in a low probability to attain maturity before the June grazing period. The timing of grazing needs to be taken into account to limit the damage on seedlings, particularly if grazing exclusion is not possible. Livestock type may influence grazing effects (Tóth et al. 2016). In our study, we focused on an overall grazing effect including sheep and cattle, and the grazer species effect was pooled with experimental site. It may be interesting for future studies to distinguish effects of cattle and sheep grazing on seedling recruitment.

The analysis of seedling survival of two focal species confirmed these conclusions. The grazing effect was small and limited to plots without soil preparation. The vegetation of these non-harrowed plots was denser and thus more attractive for grazers explaining the higher grazing effect. Grazing livestock prefers dense vegetation with higher food resource over sparse vegetation resulting in a higher biomass removal (Török et al. 2014; Meuret and Provenza 2015). *Bromopsis erecta* showed a generally high survival rate after three months suggesting that the species, characteristic of semi-dry soils and semi-natural sites, is very well adapted to the pedo-climatic conditions of our study zone and resistant to grazing (Calaciura and Spinelli 2008). *Rhinanthus alectorolophus* showed, however, a much lower survival since the abundance of potential host plants was low during the first year of restoration. The species is a hemi-parasite and its performance depends on the availability and performance of host plants (Matthies and Egli 1999; Bullock et al. 2003). It cannot

establish under low vegetation cover and its population may go locally extinct as it is an annual species whose year-to-year survival relies on the production of seeds and seedling recruitment (Coulson et al. 2001; Bullock et al. 2003). Whereas the grazing effect on the survival of *R. alectorolophus* was not significant, the effect on flower production was negative. The result suggests that the species may survive grazing, but grazing damage prevent species from flowering.

The differential effects of soil preparation and grazing on spontaneous and transferred species did not result in a change in first-year plant species composition. The abundance of spontaneously emerging species was between six and ten times higher than that of transferred species, explaining that the observed significant soil treatment effect on transferred species was not yet visible at community level. The soil of disturbed sites generally contains high numbers of seeds of annuals and/or ruderal species (Bischoff 2002; Donath et al. 2007). These species often dominate the vegetation of early successional stages before being replaced by typical grassland species that are usually perennials developing later in the succession (Kiehl et al. 2010; Valkó et al. 2016; Kiss et al. 2020). The first year after the restoration of Eastern European grasslands, Lengyel et al. (2012) observed that annual weeds were dominant, but the cover of those weeds decreased dramatically after the third year due to the growth of perennial grasses. The authors observed an increase in the cover of target species from the first to the fourth year of restoration. A similar low initial restoration treatment effect due to initial low establishment of transferred target species and a subsequent high long-term restoration success (eight years) is known for riparian mesophilous grasslands (Auestad et al. 2016; Bischoff et al. 2018). Monitoring the plant community over several years will be necessary in order to evaluate whether the observed initial differences in seedling recruitment and early survival significantly influence plant species composition and restoration success in the long run.

2.5 Conclusion

Most species of the donor site were found at the restoration sites indicating that the applied brush harvesting technique was appropriate and allowed testing soil preparation and grazing effects. Similarly to studies in mesophilous grasslands (Edwards et al. 2007, Bischoff et al. 2018), soil preparation had a clearly positive effect on the seedling recruitment. The reduction in overall seedling density dominated by spontaneously emerging species demonstrated that the reduction in competition was the principal mechanism explaining the positive effect of previous soil tillage by harrowing on the recruitment of transferred species (Edwards et al. 2007; Schmiede et al. 2012). We thus recommend soil preparation before seed-based ecological restoration even in semi-dry grasslands to improve seedling recruitment of transferred target species. Our study did not confirm a strong negative effect of grazing on seedling recruitment found by several other authors (Scotton et al. 2012;

Vidaller et al. 2019b). The marginally significant negative effect of extensive grazing may be tolerated if farmers are reluctant in putting up fences as in our study area. Further research is needed on the timing of sowing relative to grazing periods, in order to benefit from positive grazing effects by reduction of competition while avoiding seedling damage.

2.6 Supporting information

Table S2.1. Number of seedlings of transferred species from brush material measured in the greenhouse and in the field. Nomenclature follows Tison et al. (2014).

Transferred species	Greenhouse	Field
<i>Achillea millefolium</i>	6.7	0
<i>Alyssum alyssoides</i>	0	0
<i>Arabis hirsuta</i>	0.4	0
<i>Bromopsis erecta</i>	6.7	1.6
<i>Bunium bulbocastanum</i>	0	0.2
<i>Dactylis glomerata</i>	5.9	0.2
<i>Festuca marginata</i>	35.6	0.1
<i>Festuca valesiaca</i>	2.6	0
<i>Festuca sp.</i>	4.4	0
<i>Galium sp.</i>	17.8	0.4
<i>Heracleum sphondylium</i>	0	0.2
<i>Leontodon hispidus</i>	0	0
<i>Onobrychis viciifolia</i>	1.1	0.1
<i>Plantago lanceolata</i>	0.4	0
<i>Plantago media</i>	95.6	0
<i>Poa pratensis</i>	3.7	0
<i>Poa trivialis</i>	1.1	0
<i>Potentilla sp.</i>	2.6	0
<i>Ranunculus bulbosus</i>	0.7	0
<i>Rhinanthus alectorolophus</i>	0	1.3
<i>Rumex acetosa</i>	0.4	0
<i>Schedonorus pratensis</i>	6.7	0.2
<i>Silene nutans</i>	0	0.1
<i>Silene vulgaris</i>	1.1	0
<i>Taraxacum sp.</i>	2.2	0
<i>Trisetum flavescens</i>	1.5	0
<i>Veronica sp.</i>	0.4	0

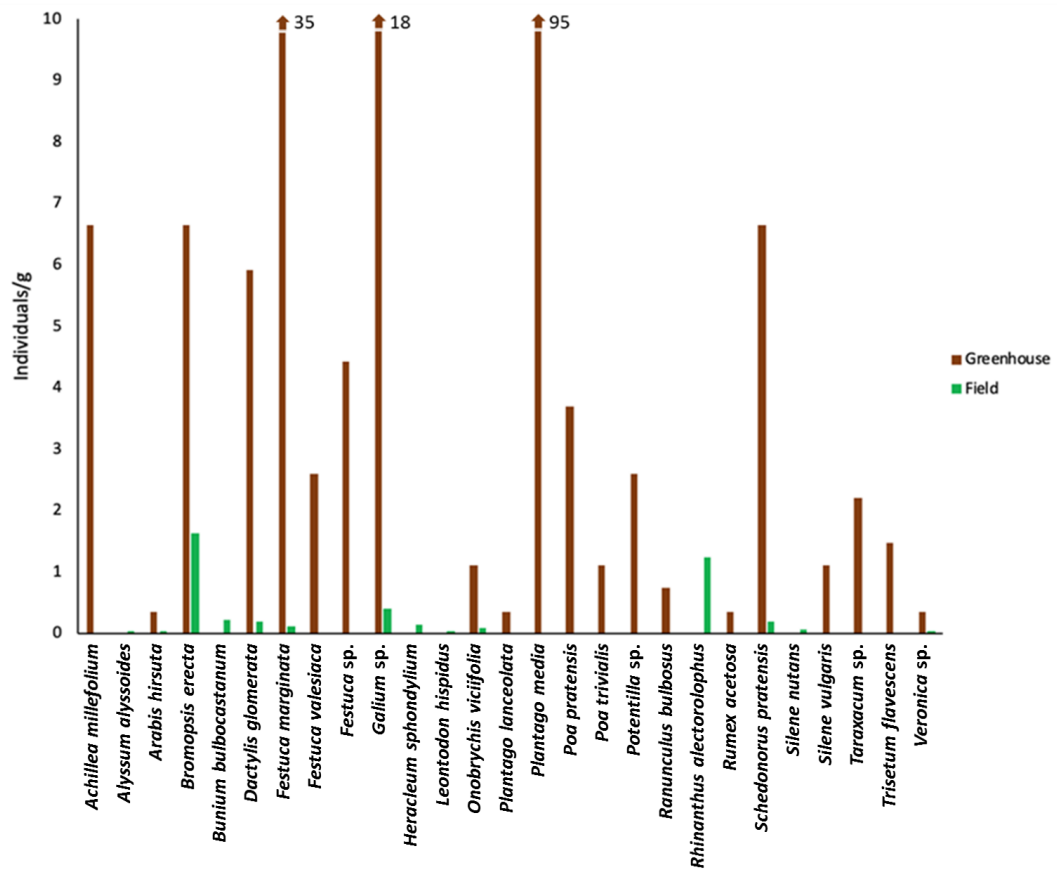


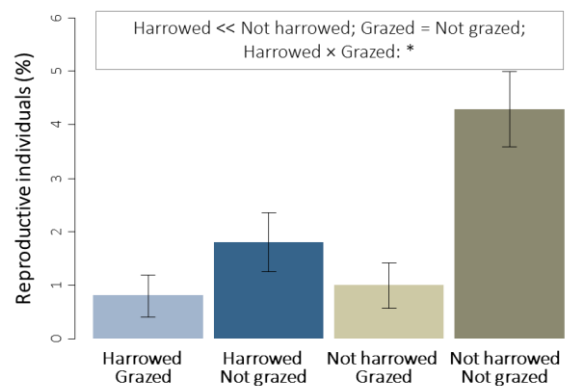
Fig. S2.1. Seedling emergence of transferred species from brush material measured in the greenhouse and in the field. For the field data, species occurring in control plots of a given site were excluded from measurements of this respective site. Number of seedlings are detailed in the table above.

Reference: Tison JM, Jauzein P, Michaud H (2014) Flore de la France méditerranéenne continentale. Naturalia Publications.



Fig. S2.2. Seedling of (a) *Rhinanthus alectorolophus* and (b) *Bromopsis erecta* after tagging with a coloured ring and a stick. The photos were taken on May 3rd, 2019.

Fig. S2.3. Percentage of reproductive individuals of *Rhinanthus alectorolophus* in the first year. The direction of main effects is indicated using “<<” ($P < 0.01$), “<” ($P < 0.05$), “(<)” ($P < 0.1$) and “=” (not significantly different). Significant grazing-by-soil preparation interactions are presented using “*” ($P < 0.05$).



Transition to Chapter 3

(2.a) Does soil preparation by harrowing before sowing improve recruitment and survival of the transferred species?

In **chapter 2**, the soil preparation (tillage) applied before addition of brush-harvested propagules largely increased the recruitment of transferred species harvested in one of the reference communities, whereas the density of spontaneously emerging species was reduced. Therefore, this technique can clearly be recommended in my study system to enhance the recruitment of target species.

(2.b) What is the effect of early extensive grazing on seedling recruitment and survival of the transferred species?

The effect of livestock grazing on transferred species recruitment was weakly negative, suggesting that fencing costs are not always justified. However, as my study sites were first grazed before seedling emergence of transferred species, and as the following grazing period occurred seven months later, the first grazing probably had a positive effect, but the second grazing period may have negatively affected young seedlings. Thus, the timing of grazing probably needs to be considered to limit seedling damage, particularly if grazing exclusion is not possible.

Chapter 3 presents a test of different seed transfer techniques and nurse plant sowing in order to overcome low seed dispersal and to understand the mechanisms favouring target species recruitment and establishment. Hay transfer, brush-harvested propagule transfer, and brush-harvested propagule transfer combined with wheat sowing were applied. Each restored plant community was compared to the best references identified in the **chapter 1**.



Hay harvest



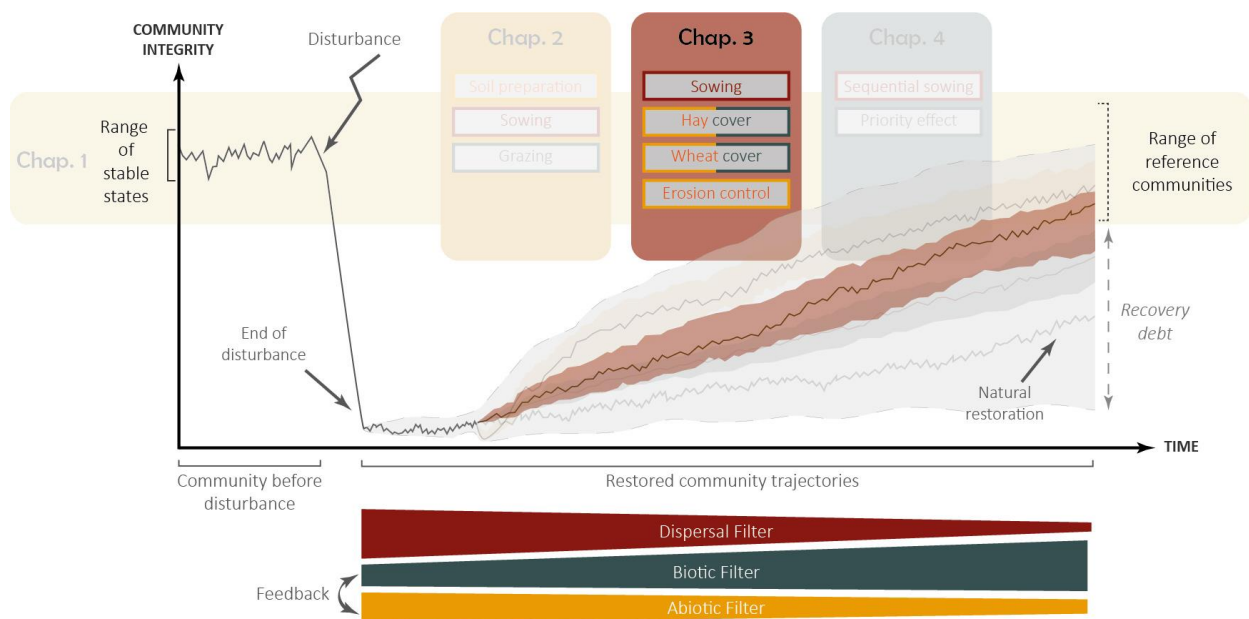
Brush-harvested material



Wheat (June 2019, 8 months after sowing)

— Chapter Three —

Mountain grassland restoration using hay and brush material transfer combined with temporary wheat cover



This chapter was submitted to Ecological Engineering on February, 26th 2021.

Mountain grassland restoration using hay and brush material transfer combined with temporary wheat cover

Abstract

Mountain grassland restoration success may be hampered by limited seed dispersal and poor soil seed banks of many grassland species. These constraints can be overcome by actively introducing propagules from nearby non-degraded communities.

We tested different restoration techniques in order to understand the mechanisms favouring target species seedling recruitment and establishment. In five degraded mountain grasslands, we analysed (i) the effect of two techniques increasingly used in ecological restoration to overcome low seed dispersal: transfer of brush-harvested seed material and hay transfer, and (ii) the potentially facilitative effect of a temporary plant cover (common wheat) on the recruitment of transferred brush-harvested propagules.

We found that both propagule transfer techniques were successful in establishing plant species of the donor community with an increase of plant species richness, cover and abundance of transferred species. Hay transfer was more efficient in transferring species of the donor grassland than brush-harvested material transfer. Brush-harvested material transfer only increased abundance and cover of donor grassland species when sown together with wheat.

The results indicated that hay mulch favoured seedling recruitment of target species, and that propagule transfer without hay mulch needs to be compensated by additional temporary plant cover in order to create favourable conditions for seedling recruitment. A comparison with best reference communities for each restoration grassland confirmed that hay transfer and brush material transfer with wheat sowing were successful in driving plant community composition towards the desired reference state.

In conclusion, restoration of mountain grasslands with shallow and stony soils clearly benefits from a facilitative effect of dead (hay) or living (wheat) vegetation cover.

Keywords: Semi-natural montane grasslands, Seedling recruitment, Nurse plant cover, Erosion control, Plant establishment, Facilitation

3.1 Introduction

Grassland ecosystems represent 25% of terrestrial biomes (Blair et al. 2014; Wilsey 2020) and are essential habitats for the conservation of biodiversity and associated ecosystem services (Stoate et al. 2009; Amiaud & Carrère 2012; Valkó et al. 2016). Since the 1980ies, the conservation of species-rich semi-natural grasslands has become a major concern in Europe. Most European semi-natural grasslands were integrated in the habitat management Natura 2000 network in order to stop habitat degradation and to re-establish grassland species diversity using ecological restoration approaches (Wilsey 2020).

Successful grassland restoration may be primarily hampered by limited dispersal capacity and poor soil seed banks of many grassland species, and by a grazing management favouring clonal reproduction over sexual reproduction (Halassy et al. 2016; Török et al. 2018). These constraints are well documented and can be overcome by actively introducing propagules (Kiehl et al. 2010; Scotton et al. 2012; Hölzel et al. 2012). The use of native plant species of local origin is recommended to assure adaptation to local environmental conditions and to maintain genetic diversity (Vander Mijnsbrugge et al. 2010; Bucharova et al. 2019). Consequently, transferring local seeds from nearby non-degraded communities is increasingly used in ecological restoration (Scotton 2019). Diverse techniques are currently applied depending on specific environmental conditions at restoration and donor sites (Kiehl et al. 2010; Hedberg & Kotowski 2010). The increasing number of studies illustrates the scientific interest in comparing restoration effectiveness of various seed harvesting and transfer techniques (Scotton & Ševčíková 2017), such as hay transfer vs. sowing of seed mixtures (Auestad et al. 2015; Kövendi-Jakó et al. 2019), brush material vs. green hay transfer (Albert et al. 2019), or sowing combined with various additional treatments (Pawluśkiewicz et al. 2019; Havrilla et al. 2020).

In our study, we compared the restoration effectiveness of two mechanical harvesting techniques increasingly used in mountain grassland restoration: the transfer of brush-harvested seed material and the transfer of dry hay both collected from the same donor site located close to the degraded grasslands. The brush harvester strips and aspirates the plant material without cutting (Scotton et al. 2012). The harvested brush material comprises seeds but also vegetative parts of fruits and, to a lesser degree, other vegetative material. Low-growing and less abundant species may be under-represented in this material (Edwards et al. 2007; Scotton et al. 2009; Wagner et al. 2020), but brush harvesting allows concentrating seeds thus reducing humidity and facilitating storage. It also reduces the mulch layer that may hamper germination (Mollard et al. 2014). Dry hay transfer involves cutting of plant material close to the soil surface, drying it on the field before storage and spreading it on the restoration site. The higher vegetative biomass compared with brush material may hamper germination but protects seedlings against high solar radiation, drought and soil erosion (Eckstein & Donath 2005; Graiss & Krautzer 2011; Havrilla et al.

2020).

As seedling recruitment and establishment are important constraints in semi-natural grassland restoration (Öster et al. 2009), we also tested whether simultaneous transfer of brush material and sowing of an annual plant species may compensate for the absence of a protecting mulch layer. Such annuals provide temporary living cover that may facilitate seedling recruitment and establishment (Padilla et al. 2006; Wright et al. 2014). Similarly to hay mulch, a living cover may, however, increase root and light competition (Donath et al. 2006). We tested a mix of two lowland varieties of common wheat *Triticum aestivum* as a facilitative species. These varieties were expected to disappear within a couple of years since climatic conditions of our mountain study sites limit their seed production and self-sowing. *Triticum* cover may reduce soil erosion, an important criterion for the functionality of restoration measures. Such soil stabilisation and erosion control are particularly important in mountain grasslands on slopes facing a high risk of soil loss and propagule run-off (Huc et al. 2018; Scotton 2019).

In order to understand the underlying mechanisms of seedling recruitment and establishment, we analysed first-year seedling abundance and second-year cover, respectively. To determine which technique is the most appropriate in mountain grassland restoration, we addressed the following questions: (1) Which method of plant material transfer is most successful in establishing species transferred from the donor community? (2) Does sowing of wheat improve the seedling establishment of brush material? (3) Do transfer techniques and wheat sowing increase the similarity between restored and reference communities? (4) What is the influence of these treatments on soil erosion?

3.2 Material and methods

3.2.1 Study area

The study area was the upper Durance valley in the Southern French Alps. The five selected restoration sites were degraded by the construction of a high-voltage transmission line involving the creation of access tracks and working platforms. The construction work locally destroyed the vegetation and affected the soil structure. The study zone extends over about 100 km, from L'Argentière-la-Bessée in the north to La-Bâtie-Neuve in the south (44°78'78"N, 6°59'41"E; 44°57'93"N, 6°20'77"E). The study sites are located on slopes above the Durance valley, at an elevation of 1060 m to 1320 m above sea level. They are characterised by a subcontinental climate, with an average annual temperature of 8.7°C and an annual rainfall of 740 mm (Embrun meteorological station, annual temperature corrected for an altitudinal difference of roughly 2°C). Dry to mesophilic grasslands occur at sites managed by grazing or mowing. Most of these surrounding non-degraded grasslands

are priority habitats of Natura 2000: 'Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on limestone'(N6210, European habitat directive 92/43/EEC; Calaciura & Spinelli 2008). The five restoration sites used for the experiments were former access tracks and working platforms, and restoration goal was the re-establishment of typical Festuco-Brometalia grasslands. Traditional management by grazing was re-introduced.

3.2.2 Donor and reference sites

One typical non-degraded Mesobromion grassland of the study area was chosen as donor site for harvesting plant material. The site was located in Freissinières (44°73'61"N; 6°56'72"E) at an elevation of 1100 m and a 3° south-west-facing slope. Two harvest techniques were applied, brush harvesting and traditional haymaking. The brush material was harvested on 13 July 2018 using a brush harvester mounted on a quad and equipped with an integrated vacuum system. On a surface of 4000 m², 2.6 kg of brush material was obtained. The harvested material comprised seeds (60% of total mass) and vegetative parts (40%). Seeds were counted in 10 subsamples of 0.5 g harvested materials revealing an average density of 887 seeds/g. Hay was cut on 15 July 2018 on an area of 800 m² using a rotary mower. The hay was dried for two days on the harvested grassland and was baled in five bales of 7.5 kg (45 × 30 × 75 cm) for a total of 37.5 kg, with approximately 1% of seeds in the total raw material. Brush material and hay were kept for three months at room temperature under dry and dark conditions.

Although the donor site reflected the medium environmental conditions of the five restoration sites we identified "best references" for each site to evaluate restoration success. Since best reference sites are not always the closest ones (Durbecq et al. 2020, **chapter 1**), we surveyed eighteen non-degraded grasslands along the new electricity line. These potential references included the donor site and were located at the same altitudinal belt as restoration sites (1000 – 1400 m). Soil and microclimatic conditions measured at restoration sites and potential reference communities were used to identify the best references of each restoration site according to Durbecq et al. (2020, methodology).

3.2.3 Experimental design

At the five restoration sites, we repeated the following five treatments in 4 m × 4 m plots: control without active seed addition (control), hay transfer ('Hay'), brush material transfer ('Seed'), wheat sowing ('Triticum') and both brush material and wheat seed addition together ('Triticum + Seed'). The restoration sites that were already colonized by spontaneous vegetation were mown before the set-up of the experiments and they were then extensively grazed in spring and autumn. The position of the five treatments was

randomised within sites.

In October 2018, 6.5 g/m² of harvested brush material was spread in the brush material treatments, corresponding to 3.75 g/m² of seed and 5765 seeds/m². The chosen seed density is a little higher than recommended for lowland grasslands and lower than usually sown in alpine grasslands (Kiehl et al. 2010; Scotton et al. 2012; Koch et al. 2015). The brush material was pressed to the ground using a lawn roller. In the hay transfer treatment, a bale of hay of 7.5 kg was spread (0.5 kg/m²) on each plot. The amount of hay was adjusted to an estimated seed content of 1% (Scotton et al. 2012) corresponding to roughly 5 g/m² of seeds. The hay was pressed to the ground by the wheels of tractor in order to maintain hay on the ground. The sown wheat treatment was a mixture of two local lowland winter wheat (*Triticum aestivum*) varieties, “Meunier d'Apt” and “Saissette de Provence”. These early lowland varieties are not well adapted to high altitude resulting in low seed production and a rapid decline during mountain grassland succession. The seed density was adjusted to 9 g/m². Like for the brush material transfer, a lawn roller was used to improve seed adhesion to the ground. Controls were not sown but they were also rolled.

3.2.4 Data collection

3.2.4.1 Vegetation relevés in donor and reference grasslands

In the donor grassland, vegetation relevés were conducted on 29 June 2018, and the occurrence of mature seeds was evaluated for each plant species at the harvest date. Relevés were used to establish a list of 37 potentially transferred species (hereafter: transferred species). The vegetation relevés of the eighteen potential reference sites were conducted between 21 June and 5 July 2018 by visual estimation of plant cover for each species in quadrats of 2 m × 2 m. In the donor grassland, five quadrats were sampled, and three in each of the other potential reference grasslands.

3.2.4.2 Germination test in a greenhouse

In November 2018, brush material and hay were spread in an unheated greenhouse to evaluate and compare the germination potential of seeds in brush material and hay. Harvested brush material and dry hay were sown to ten trays (0.03 m²) filled with standard potting soil substrate (mixture of 1/3 vermiculite and 2/3 of sterile peat). Five trays received 0.54 g of brush material corresponding to 18g/m² of seeds, and five trays received 12.5 g of hay corresponding to 4 g/m² of seed material according to an estimated seed content of 1%. The trays were regularly watered, and germinations identified and removed until April 2019.

3.2.4.3 Seedling abundance and vegetation relevés in grasslands under restoration

To measure seedling abundance, plant number was counted in three quadrats of 40 × 40 cm placed along the diagonal of each plot in June 2019, at the five restoration sites. Since it was not possible to distinguish seedlings and plants re-sprouting from belowground organs, the seedling counts may include stems or ramets of vegetative regeneration. We considered all reproducing plant species of the donor site as transferred although some of them also occurred in the control plots of sites under restoration, indicating their presence in the soil seed bank or seed rain. In June 2020, the cover of plant species was measured following the same protocol as in the potential reference grasslands: in each plot, one quadrat of 2 m × 2 m was sampled.

3.2.4.4 Erosion monitoring

To evaluate the effect of the different treatments on soil erosion, we measured erosion according to Feret & Sarrailh (2005) at four restoration sites (at one site, the permanent points were accidentally removed in autumn 2019 not allowing any analysis). The device comprised a 1.5 m long aluminium bar and two 0.5 m long iron poles fixed in the ground of each plot. These iron poles were the permanent points of the measurements. They were adjusted to provide a horizontal support for the aluminium bar before measurements. Vertical holes were drilled in the centre of the aluminium bar every 10 cm resulting in a total of 13 regularly spaced holes (**Fig. S3.1**). To take the erosion measurements, a graduated rod was introduced in each of the holes. The scale of the graduated rods allowed a direct measurement of the space between the aluminium bar and the ground. An increase in space between measurements indicates erosion whereas a decrease suggests an accumulation of soil. Measurements were taken in June 2019, October 2019, June 2020 and September 2020, in order to assess the effect of restoration treatments on soil erosion.

3.2.5 Data analysis

We used plant abundance, cover and richness of the entire plant community and of transferred species to run statistical tests and to calculate restoration indices. Major soil and microclimatic variables of restoration sites and potential reference sites were fitted to multivariate models (NMDS) to identify the best references of each restoration site. The identification was based on dissimilarity coefficients using Euclidean distances and is detailed in Durbecq et al. (2020, **chapter 1**). We compared restoration sites and these best reference sites using the restoration indices CSII_{norm} (normalized Community Structure Integrity Index) and HAI (Higher Abundance Index). The CSII allows measuring which proportion of the species abundance of the reference communities is represented in the

restored communities, and the HAI evaluates the proportions of the species abundance in the restored communities that are higher than in the reference communities (Jaunatre et al. 2013).

We ran linear and generalized mixed models with transfer treatments as fixed effect and site as random effect to analyse response variables (including restoration indices) to the restoration treatments. In the analyses of plant abundance, quadrat within treatment was additionally included as a random effect. Plant cover, species richness and restoration indices were normally distributed and thus analysed using a Gaussian distribution with identity link. Plant abundance was fitted using a Poisson distribution with log-link function. In the case of a significant treatment effect, pairwise comparisons were used to compare differences between transfer techniques (glht function in multcomp package; Hothorn et al. 2008).

A NMDS (Non-Metric Multidimensional Scaling) based on Bray–Curtis similarity was applied to compare plant species composition of the five restoration sites and their best reference sites. A Permutational Multivariate Analysis of Variance (PERMANOVA) was used to analyse whether the community composition was significantly different between treatments (R package “vegan”). All analyses were performed using R 3.5.3 (R Core Team 2019).

3.3 Results

The greenhouse tests revealed a mean density of 197.4 viable seeds/g in the brush harvest material and 2.4 viable seeds/g in the hay. This corresponds to a density of 1283 viable seeds per m² and 1200 viable seeds per m² transferred with brush material and hay to the restoration sites, respectively.

3.3.1 Effects of transfer techniques and wheat sowing on target species establishment

Seedling emergence of transferred species was only 50/m² (without wheat) to 65/m² (with wheat) in the brush material transfer and 200/m² in the hay transfer corresponding to 4%, 5%, and 17% of the number of transferred viable seeds, respectively. Transferred species abundance was significantly different between the restoration treatments ($\chi^2 = 51.85$, $df = 4$, $P < 0.001$; **Fig. 3.1**). We found the clearly highest density of transferred species in the ‘Hay’treatment although seed density was similar to brush material according to germination tests. The ‘Seed’treatment involving brush material transfer without wheat addition was neither significantly different to the control nor to the ‘Triticum’treatment.

Transferred seedling abundance in the combined ‘Triticum + Seed’ treatment was significantly higher than in the control but lower than in the ‘Hay’ treatment.

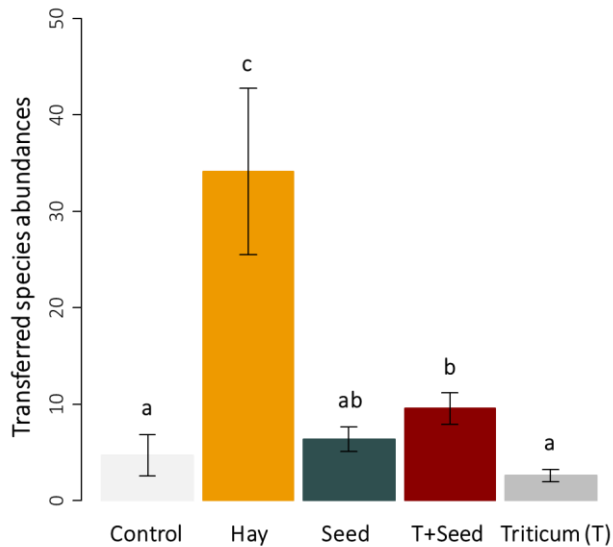


Fig. 3.1. Effect of seed transfer techniques on the abundance of transferred species estimated in quadrats of 40 × 40 cm in 2019. Error bars represent ±SE and different letters indicate significant differences between treatments ($P < 0.05$).

The treatments also had a significant effect on the second-year cover of transferred species ($\chi^2 = 16.28$, $df = 4$, $P = 0.002$; **Fig 3.2.a**). However, the strongly positive effect of the ‘Hay’ treatment vanished and the difference to the control was not significant any more. Transferred species cover was significantly higher in the combined ‘Triticum + Seed’ treatment than in the ‘Seed’ and ‘Triticum’ treatments whereas all other treatments were not significantly different.

Similarly to transferred species cover (**Fig. 3.2.a**), richness of transferred species varied significantly between treatments ($\chi^2 = 19.65$, $df = 4$, $P < 0.001$; **Fig. 2.b**). The ‘Seed’ treatment was again only significantly different from the control if combined with wheat sowing (‘Triticum + Seed’). Contrary to transferred species cover but in line with first-year abundance, species richness was significantly higher in the ‘Hay’ treatment than in the control. The ‘Hay’ and the combined ‘Triticum + Seed’ treatments comprised on average seven more transferred species (± 3 species) than the control. The ‘Seed’ treatment without wheat sowing was not significantly different from the control or the ‘Triticum’ treatment. Similar treatment effects were visible for the total species richness (**Fig. S3.2**). However, only the difference between the combined ‘Triticum + Seed’ treatment and the control was significant.

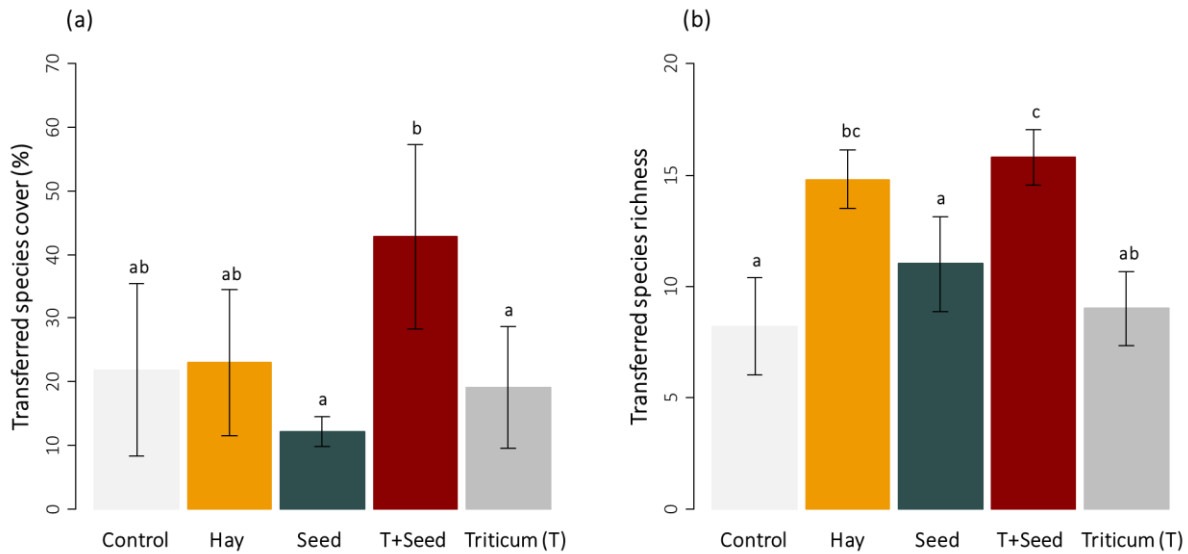


Fig. 3.2. Effect of seed transfer techniques on **(a)** transferred species cover, and **(b)** transferred species richness, both based on 2020 cover values and estimated in quadrats of 2×2 m. Error bars represent \pm SE and different letters indicate significant differences between treatments ($P < 0.05$).

3.3.2 Comparison with best references of each restoration site

The plant species composition of the restoration sites was very different from that of reference sites in all treatments (**Fig. 3.3**). Restoration and reference sites were particularly separated on the first axis. This axis represented a disturbance gradient with annual and ruderal species occurring on the left together with restoration sites (*Arctium minus* (Hill) Bernh. *Polygonum aviculare* L. *Anisantha sterilis* (L.) Nevski, etc.) and perennial grassland species on the right together with reference sites (*Stipa pennata* L. *Helianthemum nummularium* (L.) Mill. *Pilosella officinarum* F.W.Schultz & Sch.Bip. etc.) . The differences between reference sites reflect the heterogeneity of plant communities according to different environmental conditions that are independent of degradation. They were particularly separated on the second NMDS axis representing a productivity gradient from stony, xeric sites in the lower part of the biplot to more humid, mesophilic sites in the upper part. There was a large overlap between the five treatments within restoration sites, but the treatment effect was still significant ($F = 1.432$, $df = 4$, $P = 0.012$). As expected, the unsown control showed the greatest distance to the references. The ‘Hay’ and ‘Triticum + Seed’ treatments were closest to the reference sites, followed by the ‘Seed’ treatment without wheat. However, the latter treatment was not much closer to the reference communities

than wheat sowing alone. The ‘Triticum’ treatment showed the highest variation between sites resulting in a large NMDS polygon.

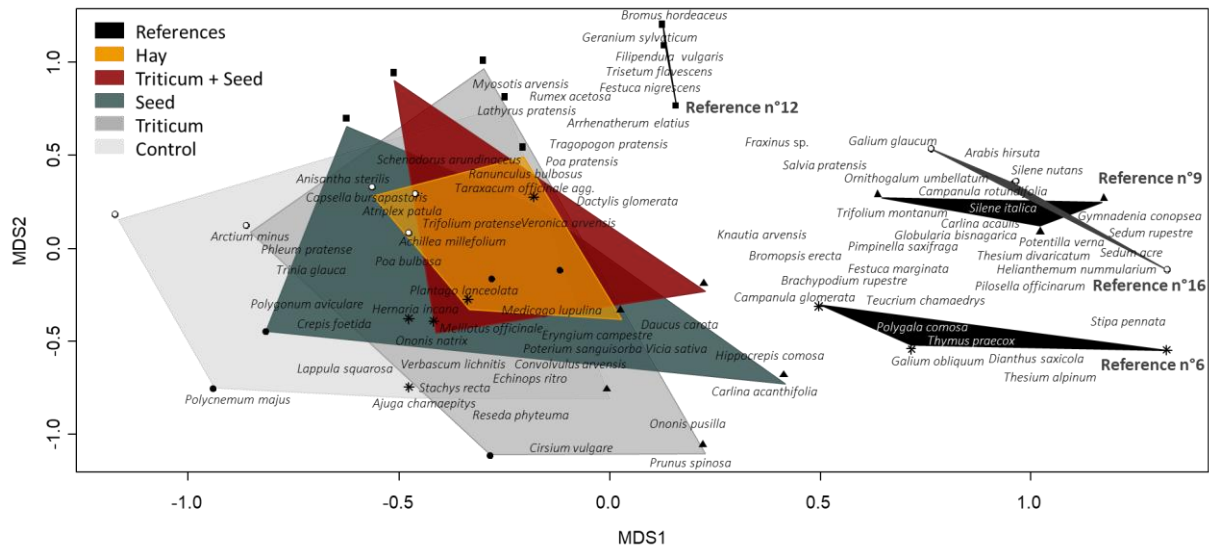


Fig. 3.3. NMDS of plant species composition in the five transfer technique treatments compared to plant species composition in the best reference for each restoration site previously identified in Durbecq et al. (2020, **chapter 1**). Polygons indicate the position of the outmost plots in each treatment (stress = 0.20). Different symbols indicate the five different restoration plots sites and corresponding references: ● and ○ for sites corresponding to reference n°16 (same reference for the two restoration sites), ■ to reference n°12, * to reference n°6, and ▲ to reference n°9.

The CSII_{norm} calculated for each restoration site compared with their respective best reference showed a significant difference between treatments ($\chi^2 = 18.02$, $df = 4$, $P < 0.01$; **Fig. 3.4a** and **3.5**). CSII_{norm} was significantly higher in the combined ‘Triticum + Seed’ treatment than in the control, the ‘Triticum’ and the ‘Seed’ treatments. The ‘Hay’ treatment was not significantly different from other treatments.

The HAI was also significantly different between treatments ($\chi^2 = 11.44$, $df = 4$, $P < 0.05$, **Fig. 3.4b** and **3.5**). The lowest HAI was found in the ‘Seed’ treatment being significantly lower than the control and the ‘Triticum’ treatment, indicating significantly lower non-target abundances in the ‘Seed’ treatment. Differences between other treatments were not significant.

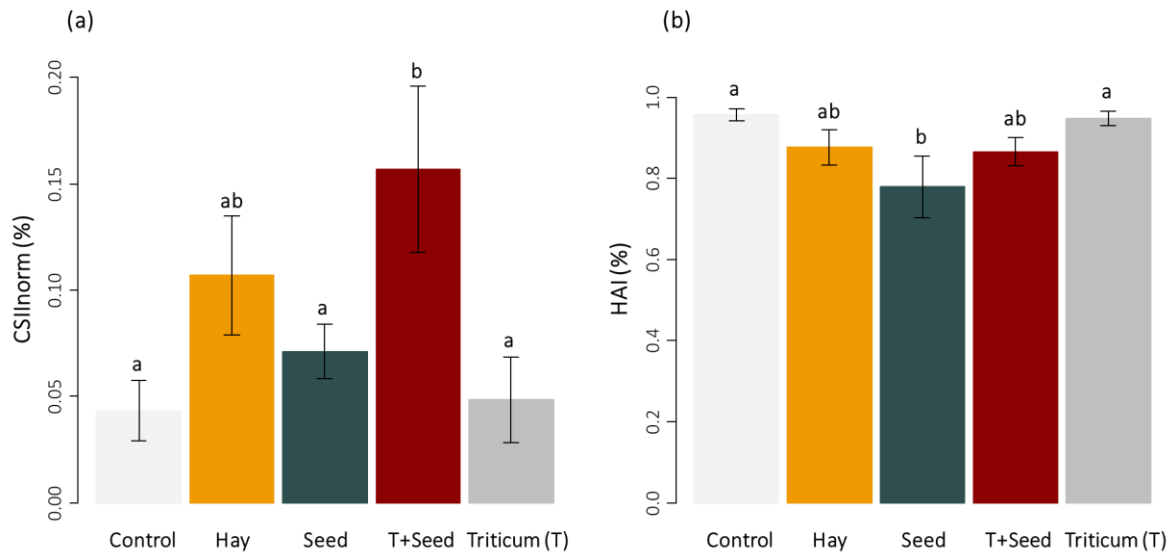


Fig. 3.4. (a) CSInorm and **(b)** HAI, comparing the plant communities of transfer technique treatments of each restoration site with their respective best reference site. Error bars represent \pm SE and different letters indicate significant differences between treatments ($P < 0.05$).

The significant differences in CSInorm were explained by a higher number of target species in the ‘Hay’ and in the combined ‘Triticum + Seed’ treatments compared to the other treatments. Target species were those occurring in the best references such as *Plantago media* L. *Festuca cinerea* Vill. *Teucrium chamaedrys* L. *Poa pratensis* L. *Hippocrepis comosa* L. *Lotus corniculatus* L. *Silene vulgaris* (Moench) Garcke, *Eryngium campestre* L. *Poterium sanguisorba* L. and *Trifolium pratense* L. (**Fig. 3.5**). In the ‘Hay’ and the ‘Triticum + Seed’ treatments, the cover of several target species was even higher than in the references, (e.g. *Poterium sanguisorba* L. *Medicago lupulina* L. *Trifolium repens* L. *Trifolium pratense* L. *Convolvulus arvensis* L.).

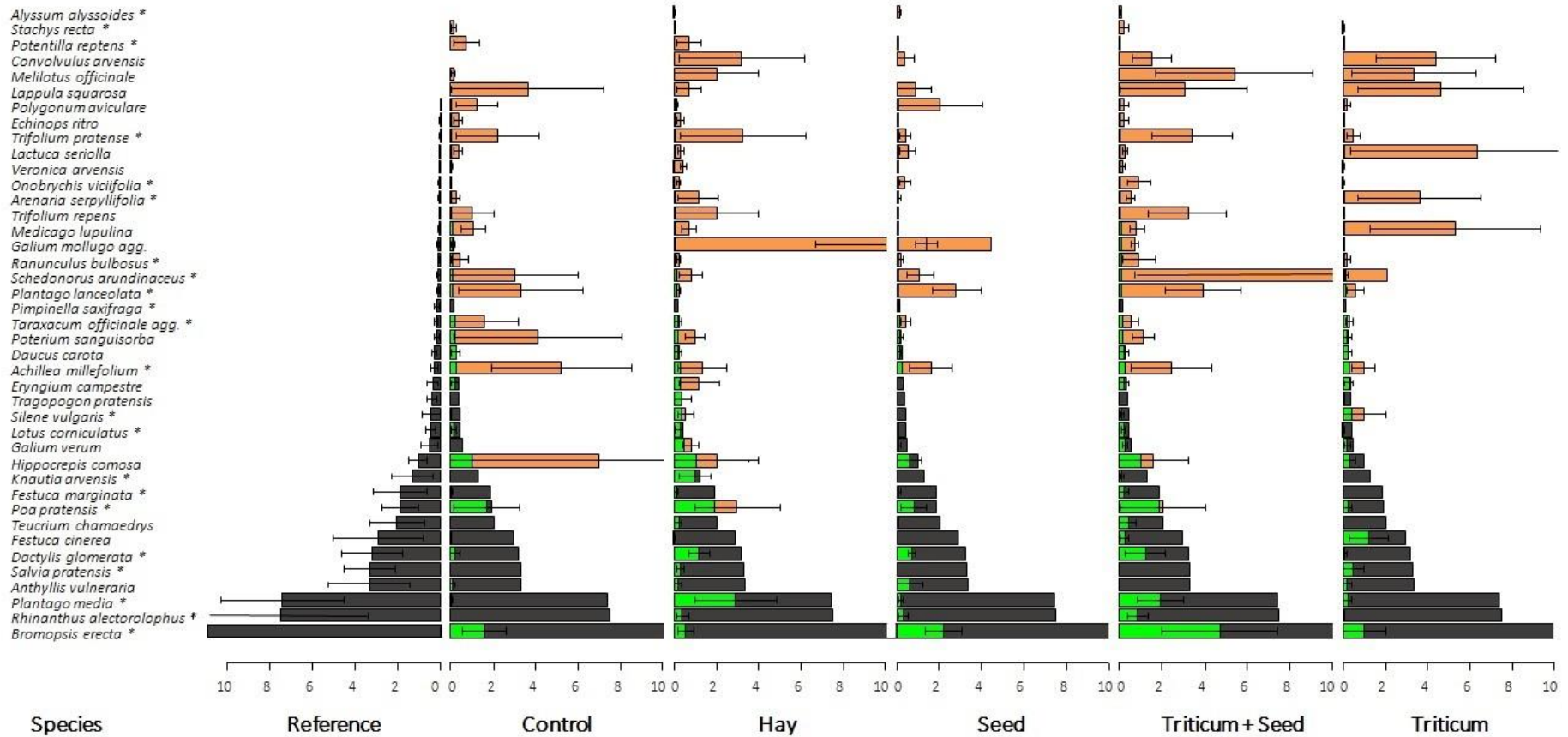


Fig. 3.5. Mean target plant cover of abundant species (> 9 samples, 41 of 259 species in total) in restoration treatments and the best reference community. Different colours represent the mean cover proportion in references (black), the mean cover proportion in communities under restoration up to the mean cover in the reference communities (green), and the mean cover proportion exceeding that of the reference communities (orange). Asterisks indicate transferred species. For readability, the x-axis is limited to 10% cover (only *Bromopsis erecta* (Huds.) Fourr. *Schedonorus arundinaceus* (Schreb.) Dumort. and *Galium mollugo* agg. exceeded).

3.3.3 Ecosystem function: erosion control

Erosion monitoring did not show significant treatment effects at the end of the observation period. Lowest erosion was observed in the 'Hay'treatment but due to high between-site variation, the difference to other treatments was not significant (**Fig. 3.6**). Intermediate measurements demonstrated a high temporal variability of erosion and of treatment effects on erosion (**Fig. S3.3**).

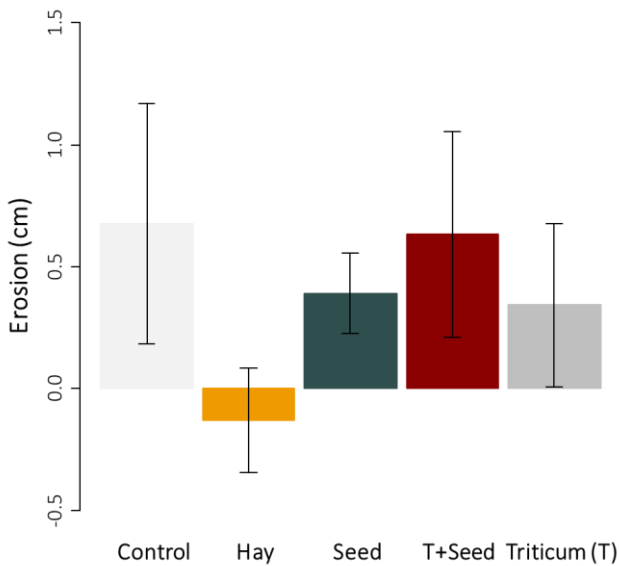


Fig. 3.6. Effect of seed transfer techniques on soil erosion. Error bars represent \pm SE. Positive values show higher erosion, thus a lesser amount of material on the ground 14 months after the beginning of the experiment.

3.4 Discussion

Both techniques, brush-harvested propagule transfer and hay transfer, allowed the introduction of high numbers of viable seeds. However, first-year seedling recruitment was much higher in hay transfer than in brush material transfer. Additional wheat sowing had a particularly positive effect on seedling establishment from brush material resulting in a second-year cover of transferred species similar to that of hay. Brush material transfer alone did neither increase transferred species cover nor plant species richness. According to transferred species abundance and cover, hay transfer and brush material transfer combined with wheat sowing were also most successful in restoring the plant species composition of the reference. Soil erosion was not affected by any restoration treatments.

3.4.1 Effect of seed-addition techniques on transferred species recruitment

Brush harvesting has many technical advantages compared to hay cutting allowing selection and cleaning of seeds, drying and particularly easy storage (lower volume than hay) under optimum temperature and humidity (Edwards et al. 2007; Vitis et al. 2020; Frischie et al. 2020). Contrary to hay cutting, it is possible to brush-harvest several times a season thus maximizing the number of transferred species (Edwards et al. 2007; Scotton & Ševčíková 2017). The possibility to use grasslands for hay making after brush harvesting is another advantage reducing losses for livestock farmers. However, several studies obtained a low efficiency in plant establishment from brush material compared to hay transfer (Edwards et al. 2007; Sengl et al. 2017; Albert et al. 2019). Albert et al. (2019) explained the lower seed number per harvested area by the higher working height of brushes compared to mowing machinery missing the seeds of low-growing species.

In our study, the brush material contained as many viable seeds as the hay suggesting that the low recruitment compared to hay transfer was due to lower germination and seedling survival. The mulch layer resulting from hay transfer may improve seedling recruitment through an increase in soil moisture retention (Donath et al. 2007; Mollard et al. 2014; Havrilla et al. 2020). Soil moisture retention and temperature buffering are important factors influencing germination in our study region characterized by shallow soils, spring frosts and high solar radiation. Furthermore, hay mulch protects seeds from predation, and animal trampling (Scotton et al. 2012; Vanderburg et al. 2020) and limits competition of ruderal species in early stages of grassland restoration (Kiehl & Wagner 2006). Additionally, a hay layer may reduce surface runoff (Graiss & Krautzer 2011) and improve seed adherence to the soil (Chambers 2000; Havrilla et al. 2020). High recruitment in the hay transfer treatment suggests that such positive effects prevailed in our study over well-known negative effects of a mulch layer, such as light limitation (Eckstein & Donath 2005).

Effects of temporary wheat cover on seedling recruitment may be similar to hay mulch effects, such as protection against drought and solar radiation (Wright et al. 2014). Additionally, belowground effects may facilitate seedling recruitment and establishment since wheat roots stabilize the soil and foster the recolonization of the soil fauna (Faivre 2000). In modifying soil structure and composition (Pohl et al. 2009; Liu et al. 2018), wheat can even be considered as an ecosystem engineer (Jones et al. 1996). Many high-altitude restoration studies aimed at improving recruitment conditions and reducing soil erosion using “nurse species” or wheat straw addition to stabilize the soil (Graiss & Krautzer 2011; Kavian et al. 2018; Scotton 2019). However, propagule run-off, germination and early survival are less likely affected by wheat sowing than by hay mulch since wheat cover develops later. In our study, this was probably the reason for an absence of a wheat sowing effect on first-year abundance of species transferred with brush material whereas the effect

on second-year cover was strongly positive. Like hay transfer, facilitative effects of wheat sowing prevailed over negative effects of competition for light and water observed in other studies on additional sowing of nurse species (Donath et al. 2006; Wright et al. 2014).

Soil preparation before sowing has often been recommended to improve seedling recruitment in sowing of brush-harvested seeds or seed mixtures (Kiehl et al. 2010; Edwards et al. 2007; Klaus et al. 2017; Shaw et al. 2020). Soil preparation increases seed and water retention as well as seed adhesion (Chambers 2000) and may compensate for the absence of a hay mulch layer limiting seed run-off. In our study area, a previous experiment testing soil preparation effects on seedling recruitment showed a strong increase in first-year abundance of transferred species (Durbecq et al. 2021, **chapter 2**). Thus, the plots that received brush material in our study may particularly benefit from previous soil tillage and a combination with additional wheat sowing may be the optimum strategy for seedling recruitment and establishment.

3.4.2 Successful restoration of reference communities and erosion protection

Seed addition using brush material and hay of the donor site was successful in increasing the similarity to best references at plant community level. As in the analysis of cover and number of species transferred from the donor site, best restoration results were obtained for hay transfer and the combination of brush material with wheat sowing, showing highest similarity to references in plant species composition and highest target abundance (higher CSInorm = abundance of species occurring in the reference). This finding confirmed that a dead (hay) or living (wheat) “nurse cover” improves seedling establishment of target (reference communities) and transferred (donor community) species (Graiss & Krautzer 2011; Kavian et al. 2018; Scotton 2019). The brush material transfer without wheat sowing did not increase similarity to references nor target abundance. However, HAI (proportion of non-target abundances) of this treatment was lower compared to the control whereas the HAI of brush material with wheat and hay transfer were not significantly different from the control. These results indicated that hay mulch and wheat cover did not only provide better conditions for target species but also for non-target species recruitment.

In mountain and alpine grasslands with more or less steep slopes, soil stabilization processes are slow, which increases soil erosion (Wiesmair et al. 2017). Erosion control is thus essential to restore these ecosystems (Pohl et al. 2009; Scotton 2019; Löbmann et al. 2020). Without plant cover, erosion, depletion of organic matter and gully formation may compromise restoration approaches (Dupin et al. 2019). Several authors have shown a negative linear relationship between vegetation cover and soil runoff (Peratoner 2003; Liu et al. 2018; Gu et al. 2020). Hence, immediate revegetation by locally adapted propagules is widely recommended (Scotton et al. 2009; Wiesmair et al. 2017; Dupin et al. 2019). Using

well-adapted temporary nurse plant cover may have a stronger positive effect on soil erosion control than dead plant material, such as hay or straw, since developing roots allow a belowground stabilisation of the soil (Pohl et al. 2009). According to Liu et al. (2018) and Gu et al. (2020), an average total vegetation cover of 60% observed in our study may reduce soil erosion by up to 80% compared to bare soil. However, our restoration treatments did not sufficiently influence total plant cover compared to control and thus the treatment effect on soil erosion was not significant. Contrary to our results, Kavian et al. (2018) found a long-term reduction in soil runoff using wheat residues as “nurse cover”. Our restoration sites were not characterized by steep slopes reducing soil run-off and the probability to find differences between treatments. Additionally, spontaneous vegetation emerging from the soil seed bank dominated in the beginning limiting the treatment effect.

3.5 Conclusions

All applied transfer techniques were successful in establishing plant species of the donor community. However, the transfer of brush-harvested seed material was only efficient together with wheat sowing. Our results demonstrated that restoration of grasslands of the mountain altitudinal belt with shallow and stony soils clearly benefit from dead (hay) or living (wheat) vegetation cover providing a nurse effect. Facilitation effects prevail over competition and/or reduction in resources such as light (Eckstein & Donath 2005; Padilla et al. 2006; Wright et al. 2014). Hay transfer was the best method to increase germination and seedling recruitment but the difference to brush material transfer with wheat sowing vanished in the second year indicating higher seedling mortality in the hay mulch (Eckstein & Donath 2005). The lower seedling recruitment in brush material transfer may be compensated by harrowing the soil before brush material transfer to improve recruitment conditions (Kiehl et al. 2010; Klaus et al. 2017; Durbecq et al. 2021, **chapter 2**). Propagule transfer did not improve the soil erosion control. Short-lived species spontaneously emerging from the soil seed bank dominated in the beginning reducing the magnitude of treatment effects. Long-term monitoring is required to evaluate the replacement of short-lived ruderals by the transferred perennial species often observed in restoration studies (Albert et al. 2019; Kiss et al. 2020; Shaw et al. 2020).

3.6 Supporting information



Fig. S3.1. Picture of erosion measurement device

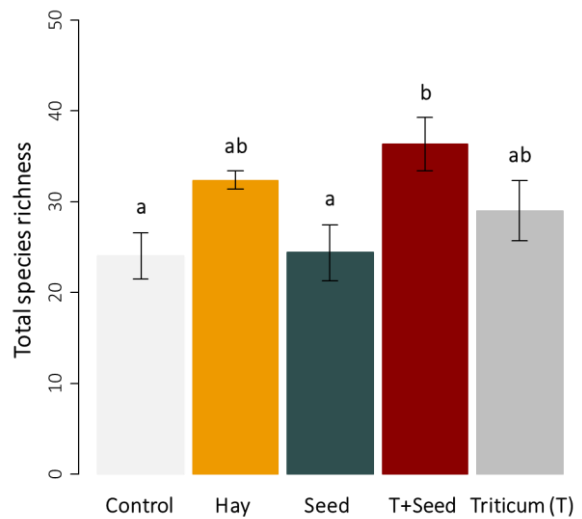


Fig. S3.2. Total species richness estimated in quadrats of 2×2 m in June 2020. Error bars represent \pm SE and different letters indicate significant differences between treatments ($P < 0.05$).

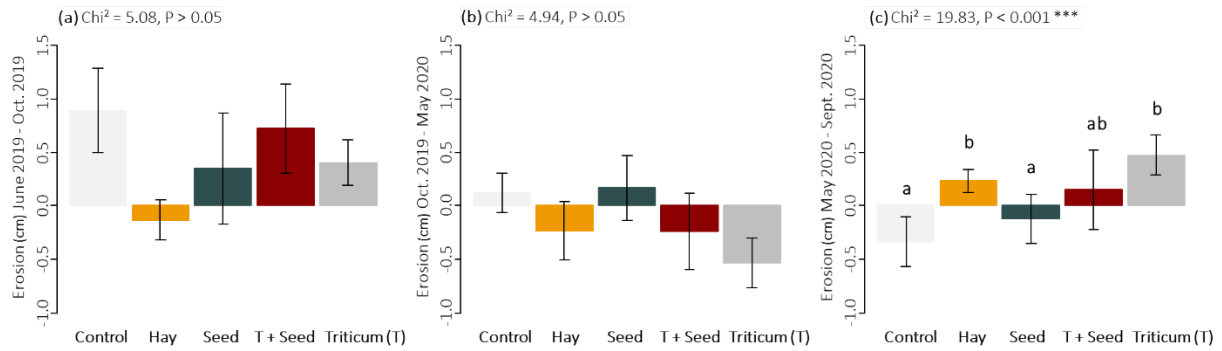


Fig. S3.3. Temporal differences in the effect of seed transfer techniques on soil erosion between (a) June 2019 and October 2019, (b) October 2019 and May 2020, and (c) May 2020 and September 2020. Error bars represent \pm SE and letters indicate significant differences between treatments ($P < 0.05$). Positive values show a lesser amount of material on the ground, thus higher erosion.

Transition to Chapter 4

(3.a) Which restoration technique is most successful in establishing species transferred from the donor community?

In **chapter 3**, hay transfer was the most efficient technique in transferring species of the donor grassland. The transfer of brush-harvested material was only efficient when combined with wheat sowing.

(3.c) Do transfer techniques and wheat sowing increase the similarity between restored and reference communities?

Hay transfer and brush material transfer combined with wheat sowing were the most successful techniques in restoring mountain grasslands, showing highest similarity to references in plant species composition and highest target abundances.

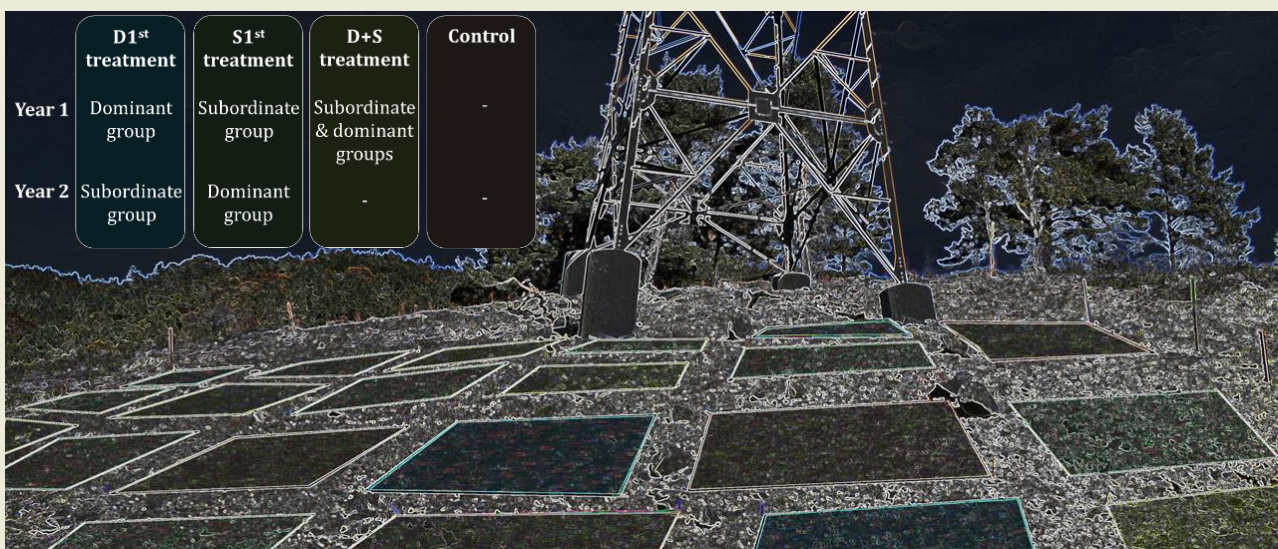
(3.b) Does sowing of wheat facilitate the establishment of brush-harvested seeds?

Wheat sowing clearly facilitated seedling establishment of seeds transferred with brush material. In mountain grasslands with shallow and stony soils, recruitment of harvested seeds clearly benefited from a facilitative effect of living (wheat) or dead (hay) vegetation cover.

(3.d) Does propagule addition limit soil erosion?

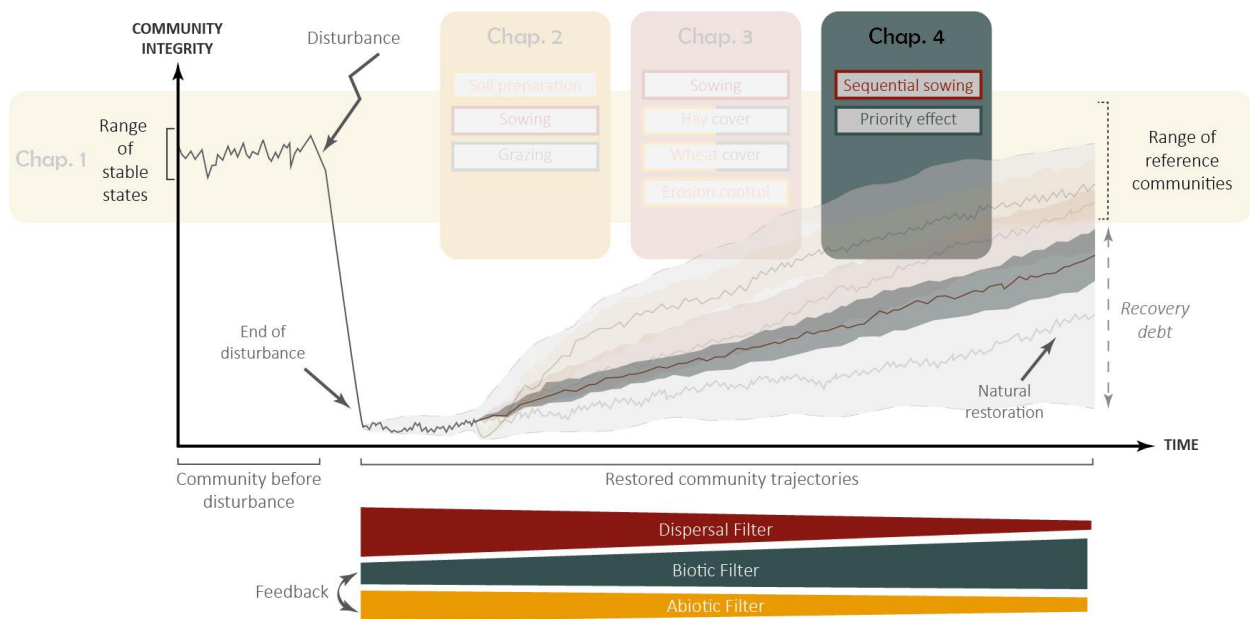
Soil erosion was not affected by any restoration treatment at the end of the observation period. Intermediate measurements demonstrated a high temporal variability of erosion, and of treatment effects on erosion.

As in the **chapters 2 and 3** experiments, most restoration projects using seed addition involved transferring all target species at the same time, resulting in strong plant-plant interactions between transferred species, and therefore, the prioritization of the most competitive among them. In **chapter 4**, I focus on the analysis of priority effects varied by sequential sowing of different species groups (Dominants or Subordinates), each comprising three species.



— Chapter Four —

Consider sequential sowing to use priority effects in plant community restoration



Aure Durbecq, Armin Bischoff, Elise Buisson, Emmanuel Corcket, Renaud Jaunatre

Consider sequential sowing to use priority effects in plant community restoration

Abstract

Priority effects have been identified as a core concept in restoration ecology since their influence in early community succession may have long-term consequences for plant succession and community assembly. Therefore, the trajectory of the restored community can be modified by the sequence of plant species arrival, thus modifying such priority effects.

In order to test the effect of species arrival on community assembly after soil disturbance in French mountain grasslands, we applied sequential sowing using two communities, each comprising three different perennial plant species: one community of species dominant in the study area, the other one comprising subordinate species. We tested four sowing treatments: control (without any sowing), two sequential sowing treatments and simultaneous sowing. In the first sequential sowing treatment the three dominants were sown in 2018 and the three subordinates in 2019, in the second treatment the three subordinates were sown in 2018 and the three dominants in 2019. We analysed plant cover, abundance and aboveground biomass, and calculated priority and secondary indices for each sown species.

Our results showed that manipulating the order of arrival shaped the community composition. Both the dominant Poaceae *Bromopsis erecta* and the subordinate Fabaceae *Onobrychis viciifolia* showed significantly negative secondary index. The late-arriving species recruitment of *B. erecta* and *O. viciifolia* was probably hampered by niche modification and size asymmetric competition of the pre-established vegetation. Interestingly, the dominant Fabaceae *Anthyllis vulneraria* was clearly favoured when sown in second, demonstrating a high recruitment capacity even when arriving late. We suggested that the strength of priority effects varies according to the niche characteristics which differ between species and may influence (negatively or positively) the late-arriving species establishment. Our study provided evidence that plant community assembly was influenced by the order of arrival but highlighted strong response variations between species suggesting high species-specific priority effects.

Keywords: dominant species, subordinate species, time-advanced, niche modification, stepwise sowing, grassland restoration, late-arriving species, early-arriving species

4.1 Introduction

Priority effects (hereafter PEs) occur when the order of species arrival in a habitat modifies biotic and abiotic properties of the micro-environment and thus affects the recruitment, establishment, growth and/or reproduction of late-arriving species (Gillhaussen et al. 2014; Temperton et al. 2016; Weidlich et al. 2018). PEs can be either positive (i.e. facilitative; Bertness & Shumway 1993) or inhibitory (i.e. competitive; Cole 1983; Fukami 2015) but usually, a competitive advantage for the first arriving species prevails because of their greater size or density compared with late-arriving species (i.e. size-asymmetric competition; Grman & Suding 2010, Wainwright et al. 2012). Many other mechanisms can also drive PEs, such as niche pre-emption or niche modification (Helsen et al 2015; Fukami 2015), plant-soil feedbacks and soil legacies (Bever 2003; Van der Putten et al. 2013; Fukami & Nakajima 2013; Grman & Suding 2010) or even allelopathic effects (Levine 2004). Furthermore, the strength of PEs can be mediated by both biotic and abiotic factors, such as species identity (Cleland et al. 2015; Stuble & Souza 2016; Gillhaussen et al. 2014; Wilsey et al. 2015; Werner et al. 2016) or overlapping niches (Vannette & Fukami 2014), predation pressure (Chase, et al. 2009) and habitat size or productivity (Fukami 2004, Orrock & Fletcher 2005; Chase 2010, Kardol, et al. 2013). In productive environments, PEs may be very strong resulting in alternative stable states (Chase 2003; Hobbs & Norton 2004; Kardol et al. 2013; Weidlich et al. 2017). PEs are, however, poorly studied in unproductive environments (Wisley 2020). Finally, PEs do not only influence the early community assembly but also shape the potential future community trajectories, including their composition and associated functions (Grmann et al 2010; Gillhaussen et al. 2014; Wermer et al 2016; Stuble & Young 2020).

Translating the concepts of community assembly and trajectories into relevant methods to drive ecosystem dynamics is a core approach in restoration ecology. If a habitat has been damaged or destroyed, restoration managers try to re-establish the pre-disturbance ecosystem including species composition and ecosystem functions (Gann et al. 2019). Changing initial abiotic conditions and species pools may help to accelerate restoration towards the reference plant community. Despite growing evidence of the importance of PEs in early plant succession (Van der Putten et al. 2000; Vaughn & Young 2015) and their long-term consequences (Fukami 2004; Švamberková et al. 2019; García-Girón 2021) most restoration approaches using seed-addition involved transferring all target species at the same time (Török et al. 2018). However, using PEs by establishing different sequences of species arrival has been identified as a promising tool to increase the establishment success of target species for ecological restoration (Fukami et al. 2011; Young et al. 2001, Vaughn and Young 2015; Wermer et al. 2016; Young & Stuble 2017).

Consequently, there is an increasing number of field and greenhouse studies testing PEs as a restoration tool. Tests of PEs involve sequential sowing of different species or species groups in order to test their influence on plant community dynamics. Species groups may be based on their origin (native vs. exotic, Grman & Suding 2010; Hess et al. 2019; Goodale & Wilsey 2019; Ploughe et al. 2020; Yu et al. 2020), on their life cycle type (annual vs. perennial, Schantz et al. 2015; Vauchn & Young 2015), or on their functional group (grasses vs. forbs vs. legumes, Gillhaussen et al. 2014; Stuble & Souza 2016; Stuble et al. 2017; Weidlich et al. 2017, 2018; Delory et al. 2019). Other studies examined PEs by mixing these groups (Werner et al. 2016; Shantz et al. 2018; Mason et al. 2013; Young et al. 2017; Cleland et al. 2014; Lang et al. 2017; Stuble & Young 2020; Wisley et al. 2020). Alternatively, species may be assigned to groups according to their importance in reference plant communities, for example in distinguishing dominant (species frequently occurring in high abundance) and subordinate species (frequently occurring in low abundance; Grime 1998). Subordinate species may have a greater impact on ecosystem functions than dominant species (Mariotte et al. 2013, 2014), particularly in semi-natural grasslands (Gibson 2009; Werner et al. 2016). They can increase plant community resistance against drought (Mariotte et al. 2013), probably via mycorrhizal fungal associations that improve plant productivity and positive plant-soil feedback (Mariotte et al. 2012; De Vries et al. 2011, 2018). Furthermore, dominant species are competitive and thus less sensitive to PEs than subordinate species (Sarneel et al. 2016). Delaying dominant species arrival may thus improve the establishment of the subordinate species (Mariotte et al. 2012; Young et al. 2017) and promote the coexistence of both. Contrary to dominant species, the subordinate species are expected to show a low competitive response (tolerance to competition of other species) and low competitive effect (competition pressure exerted on other species; Goldberg et Landa 1991; Wang et al. 2010; Keddy et al. 2002).

We first identified sets of dominant or subordinate species in our study area using abundances in vegetation releves. Then we tested PEs by sowing dominants and subordinates at the same time and sequentially. According to the theoretical competitive abilities exerted for subordinate and dominant species, we hypothesized that the order of arrival of the dominants and subordinates changes the species composition of the plant community (**Fig. 4.1a**). More specifically, as dominants have a strong competitive ability (both competitive effect and response), we expected that sowing dominants first or together with subordinates leads to competitive exclusion of subordinates. In contrast, subordinates are expected to be less competitive than dominants. Therefore, sowing subordinates first should favour their establishment without preventing the establishment of dominants.

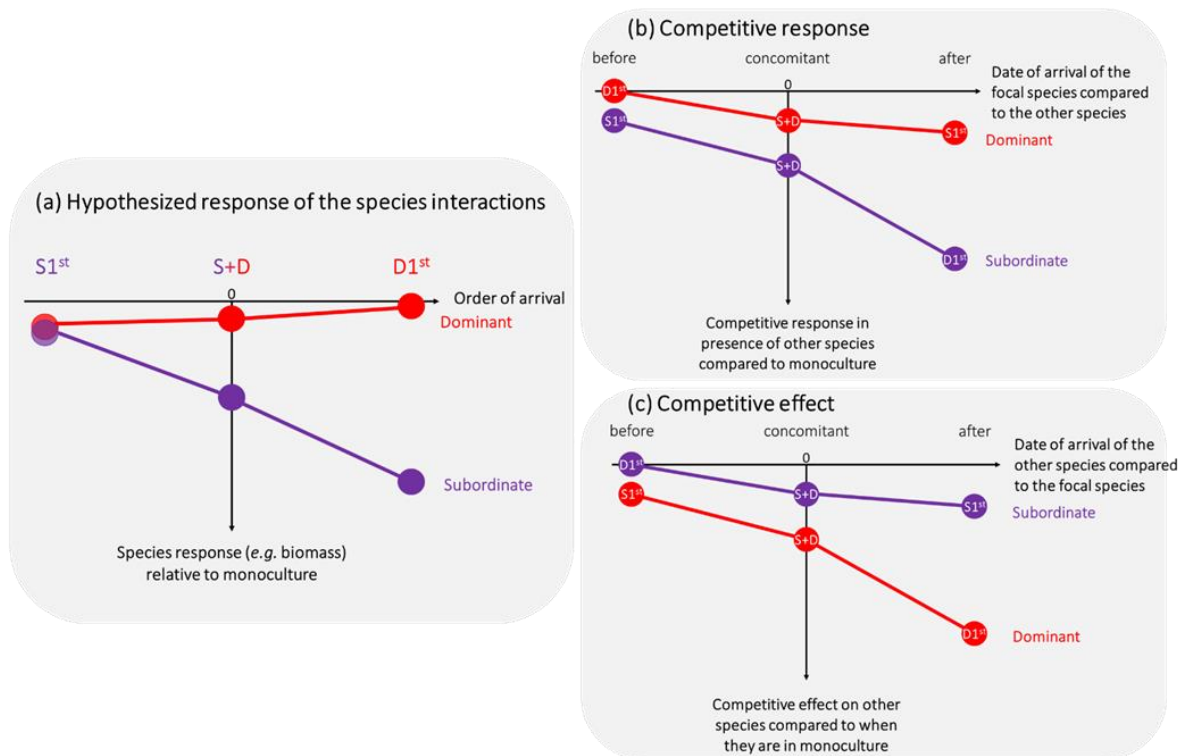


Fig. 4.1. Hypothesized priority effects. (a) Hypothesized response of the species interactions according to both (b) competitive response and (c) competitive effect (Goldberg and Landa 1991). Dominants are displayed in red and subordinates in purple. Competitive response (proportion of focal species cover or biomass when grown with other species compared to monoculture) and effect (i.e. proportion of cover or biomass of other species when they are grown with the focal species compared to monoculture) are displayed according to the hypotheses drawn for each species group (dominant or subordinate). **Hypothesis n°1:** Subordinates show a low competitive response and therefore establish less well in pre-established communities, but resist if sown at first (b, purple line). **Hypothesis n°2:** Subordinates show a low competitive effect and therefore, do not or hardly affect other species regardless of arrival date (c, purple line). **Hypothesis n°3:** Dominant species show a highly competitive response and are therefore not affected by other species, even if sown later (b, red line). **Hypothesis n°4:** Dominant species show a strong competitive effect and therefore strongly affect other species when arriving first, but to a lesser degree when arriving later (c, red line).

4.2 Material and Methods

4.2.1 Site description

The experiment was set up in a degraded montane grassland at La-Bâtie-Neuve, in the upper Durance valley ('Haute-Durance') of the Southern French Alps (44°57'93"N, 6°20'77'E). It is located at 1270 meters above sea level (asl), on an approximately 20° west-facing slope. The annual precipitation is 947 mm on average. July is the warmest month with an estimated mean temperature of 15.5°C, and January is the coolest with a mean temperature of -3°C. Frost can occur from September to May (estimated from Charges meteorological station, 863 m asl, 10 km from La-Bâtie-Neuve). The soil is stony on calcareous bedrock. Grasslands are extensively grazed by cattle. The recent construction of a high-voltage transmission line in the valley involved a degradation of the soil structure affected by stripping, compacting and decompacting the soil to create transitory access tracks and construction platforms. At the end of the work, the stockpiled soil was moved back to recreate the original slope.

4.2.2 Experimental set up

To test the effect of date of species arrival on community assembly, we applied sequential sowing using two communities of different perennial plant species. Both communities included one Poaceae, one Plantaginaceae and one Fabaceae representing different functional groups. The first community comprised one dominant species of each family, and the second one subordinate species of each family (**Table 4.1**). These sown species are common in grasslands of the study area belonging to the habitat type "Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates" (N6210, EU directive habitat 92/43/EEC, Calaciura & Spinelli 2008). The species were assigned to the community of dominant and subordinate species according to their relative rank of plant cover in 54 plots of typical undisturbed grasslands of the 'Haute-Durance' valley (Durbecq et al. 2020, **chapter 1**).

Table 4.1. Characteristics of dominant and subordinate species sown in the experiment. Competitive, stress-tolerant and ruderal strategies according to Grime (1998), Erschbamer (2007), and Pierce et al. (2013). Number of seeds sown/m² adjusted according to seed producer expertise on field germination (Phytosem, Gap, France). Frequency of occurrence and average cover according to Durbecq et al. (2020, **chapter 1**).

Species	Composition group	Family	CSR strategy	No. of seeds/m ²	Frequency of occurrence	Average cover (%)
<i>Bromopsis erecta</i>	Dominant (D)	Poaceae	S/SC	555	0.93	32.13
<i>Festuca cinerea</i>	Subordinate (S)	Poaceae	S/SR	2000	0.26	21.51
<i>Plantago media</i>	Dominant (D)	Plantaginaceae	C/CR	1250	0.68	5.42
<i>Plantago lanceolata</i>	Subordinate (S)	Plantaginaceae	C/SC	533	0.40	1.22
<i>Anthyllis vulneraria</i>	Dominant (D)	Fabaceae	CS/CSR	400	0.28	3.07
<i>Onobrychis viciifolia</i>	Subordinate (S)	Fabaceae	CS/CSR	710	0.14	1.04

4.2.3 Experimental design

We tested four treatments:

- (i) **control** (without any sowing)
- (ii) **S+D**: simultaneous sowing (the six species were sown together in 2018)
- (iii) **D1st**: the three dominant species (D) were sown in 2018 and the three subordinate species (S) in 2019
- (iv) **S1st**: the three subordinate species were sown in 2018 and the three dominant species in 2019.

Each treatment was replicated in ten spatially randomized plots (total n = 40; **Fig. 4.2a**). The 2018 sowing date was October 19th, and the 2019 sowing date was October 9th. Autumn sowing was chosen to allow dormancy breaking by cold stratification. Seeds of local origin according to the French label 'Végétal local' (Malaval et al. 2018) were obtained from a local seed company (Phytosem, Gap, France). The number of sown seeds was adjusted to a potential density of 100 individuals/m² for each sown species, based on seed producer expertise (**Table 4.1**). Plot size was 2m × 2m plots, spaced by 50 cm

from one another. To prepare the seed bed and to cover seeds the plots were manually raked before and after each sowing. The experimental site was fenced to prevent seedling damage by cattle grazing. The plots were neither watered nor fertilized.

4.2.4 Data collection

Plant community surveys were carried out on all plots. The percentage cover of all vascular species was estimated in sub-plots of 1 m × 1 m placed in the centre of each experimental plot (n = 40; Fig. 4.2a) in June 2019 and 2020.

To assess individual growth response, we harvested aboveground biomass of each sown species in two separate 30 × 53.5 cm sub-plots (0.16 m²; Fig. 4.2b), one in 2019 and one in 2020. We also counted the number of individuals. The harvest dates were 278 days and 264 days (9 months) after the first and the second sowing, respectively. To avoid an impact on plant community surveys, biomass was sampled outside the 1 × 1 m sub-plots (Fig. 4.2b). Biomass samples were oven-dried at 80°C for 72 hours and weighed.

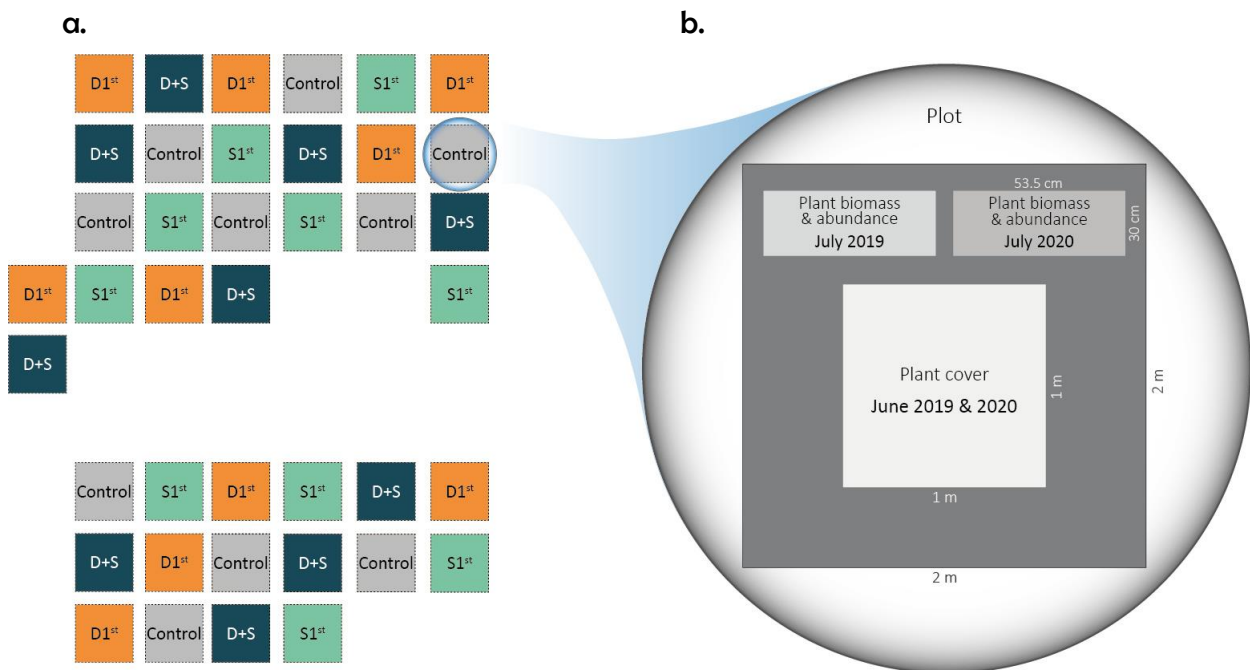


Fig. 4.2. Experimental design with a. the 40 plots including the ten replicates of the four randomly distributed treatments: D+S: dominant and subordinate species were sown simultaneously, D1st: dominant species were sown 1st (subordinates 2nd), S1st: subordinate species were sown 1st (dominants 2nd) and Control (no sowing); b. Zoom on one 2 × 2 m plot (and its sub-plots).

4.2.5 Statistical analyses

To explore the effect of sequential sowing on the community assembly, linear models (LM) were fitted with sowing treatment (control, D+S, D1st, S1st) and year as fixed effects and plant species cover as response variable. We tested the significance of the LM using the 'anova' function of the R package 'stats' with F-test (Chambers & Hastie 1992). In case of a significant treatment effect, multiple least-square mean comparisons were run using a Tukey adjustment in order to test differences between treatment levels ('emmeans' package, Lenth 2020). Data that did not comply with the assumptions of linear models (normal distribution, homoscedasticity) were sqrt-transformed before analysis.

In order to compare plant communities between the four treatments, Non-Metric Multidimensional Scaling (NMDS) was performed based on Bray-Curtis distance (Borcard et al. 2011) using R package 'vegan' (Oksanen et al. 2019). A permutation multivariate analysis of variance (PERMANOVA, Anderson 2001) with 9,999 permutations was used to analyse whether the community composition was significantly different between treatments (R package 'vegan').

We then tested for each sown species and the group of unsown species the differences between sowing one year before other species and simultaneous sowing. The proportion of individual species cover to total plant cover was used as response variable. Since transformation were not successful to comply with the assumptions of linear models, differences between the three sequential sowing treatments were analysed using Kruskal-Wallis and pairwise Wilcoxon tests. P-values of pairwise Wilcoxon tests were adjusted for multiple testing using the conservative Bonferroni correction (Jafari & Ansari-Pour 2019).

To evaluate facilitative versus competitive advantage, we calculated a priority index (PI) and a secondary index (SI) comparing the performance effects of arrival before, after and at the same time as other species (Cleland et al. 2015). Both PI and SI were calculated for aboveground biomass, abundance and cover of each sown species. For PI, we compared sowing first and simultaneous sowing, and for SI, sowing second and simultaneous sowing. 'Focal individual' (Fi) is the individual sown species considered in the calculation. The two indices are defined as follow:

$$PI = \frac{\text{Biomass}_{2020}(\text{Fi})_{1\text{st}} - \text{Biomass}_{2020}(\text{Fi})_{\text{simultaneously}}}{\text{Biomass}_{2020}(\text{Fi})_{1\text{st}} + \text{Biomass}_{2020}(\text{Fi})_{\text{simultaneously}}}$$

$$SI = \frac{\text{Biomass}_{2020}(\text{Fi})_{2\text{nd}} - \text{Biomass}_{2019}(\text{Fi})_{\text{simultaneously}}}{\text{Biomass}_{2020}(\text{Fi})_{2\text{nd}} + \text{Biomass}_{2019}(\text{Fi})_{\text{simultaneously}}}$$

The PI and SI results calculated on individual aboveground biomass are shown in the main document (**Fig. 4.6**) while indices on abundance and cover are presented in supporting information (**Fig. 54.3** and **54.4**). Individual biomass production is a particularly reliable indicator to assess plant competitive ability, particularly in studies with few species and with small biomass range (Gaudet and Keddy 1988). Positive values of PI and SI indicate that the Fi benefited from being sown first, or second, respectively (i.e. before or after the other species group) compared to being sown simultaneously with the other group (for PI: $\text{Biomass}_{2020}(\text{Fi})_{1\text{st}}$ exceeding mean $\text{Biomass}_{2020}(\text{Fi})_{\text{simultaneously}}$). Negative values indicate that the Fi performed less well when being sown first or second. According to criteria for linear models (normality, homoscedasticity), the difference between the indices and zero of each was either determined using t-test for PI or Mann–Whitney U test for SI, with a Bonferroni p-value adjustment (Jafari & Ansari-Pour 2019). All analyses were performed using R 3.5.3 (R Core Team 2019).

4.3 Results

4.3.1 Community assemblages

Total species cover significantly increased between 2019 and 2020 in the four treatments ($df = 1$, $F = 105.5$, $p < 0.001$, **Fig. 54.1a**), with a lower species cover in the control than in the other treatments in 2020 ($df = 3$, $F = 5.589$, $p < 0.001$). Compared to the control, unsown species cover was not significantly affected by sequential sowing, neither in 2019 nor in 2020. As the total species cover, unsown species cover significantly increased in 2020 ($df = 1$, $F = 13.25$, $p < 0.001$, **Fig. 54.1b**). The NMDS ordination showed a clear separation of the community composition between the four treatments ($df = 3$; $F = 11.29$; $p < 0.001$, **Fig. 4.3**). Along the first axis of the NMDS, the three sequential sowings were characterized by the sown species on the far right, while ruderal species such as *Daucus carota* L. *Lactuca serriola* L. *Convolvulus arvensis* L. *Stachys recta* L. *Arenaria serpyllifolia* L. dominated the control (left). The second axis separated the three sowing treatments, placing the simultaneous sowing (D+S) in the middle of the two sequential sowings with D1st on the upper and S1st on the lower part of this axis.

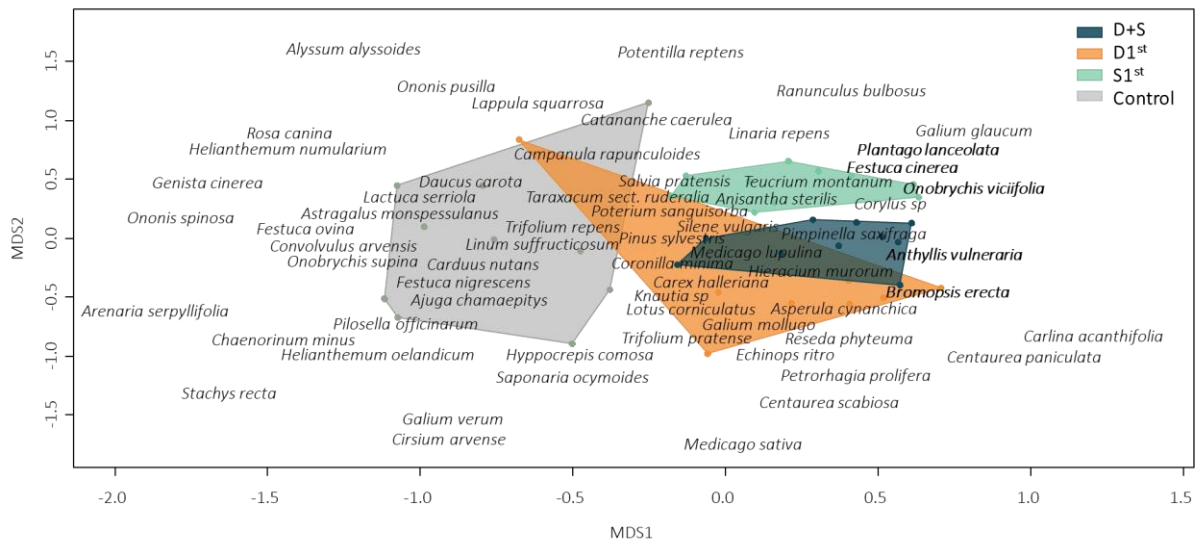


Fig. 4.3. NMDS of plant species composition in the four sowing treatments in July 2020. Polygons indicate the position of the outmost plots in each treatment with D+S: dominant and subordinate species sown simultaneously, D1st: dominant species sown 1st (subordinates 2nd), S1st: subordinate species sown 1st (dominants 2nd) and Control (no sowing). Sown species are in bold. NMDS stress = 0.24.

4.3.2 Community composition driving by time-advanced

The dominant Plantaginaceae *P. media* did not germinate in any treatment. However, the dominant Poaceae *B. erecta* dominated when sown first (two years prior to the final survey) in both D+S and D1st treatments, contributing 45% to total community cover ($\pm 2\%$). Its contribution to total cover was less than 5% when sown secondly (S1st treatment; **Fig. 4.4**). The contribution of the dominant Fabaceae *A. vulneraria* was similar ($\pm 10\%$) when sown secondly (S1st) or simultaneously with the other group (D+S), and 20% greater when sown first (D1st). The contribution of the three subordinate species (i.e. *F. cinerea*, *P. lanceolata* and *O. viciifolia*) reached each approximately 20% when sown first (S1st) whereas it did not exceed 2% when sown secondly (D1st). The cover of the Poaceae *F. cinerea* and the Plantaginaceae *P. lanceolata* did not exceed 6% when sown simultaneously with the other species (D+S), but the subordinate Fabaceae (*O. viciifolia*) contributed more than 15% to total cover in the D+S treatment. Total cover contribution of unsown species was 80% in the control and drastically decreased in D+S, D1st and S1st treatments to reach respectively 16%, 26% and 24%.

The total cover of the dominant Poaceae *B. erecta* was significantly higher in both D+S and D1st treatments with an average cover of 25%, compared to the S1st treatment showing a cover of less than 5% (Fig. 4.5, Table S4.1). No significant difference was found between the three sequential sowing treatments for the dominant Fabaceae *A. vulneraria*. The three subordinate species (*F. cinerea*, *O. viciifolia* and *P. lanceolata*) performed significantly better in S1st than in D1st. However, the cover of the subordinate Fabaceae *O. viciifolia* cover was not significantly different between the D+S and S1st treatment whereas the cover of *F. cinerea* and *P. lanceolata* was significantly lower in the D+S than in the S1st treatment.

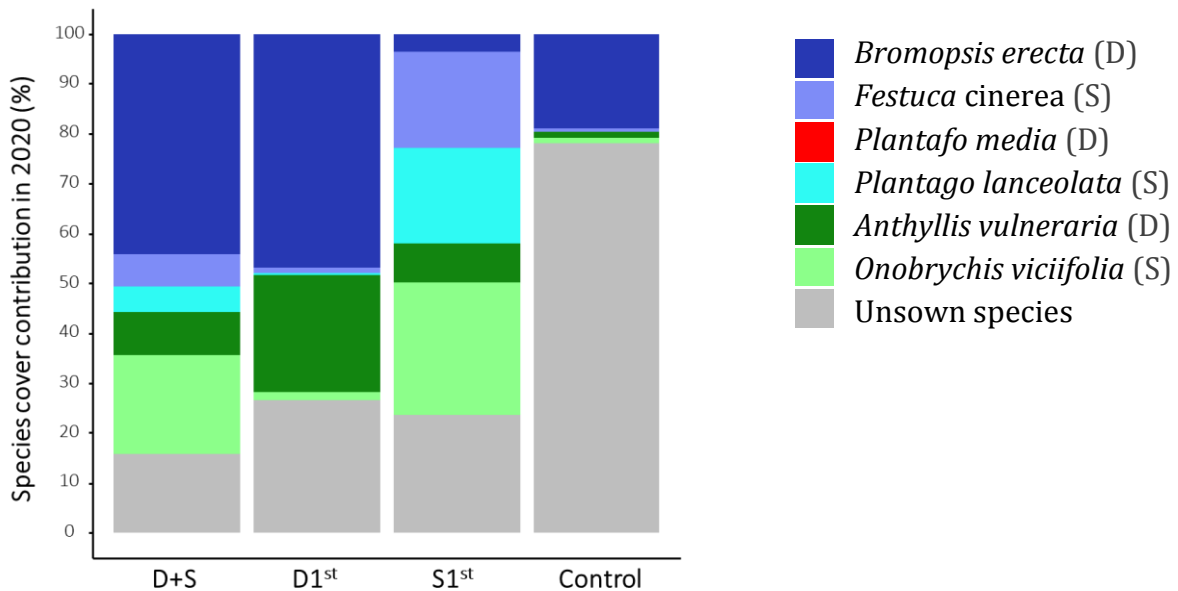


Fig. 4.4. Species cover contribution to the total cover in the four treatments. (D): dominant species; (S): subordinate species. D+S: dominant and subordinate species sown simultaneously, D1st: dominant species sown 1st (subordinates 2nd), S1st: subordinate species sown 1st (dominants 2nd) and Control (no sowing). *P. media* did not germinate.

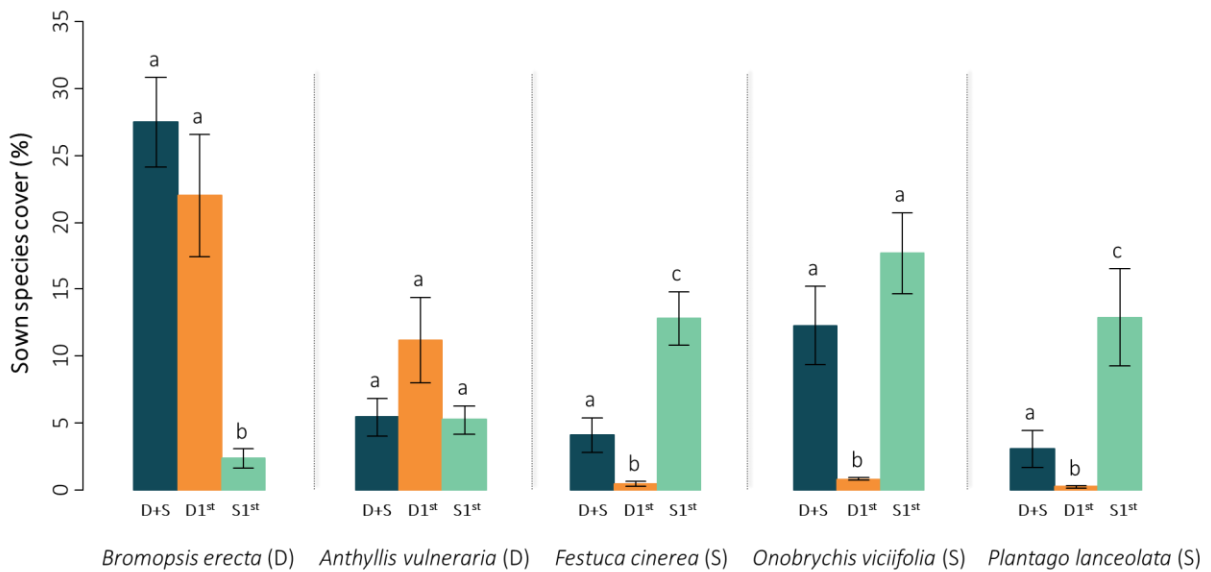


Fig. 4.5. Sown species cover in the three sequential sowing treatments with D+S: dominant and subordinate species sown simultaneously, D1st: dominant species sown 1st (subordinates 2nd), and S1st: subordinate species sown 1st (dominants 2nd). (D): dominant species, (S): subordinate species. Error bars are \pm SE and a common letter indicates absence of significant difference between treatments (a separate analysis was run for each species with Bonferroni adjustment of p-values).

4.3.3 Priority and secondary indices

For none of the species, priority indices were significantly different from zero, neither for aboveground biomass (Fig. 4.6a, Table S4.1), nor for species abundance (Fig. S4.3a) or cover (Fig. S4.4a). However, significant differences in the secondary index showed that the establishment of the dominant *B. erecta* and *A. vulneraria*, and the subordinate *O. viciifolia* were significantly affected by sequential sowing. *O. viciifolia* biomass and cover were lower (Fig. 4.6b; S4.3b, S4.4b) and *B. erecta* biomass was lower when sown second (Fig. 4.6b, Table S4.1). In contrast, the dominant *A. vulneraria* significantly benefited from being sown second (Fig. 4.6b, S4.3b, S4.4b, Table S4.1). The secondary index of *F. cinerea* and *P. lanceolata* was not significantly different from zero.

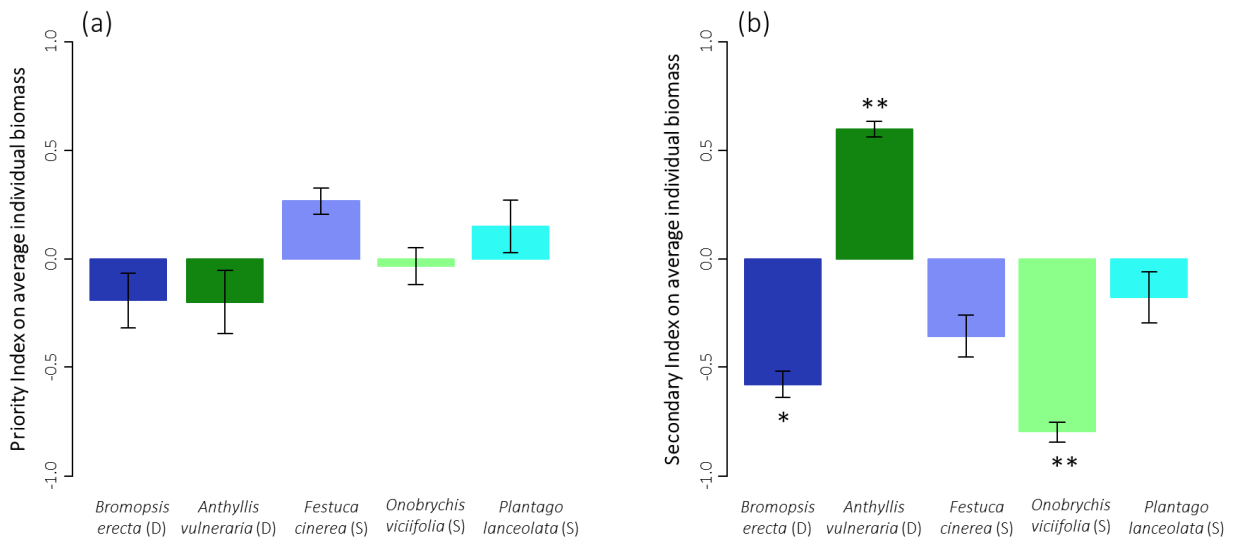


Fig. 4.6. (a) Priority Index (PI), and **(b)** Secondary Index (SI), both calculated on aboveground biomass of sown species. Error bars are \pm SE and *asterisks* indicate significant effects with * (p-adj < 0.05) and ** (p-adj < 0.01). p-adj are p-values with Bonferroni adjustment. (D): dominant species, (S): subordinate species.

4.4 Discussion

Manipulating the order of arrival by simultaneous or sequential sowing influenced the community composition two years after first sowing. As expected, subordinate species (i.e. *F. cinerea*, *O. viciifolia* and *P. lanceolata*) were favoured by priority effects (hereafter PEs) as they all best developed when sown first. However, dominant species (*B. erecta*, *A. vulneraria* and *P. media*) showed differences in their establishment. *P. media* did not germinate at all, regardless of the treatment. *B. erecta* known as a stress-tolerant competitor (Grime 1979) showed a significant disadvantage when sown one year later and performed significantly better when sown before or at the same time as the subordinates (i.e. D1st or D+S treatments respectively). This result suggests that *B. erecta* was affected by direct competition or indirect effects of already established vegetation. Similarly, Corcket et al. (2003) found a strong effect of competition on this species, and Harper (1961) identified a competitive priority effect of *Bromus madritensis* on *Bromus rigidus* when sown three weeks before. In contrast, the dominant Fabaceae *A. vulneraria* showed a higher performance when sown after other species, which indicated that it benefited from the presence of already established vegetation. Thus, we did not find a general pattern of priority effects in dominant versus subordinate species. However,

dominant species were on average less affected by sowing together with subordinate species than subordinate species sown together with dominants.

PEs demonstrated interesting contrasted effects between species, which can be explained by the niche components hypothesis (Vanette & Fukami 2014). This hypothesis is a robust tool to analyse priority effects even if large differences in species-specific strategies and interactions among environments increase unexplained variation. In this study, the dominant Poaceae *B. erecta* and the subordinate Fabaceae *O. viciifolia* showed the same trends. The two species both established well, when sown at the same time as species of the other group (simultaneously with subordinates or dominants) or only with the other two species of the same group (D1st for *B. erecta* and S1st for *O. viciifolia*). Both species were clearly disadvantaged when sown in 2019 (as second species group) suggesting that both species show a strong recruitment ability if the niche is not occupied. However, the priority index did not confirm this result. Both species, *B. erecta* and *O. viciifolia*, showed a significant secondary effect. These both late-arriving species recruitment were probably hampered by niche modification and size-asymmetric competition of the pre-established vegetation (Vanette & Fukami 2014; Wisley 2020), or by niche pre-emption (Fukami 2015).

The dominant Fabaceae *A. vulneraria* well established in the three seed-addition treatments and was not negatively affected by the pre-established community when sown after subordinate species. *A. vulneraria* even benefited from a secondary effect, demonstrating thus a high recruitment capacity in established vegetation (Vanette & Kukami 2014). Positive effects of already established vegetation on the recruitment niche (*e.g.* living cover protecting young seedlings and improving water retention, Donath et al. 2006; Wright et al. 2014; **Chapter 3**) may have favoured *A. vulneraria*. Alternatively, a positive plant-soil feedback may have contributed to the secondary effect (Bever 2003; De Vries et al. 2011, 2018; Fukami 2013, 2015; Goldstein & Suding 2014). The association of plant and soil organisms may have been favoured by the previous establishment of the Fabaceae *O. viciifolia*. Species-specific functional traits were also suggested to play an important role in the establishment of the subordinate Fabaceae. As a late-successional species, *A. vulneraria* is a dispersal-limited but fast-growing species that may take advantage from being sown in a pre-established plant community (Erschbamer 2007; Marcante et al. 2009). Furthermore, its roots are longer and deeper than those of the sown subordinate Fabaceae *O. viciifolia* and of the other sown species (Jungk 1993), avoiding thus competition for soil resources. Our results are similar to that of Hess (2020) who found a particularly weak competitiveness of *O. viciifolia* when sown after exotic species in an experiment on priority effects. Furthermore, our results are in agreement with the hypotheses of Vanette & Fukami (2014) who suggested that the strength of PEs varies according to the niche components (overlap, impact and requirements). Such niche requirements differ between species, and may influence (negatively or positively) late-arriving species recruitment and/or establishment.

However, the mechanisms of underlying PEs need to be better investigated, with a particular focus on plant-soil feedback and belowground interactions (Bever 2003; Fukami & Nakajima 2013; Weidlich et al. 2018; Hess 2020).

To conclude, our study provides evidence that plant the community assembly was influenced by the order of arrival, potentially changing community composition and trajectories. Although our results did not show consistent responses for dominant and subordinate species, they were in agreement with other studies indicating strong variations between species and thus high species-specific PEs (Cleland et al. 2015; Stuble & Souza 2016). Using the priority and secondary indices of target species sown in ecological restoration may be a relevant method to improve management actions. Species showing a strong priority effect but a low recruitment in early succession may be sown in the beginning, and species that become dominant when sowing first or species showing a positive secondary effect may be sown later. To be applied in a restoration context, further studies are needed to assess both priority and secondary indices of restoration target species in different habitat types. This would thus enable practitioners to establish lists of species to be sown first and second in order to develop plant community structure and composition towards references identified prior to restoration.

4.5 Supporting information

Table S4.1. Analysis of sown species cover, their priority and secondary indices. Results of Wilcoxon-test (χ^2) or t-test (t) with Bonferroni p-value adjustment (p-adj). Significant p-values are in bold. (D): dominant species, and (S): subordinate species.

Species	Total cover			Priority Index			Secondary Index		
	df	χ^2	p-adj	df	t	p-adj	df	χ^2	p-adj
<i>B. erecta</i> (D)	3	23.56	< 0.001	9	0.375	1.000	9	0.004	< 0.05
<i>A. vulneraria</i> (D)	3	20.03	< 0.001	9	0.356	1.000	9	0.002	< 0.01
<i>F. cinerea</i> (S)	3	31.15	< 0.001	9	0.049	0.168	9	0.084	0.420
<i>O. viciifolia</i> (S)	3	32.27	< 0.001	9	1.000	1.000	9	0.002	< 0.01
<i>P. lanceolata</i> (S)	3	30.04	< 0.001	9	0.492	1.000	9	0.414	1.000

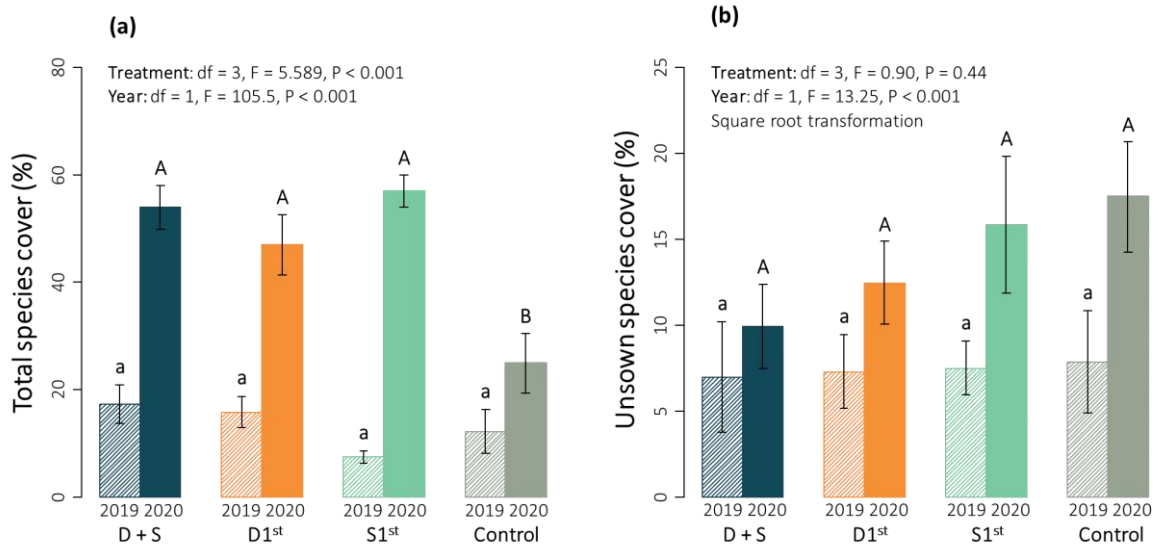


Fig. S4.1 (a) Total species cover and **(b)** Unsown species cover (species from the seed bank/seed rain) in the four treatments. D+S: dominant and subordinate species sown simultaneously, D1st: dominant species sown 1st (subordinates 2nd), and S1st: subordinate species sown 1st (dominants 2nd). (D): dominant species, (S): subordinate species. Error bars are ±SE and different letters indicate significant differences between treatments.

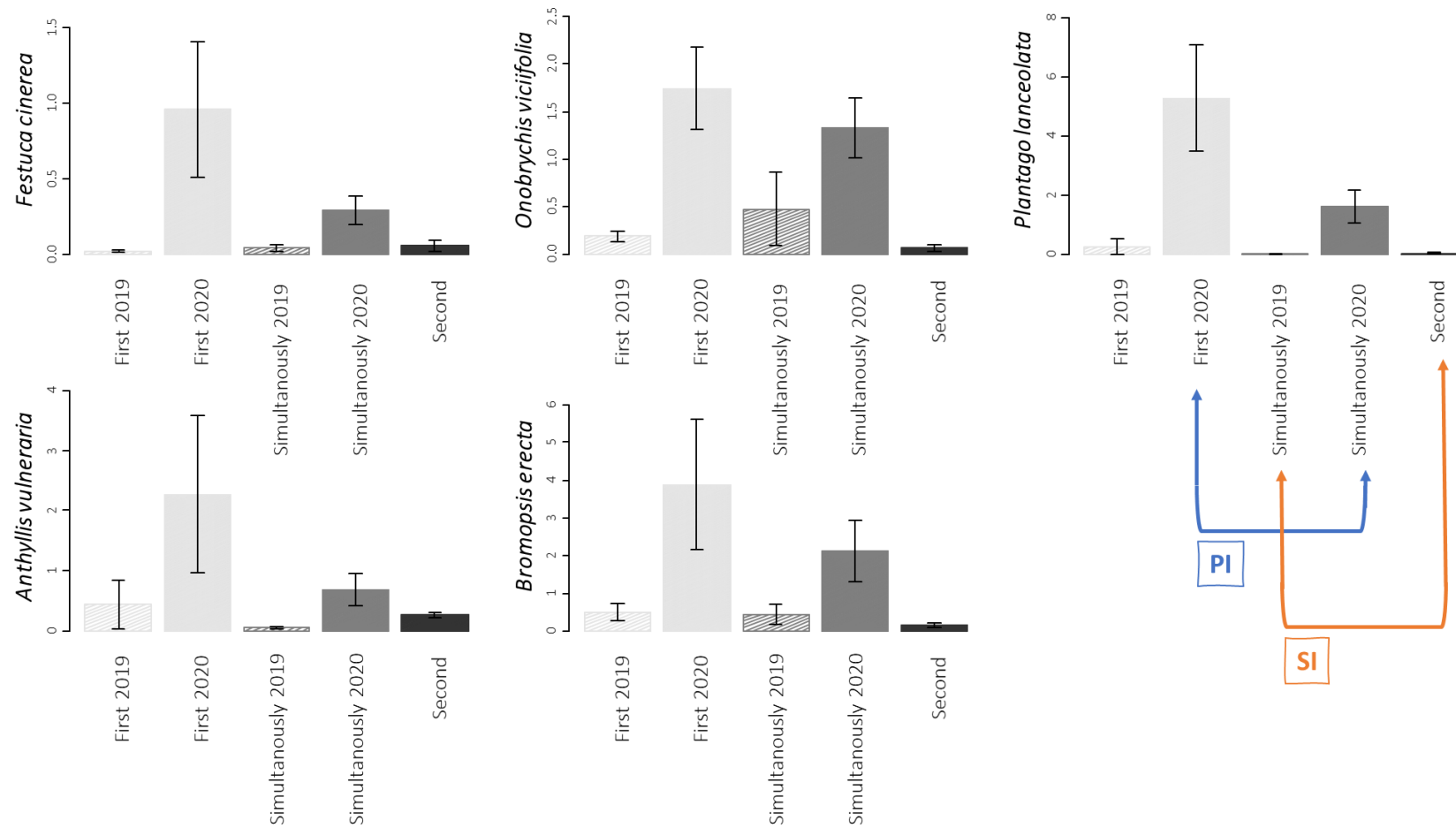


Fig. S4.2. Species biomass when sown alone one year earlier (First 2019) or simultaneously with the other group (Simultaneously 2019), and when sown two years alone two years earlier (First 2020) or simultaneously with the other group (Simultaneously 2020). The years correspond to the year of the biomass survey (not to the year of sowing). PI: Priority Index, SI: Secondary Index.

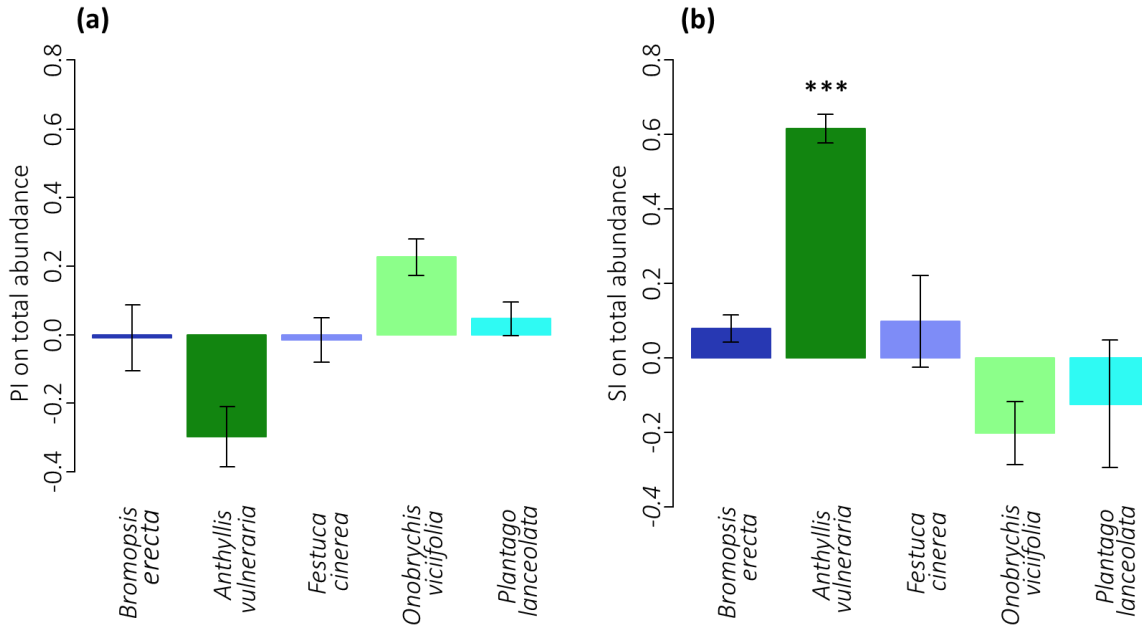


Fig. S4.3. (a) Priority Index (PI), and (b) Secondary Index (SI), both calculated on the abundance of the sown species. Error bars are \pm SE and *asterisks* indicate significant effects with * ($p\text{-adj} < 0.05$) and ** ($p\text{-adj} < 0.01$). $p\text{-adj}$ are p -values with Bonferroni adjustment.

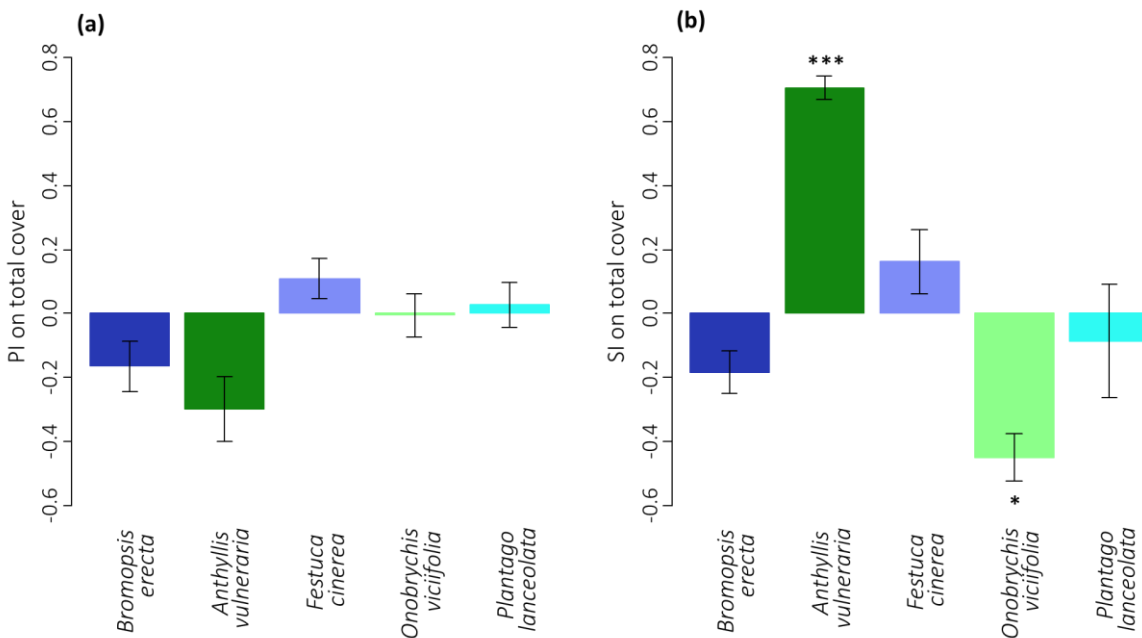


Fig. S4.4. (a) Priority Index (PI), and (b) Secondary Index (SI), both calculated on sown species cover. Error bars are \pm SE and *asterisks* indicate significant effects with * ($p\text{-adj} < 0.05$) and ** ($p\text{-adj} < 0.01$). $p\text{-adj}$ are p -values with Bonferroni adjustment.

— General discussion —

5 Discussion

The thesis aimed at better understanding the factors limiting the restoration of mountain grasslands after soil disturbance, and to identify successful restoration techniques to compensate the degradation. In the first part of the general discussion, I relate the main findings of the thesis in terms of seed-addition technique success (5.1.1) and their underlying ecological mechanisms and processes (5.1.2), and I conclude on the importance of priority effects in shaping plant community assembly (5.1.3). The second part of the discussion focuses on the limits and perspectives of the framework used to identify reference communities (5.2.1), and on the trade-off between extensive grazing as a requirement to maintain systems open and as a constraint in seedling recruitment (5.2.2). Finally, given the outcomes of chapters 3, and of both chapters 2 and 4, respectively, I develop suggestions to improve our understanding in soil control measures (5.2.3) and in plant-soil interactions (5.2.4).

5.1 General outcome

5.1.1 Success of seed-addition techniques to accelerate restoration

Using a filter-based integrated community concept is a useful and reliable tool to improve knowledge in restoration ecology (Hulvey and Aigner 2014; Halassy et al. 2016; Temperton et al. 2016; Chang and HilleRisLambers 2016). Examining dispersal, biotic and abiotic filters allow identifying different factors driving community assembly and dynamics of mountain grasslands. **Chapters 1 and 2** mainly focused on abiotic drivers. In agreement with (Pykälä et al. 2005) and (Srinivasan et al. 2005), both slope and aspect driving direct incident solar radiation were identified as major factors influencing species richness and composition in mountain grasslands. Elevation is also known as an important factor influencing microclimate and plant community composition (Callaway et al. 2002; Körner et al. 2008; Sundqvist et al. 2013), but in my study, elevation was standardized according to the restoration project (difference < 400 m) resulting in a non-significant effect. Edaphic factors such as soil moisture, soil pH and exchangeable phosphorus were also important parameters structuring plant communities of the 'Haute-Durance' valley.

The existence of a dispersal filter was demonstrated in **chapters 2 and 3**. Adding seeds from a nearby source community clearly improved ecological restoration of mountain

grasslands, largely promoted by additional treatments influencing both the biotic and abiotic filters. These additional treatments were successful in accelerating restoration since they increased the recruitment of transferred species (**chapters 2 and 3**) and shaped the restored community towards the references identified in **chapter 1**, suggesting that the restoration trajectories are on the right track. Despite several technical advantages (harvest, storage, spread), the transfer of brush-harvested propagules was poorly successful in establishing species of the source community when sown alone (without any other treatment, **chapters 2 and 3**). However, its combination with soil preparation in **chapter 2**, and the addition of wheat in **chapter 3**, largely increased the recruitment and establishment of brush-harvested propagules. I also observed significant differences in the recruitment rate of transferred species between brush and hay transfer (**chapter 3**). When both harvest techniques were applied at the same donor site and the quantity of sown seeds was similar, the seedling number of transferred species was much higher in the hay transfer treatment. Moreover, a comparison with greenhouse germination tests of propagules in hay and brush material demonstrated a low field emergence, suggesting that seedling recruitment is a bottleneck in ecological restoration of mountain grasslands (Öster et al. 2009). Brush material transfer alone did neither increase transferred species cover nor plant species richness, suggesting that the dispersal limitation filter of mountain grasslands can only be overcome, if this transfer is combined with other community assembly filter manipulations to improve the recruitment of transferred species.

5.1.2 Towards a better understanding of community assembly filters to improve restoration success

Manipulating abiotic (soil preparation, **chapter 2**) and biotic factors (hay mulch and wheat addition, **chapter 3**) increased the recruitment and establishment of transferred species. Several underlying mechanisms may explain this improvement, suggesting a modification of the niche in favour of the transferred species establishment: (i) Positive effects of treatments on soil conditions; (ii) Positive effects of treatments on seedling recruitment (germination, seedling growth and survival); (iii) Negative effects of treatments on the recruitment of spontaneous species (seed bank and rain) limiting competition with transferred species.

Both the mulch layer of the hay transfer and the temporary living cover of the sown wheat (hereafter collectively referred to as VC for ‘Vegetation cover’) may facilitate seedling recruitment by protecting seeds and seedlings. VC reduces direct solar radiation that may damage seedlings and prevents evaporation reducing drought stress (Donath et al. 2007; Wright et al. 2014; Mollard et al. 2014; Havrilla et al. 2020). VC may also limit seed removal by granivores such as arthropods and vertebrates, predation of young seedlings, damage by livestock herbivory and trampling, because seeds and seedlings are less visible (Scotton et

al. 2012; Linabury et al. 2019; Vanderburg et al. 2020). Moreover, VC is increasingly recognized to provide a shield against wind pressure and seed run-off, particularly in mountain grassland with steep slopes (Huc et al. 2018; Scotton 2019). Several restoration techniques have been developed in alpine grasslands to limit propagule run-off such as hydroseeding, hydromulching or geotextiles (Fattorini Marzio 2001; Krautzer et al. 2012; Shao et al. 2014; Tamura et al. 2017). However, these techniques are expensive and less cost-efficient in mountain grasslands. VC improves seed adhesion to the soil (Chambers 2000; Havrilla et al. 2020), particularly the hay layer that contains cellulose promoting mucilage adhesion (Western 2012). Similarly, soil preparation before sowing had a facilitative recruitment effect as it significantly favoured transferred species establishment suggesting seed adhesion improvement by micro-relief creation (Chambers 2000; Isselin-Nondedeu et al. 2006). VC and tillage affected the soil component indirectly and directly suggesting strong niche modification effects (Fukami 2015). Acting as a windscreen, VC stabilizes also the soil by decreasing surface run-off (Graiss and Krautzer 2011; Kavian et al. 2018; Scotton 2019), while increasing soil moisture retention by reducing solar radiation (Donath et al. 2007; Wright et al. 2014; Mollard et al. 2014; Havrilla et al. 2020).

All the three techniques may also have negative effects on seedling establishment via reduced vegetation cover (soil preparation) or increased competition (hay and brush material transfer). However, facilitation effects prevailed over competition and/or reduction in resources such as light or water (Eckstein & Donath 2005; Donath et al. 2006; Padilla et al. 2006; Wright et al. 2014), indicating that parameters of the chosen methods were well adapted (thickness of hay layer, depth of harrowing, date of wheat sowing). Soil tillage increased transferred species abundance but reduced the abundance of ruderal and competitive early-successional species (Grime 1998) that may have hampered the establishment of the transferred late-successional species (Edwards et al. 2007; Donath et al. 2007; Schmiede et al. 2012; Jaunatre et al. 2014; Harvolk-Schöning et al. 2020). Contrary to Kiehl and Wagner (2006) who suggested that a mulch layer limits competition of ruderal species in early stages of grassland restoration, VC did not have negative effects on spontaneous colonisation while simultaneously promoting establishment of transferred species in my study. The proportion of non-target abundances of brush material combined with wheat and hay transfer were not significantly different from the control, indicating that VC did not only provide better conditions for transferred species but also for non-transferred species recruitment, probably due to their general protection effect against drought stress, solar radiation, predation, and soil/propagule run-off. As these techniques are complementary, soil preparation may be even beneficial in combination with VC and this combined effect of soil preparation and VC is worth testing in future studies.

5.1.3 Priority effects shape community assembly and influence restoration outcome

In **chapter 4**, I tested priority effects by sequential sowing using a one-year time difference. Soil preparation and the addition of vegetation cover in **chapters 2 and 3** also shaped the communities to prioritize transferred species by modifying the conditions of germination. Soil tillage reduced the abundance of species spontaneously emerging from the seed bank, and wheat sowing had a nurse effect due to the rapid development of its belowground and aboveground organs, thus modifying the niche pre-emption (Fukami 2015). Furthermore, comparing species *ex situ* versus *in situ* (**chapter 2**) revealed differences in germination patterns. For example, *Plantago media* L. *Festuca marginata* (Hack.) K.Richt. and *Schedonorus pratensis* (Huds.) P.Beauv. showed a much higher greenhouse germination whereas the difference between greenhouse and field was much smaller in *Bromopsis erecta* (Huds.) Fourr. *Rhinanthus alectorolophus* (Scop.) Pollich did not germinate in the greenhouse whereas it dominated in the early stages of succession after brush-harvested propagule transfer in the **chapter 2** experiment. However, in **chapter 4**, I did not find a general pattern of priority effects neither in dominant versus subordinate species groups nor within the same family (i.e. *A. vulneraria* benefited from sowing after other species whereas *O. viciifolia* and *B. erecta* were disadvantaged). In agreement with other studies (Cleland *et al.* 2015; Stuble and Souza 2016), these results indicate that priority effects are highly species-specific (**chapter 4**). Thus, transferred species may have changed the community trajectory in **chapter 2 and 3** experiments according to the effects of soil tillage or different VC on seedling recruitment.

In conclusion, the manipulation of filters in the three experiments created or modified priority effects and feedback loops with abiotic conditions, which in turn changed community trajectories towards reference communities. As suggested in the last thesis chapters, several factors may have promoted the seedling recruitment, such as positive effects of already established vegetation on niche modification (e.g. CV), or on the soil compartment (e.g. plant-soil feedback; Bever 2003; De Vries *et al.* 2012, 2018; Fukami and Nakajima 2013; Goldstein and Suding 2014; Fukami 2015). Furthermore, as in the **chapters 2 and 3** experiments, most restoration projects using seed addition involved transferring all target species at the same time (Török *et al.* 2018), resulting in strong plant-plant interactions between transferred species, and therefore, the prioritization of the most competitive among them. Long-term monitoring would allow the identification of species that survive and establish well, and on the opposite those species that disappear over time. Another implication of priority effects would be the identification of key facilitator species (ecological engineers, nexus or keystone species) Jones *et al.* 1996; Power *et al.* 1996; Lockwood and Samuels 2004). Such species should be sown first to favour the establishment of other species that may be added later. Finally, monitoring the plant community over several years is necessary to evaluate whether the observed initial differences in seedling recruitment significantly influence plant species composition and restoration success in the

long run, and to analyse the replacement of ruderals by the transferred perennial species (Auestad *et al.* 2015; Albert *et al.* 2019; Shaw *et al.* 2020; Kiss *et al.* 2020).

5.2 Perspectives

5.2.1 Integrating theory for a more generalised application of restoration strategies

Researchers stressed the importance of integrating ecological theory in ecological restoration to allow the transfer of knowledge and methodologies between different restoration contexts (Walker *et al.* 2007; Temperton *et al.* 2016; Palmer *et al.* 2016; Lindenmayer 2020). Hence, recent studies suggested extending the surveys beyond the close vicinity of restoration sites and using statistical tools to identify and to rank structuring variables in order to select appropriate reference communities (Suganuma and Durigan 2015; McManamay *et al.* 2018; Erskine *et al.* 2019). In **chapter 1**, I propose a methodological framework in four steps to identify the best references for ecological restoration. This framework may be appropriate for various ecosystems, provided that the restoration towards non-degraded references is possible. The aim of this framework is, first, to obtain a good understanding of the environmental factors structuring the plant communities of the study system, second, to analyse environmental similarities between several potential reference sites around the degraded sites, and finally, to conclude on the choice of the best references as target or source communities for ecological restoration. This method can be easily applied to restoration practice but requires a good knowledge of ecosystem ecology. As it focuses on environmental drivers of plant community assembly, limitations of the evaluation approach can be the natural variability of the factors driving the system and/or the identification of appropriate drivers that are resistant to degradation. Moreover, main drivers of the system may be overlooked. For instance, soil structure, soil fauna and the history of land management may be important but their measurement is time-consuming. Additionally, historical data (e.g. wildfire records, historical land use changes) that complement data collected *in situ* (Asbjornsen *et al.* 2005; Urgenson *et al.* 2018) are not always available at the study site scale. In my study, it would be useful to examine the evolution of community trajectories over time (for each treatment; **Chapters 2, 3, 4**) in order to check whether restored communities really develop towards “best references” identified in the **chapter 1**. Moreover, it would be interesting to (i) test whether the use of propagules harvested in best reference communities is more successful than the use of those harvested in other source grasslands, and (ii) to analyse the effectiveness of the method in other systems. For instance, if this method is applied to a lagoon restoration project, the water

level, the duration of flooding and the degree of salinity would be major factors that may be used to identify the references (Bouzillé 2007).

5.2.2 Grazing: trade-off between seedling damage and grassland preservation

In **chapters 2 and 3**, the applied restoration techniques would be useful for mountain as well as for low altitude grasslands under extensive grazing, but also for the restoration of other systems that need to be revegetated, such as quarries (Kirmer 2004; Sheoran et al. 2010; Chenot et al. 2017). In **chapter 2**, the effect of livestock grazing on transferred species recruitment was weakly negative. Grazing may hamper the establishment by biomass removal (Scotton et al. 2012; Buisson et al. 2015; Vidaller et al. 2019) but also increase seed adhesion by trampling (Chambers 2000; Isselin-Nondedeu et al. 2006). As our study sites were first grazed before seedling emergence of transferred species, and as the following grazing period occurred seven months later, the first grazing probably had a positive effect (i.e. in reducing competition by pre-existing vegetation and creating micro-reliefs), but the second grazing period may have negatively affected young seedlings. Thus, the timing of grazing needs to be taken into account to limit the damage to seedlings, particularly if grazing exclusion is not possible.

While the livestock type may influence grazing effect (Tóth et al. 2018), I pooled the grazer species effect in an overall grazing effect including sheep and cattle. It would be useful for future restoration studies to distinguish cattle and sheep grazing effects on seedling recruitment and to separate positive microsite creation effect and negative seedling damage effects for both grazers. Moreover, it would be interesting to include effects of stocking rate (livestock density), grazing duration (Boschi and Baur 2007; Critchley et al. 2008), and to analyse whether their effects on seedlings translate into mid- or long-term effects on the plant community (McDonald et al. 1996).

5.2.3 How to measure erosion control in restoration studies?

In addition to the dispersal filter limiting the natural restoration of European semi-natural grasslands (Münzbergová and Herben 2005; Török et al. 2012; Muller et al. 2014; Halassy et al. 2019; Kövendi-Jakó et al. 2019), further important aims of mountain grassland restoration are the stabilisation of bare soils and erosion control. A rapid vegetation recovery helps to reduce erosion (Peratoner 2003; Liu et al. 2018; Scotton 2019; Gu et al. 2020), and many revegetation techniques focus on anti-erosion efficiency and persistence of vegetation cover (Graiss & Krautzer 2011; Fattorini 2001; Krautzer et al. 2012; Shao et al. 2014; Tamura et al. 2017; Scotton 2019). However, while immediate revegetation of

degraded mountain grasslands by locally adapted propagules is widely recommended to avoid erosion (Scotton et al. 2009; Wiesmair et al. 2017; Dupin et al. 2019), few studies have investigated the direct effect of restoration techniques on the reduction of soil erosion. In **chapter 3**, I used a device adapted from Feret & Sarrailh (2005) in order to evaluate the effect of VC on soil erosion (**Fig. S3.1 chapter 3**). However, our restoration treatments did not sufficiently influence total plant cover compared to the control and thus the treatment effect on soil erosion was not significant. Contrary to our results, Kaviani et al. (2018) found a reduction in soil runoff using wheat residues as “nurse cover” in a simulation experiment on a slope of 30%. In another *in situ* study, (Chenot et al. 2017) measured soil erosion by placing empty boxes on slopes below restoration plots to collect eroded soil over several months. In mountain grasslands, such devices need to be fixed strongly and protected from grazing which complicates its application. Photogrammetry or terrestrial laser scanning are alternative indirect measurement techniques providing topographic models of soil erosion and their temporal dynamics (Nadal-Romero et al. 2015). In my study, intermediate measurements demonstrated a high temporal variability of erosion (**Fig. S3.3 chapter 3**), suggesting a possible compensatory effect of hay and wheat decomposition increasing the humus layer and thus potentially raising the soil level. Finally, as wheat (**chapter 3**) shows a rapid development of roots and clonal structures such as rhizomes, wheat sowing may contribute to limit erosion (Löbmann et al. 2020) and to improve restoration success in favouring target species establishment. Moreover, as my **chapter 3** experiment was not fenced, it would be interesting to analyse the effect of wheat addition without the potential negative effect of livestock grazing.

5.2.4 Ecological restoration overlooks soil interactions: trade-off between positive effects on vegetation and negative effects on soil organisms

Plant-soil interactions (and feedbacks) operate during all stages of the plant life cycle, as well as during plant succession (Bever 2003; Wardle 2006; Voorde et al. 2011; van der Putten et al. 2013). In grassland restoration studies, soil preparation is increasingly recommended to improve seedling recruitment (**chapter 2**). Therefore, different techniques of soil treatment have been tested, such as topsoil removal or harrowing, to remove the seed bank or belowground plant organs of undesired plant species (Hölzel et al. 2003; Klimkowska et al. 2007; Schnoor and Olsson 2010; Schmiede et al. 2012; Jaunatre et al. 2014; Muller et al. 2014; Bischoff et al. 2018). However, few studies consider the consequences of these soil treatments on the communities of soil organisms (Young et al. 2005; Wardle 2006; Bever et al. 2010). Yet, there is growing evidence that soil legacies and plant-soil interactions are major drivers of the community assembly dynamics (Dam 2009; Grman and Suding 2010; Bever et al. 2010; Fukami and Nakajima 2013; Kardol et al. 2013; van der Putten et al. 2013). Moreover, interactions between wheat roots (**chapter 3**) and soil organisms such as

bacteria, nematodes or mycorrhiza have been shown to modulate aboveground interactions (Dam 2009). Thus, soil-mediated effects of wheat sowing on restoration would be worth studying. As plant-soil organism interactions range from mutually positive (e.g. mycorrhizal fungi, nitrogen-fixing bacteria) to strongly negative (e.g. pathogenic microbes, root-feeding invertebrates), and as soil organisms play an important role in determining the outcome of plant-plant interactions, they are essential for early plant colonization in grassland restoration (Bever 2003; Fukami and Nakajima 2013). Since soil treatments suggested to improve plant colonisation have a generally negative effect on soil organisms (Chan 2001; Andrade et al. 2002; Holland 2004) positive plant-soil interactions may be compromised. This trade-off between positive effects on seedling recruitment and negative effects on soil organisms needs to be considered in ecological restoration. However, consequences of soil treatments on soil organisms are less well studied and the final outcome of this trade-off is unknown.

Finally, as plant-soil interactions are species-specific (Bezemer et al. 2006; Voorde et al. 2011), and as the strength of priority effects depends on both the order of arrival and the identity of the species (**chapter 4**; Gillhaussen et al. 2014; Cleland et al. 2015; Wilsey et al. 2015; Stuble and Souza 2016; Werner et al. 2016), one year advance of a particular species may change soil biota and productivity by niche modification. Such modification may subsequently affect ecological processes and restoration success (e.g. biogeochemical cycling, plant community assembly; Fukami 2015; Weidlich et al. 2018; Gundale et al. 2019; Hess 2020). Therefore, future research needs to pay greater attention to belowground effects of restoration actions and subsequent plant-soil interactions during early community assembly. This research would improve our understanding of plant community succession and represents a potential for increasing restoration success (Young et al. 2005; Kardol and Wardle 2010; Kardol et al. 2013).

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— **Appendix** —

LA RESTAURATION DES PRAIRIES DE MONTAGNE

Le transfert de foin et le transfert de graines récoltées à la brosseuse comme techniques de restauration écologique



Prairie de référence pour la restauration écologique – Embrun (05) – © A. Durbecq

AU PRÉALABLE

Plusieurs techniques de restauration écologique sont couramment employées, en fonction des superficies à traiter, de leurs contraintes topographiques et des moyens techniques à disposition.

Il est préférable d'envisager la récolte des graines localement.

Dans ce cas, deux zones sont impliquées dans la mise en œuvre des travaux de restauration de prairie :

- la zone à restaurer, et
- la zone source pour la récolte des graines ou du foin.

Le choix de la technique de restauration va dépendre de la surface et des contraintes de ces deux zones, à savoir, *sont-elles mécanisables ?*

Le transfert de foin est préférable si les surfaces de récolte et de restauration sont à proximité, mécanisables, grandes (>1ha) et peu pentues. Par contre, si les surfaces sont petites ou présentent de fortes pentes, la récolte des graines via la brosseuse sera plus adaptée.



Revégétalisation des zones ouvertes remaniées

Programme de rénovation électrique de la Haute-Durance

Par Aure Durbecq
Et Yvon Sindzingre

Comment restaurer les prairies montagnardes ?

EN PRATIQUE

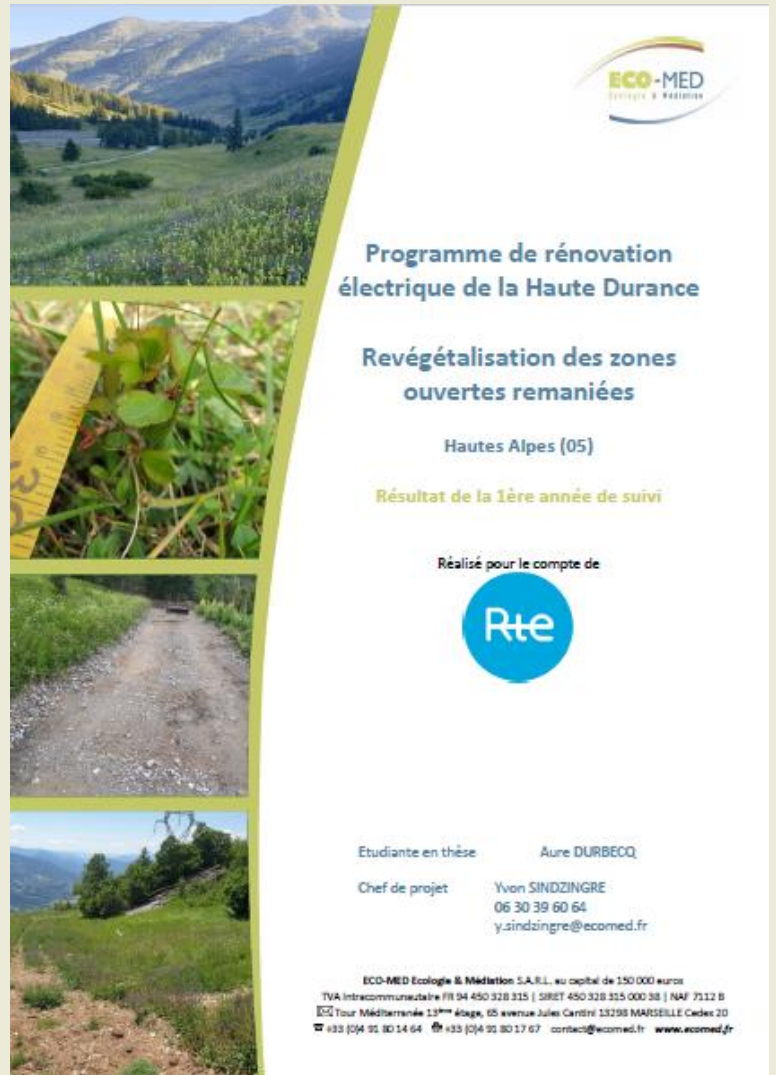
Le transfert des graines récoltées à la brosseuse et le transfert du foin sont des techniques efficaces pour restaurer les prairies de montagne. Cependant, les résultats de la thèse ont mis en évidence que le transfert de graines récoltées à la brosseuse est amélioré par un léger hersage antérieur au semis, ou par l'ajout d'un semis supplémentaire de blé. Cela permet de favoriser le recrutement et l'établissement de plantules cibles pour la restauration, à condition que le blé ne perdure pas dans l'écosystème.

Par ailleurs, bien que le transfert de foin semble mieux convenir aux surfaces étendues et peu pentues, la contractualisation avec les agriculteurs pour ramasser le foin, le stockage et le transport des bottes de foin compliquent la mise en œuvre de cette technique, en particulier sur les projets de restauration à grande échelle (cf. rapport Revégétalisation des zones ouvertes remaniées, ci-après).

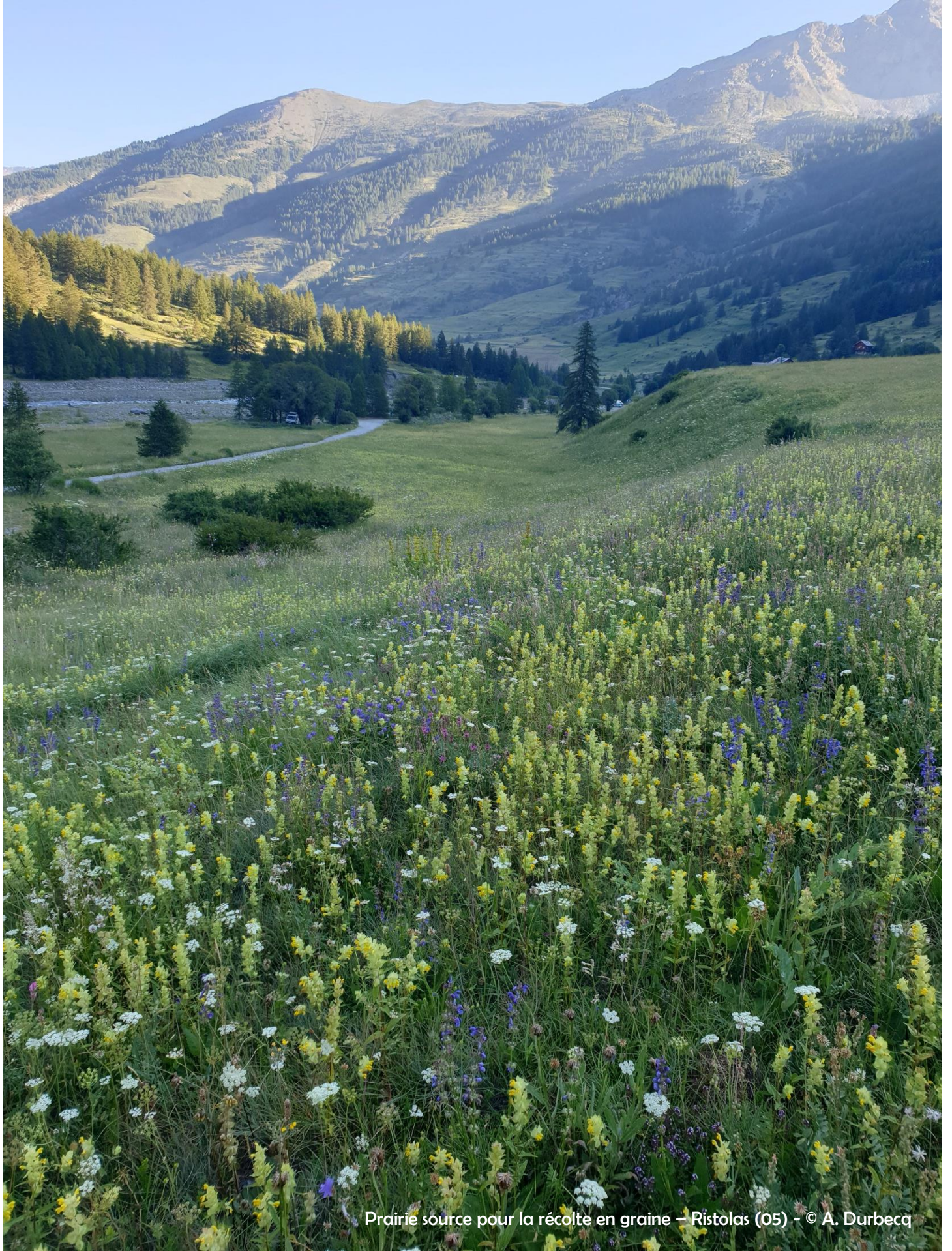
Le choix de la technique à sélectionner doit donc tenir compte de ces facteurs.

PERSPECTIVES

L'utilisation des effets de priorité pourrait être un atout en restauration écologique. Bien que cette approche soit encore préliminaire, il serait intéressant de poursuivre les recherches pour établir les listes d'espèces qui bénéficieraient à être semées de manière séquentielle pour restaurer la structure de la communauté.



En 2009, RTE a lancé le 'Programme Haute-Durance' consistant à la rénovation des lignes électriques à haute tension dans les Hautes-Alpes. Ce programme ayant fait l'objet d'une demande de dérogation à l'interdiction de destruction d'espèces protégées, RTE s'est engagé sur la mise en œuvre de mesures compensatoires, dont la mesure intitulée 'Restauration expérimentale de zones ouvertes remaniées'. Le présent rapport (annexe #) présente l'état d'avancement de la mesure et les résultats de la première année de suivi du dispositif de déploiement avec transfert de foin et semis, projet de restauration mis en œuvre par le bureau d'étude ECO-MED, sur une surface d'environ 8 ha.



Prairie source pour la récolte en graine – Ristolas (05) - © A. Durbecq



Programme de rénovation électrique de la Haute Durance

Revégétalisation des zones ouvertes remaniées

Hautes Alpes (05)

Résultat de la 1ère année de suivi

Réalisé pour le compte de



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2
3
4

Illustrations page de garde :

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2 – Plante et quadrat, B. TEUF, 03/07/2020, Embrun (05)
3 – Piste ouverte, Y. SINDZINGRE, 01/06/2020, Saint-Clément-sur-Durance (05)
4 – Piste refermé en cours de revégétalisation, A. DURBECQ, 02/07/2020, Chorges (05)

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CONTEXTE

En 2009, le gestionnaire du Réseau public de Transport d'Électricité (RTE) a lancé le 'Programme Haute-Durance' consistant à la rénovation des lignes électriques à haute tension dans les Hautes-Alpes. Ce programme ayant fait l'objet d'une demande de dérogation à l'interdiction de destruction d'espèces protégées, RTE s'est engagé sur la mise en œuvre de mesures compensatoires, dont la mesure de compensation MC2 intitulée 'Restauration expérimentale de zones ouvertes remaniées', élaborée suivant une convention de collaboration de recherche quadripartite entre RTE, ECO-MED, Avignon Université-IMBE et INRAE. Cette mesure a pour but la restauration écologique des prairies impactées par les travaux, suivant deux formes :

- 1) Un dispositif expérimental visant à évaluer et comparer l'efficacité de plusieurs itinéraires techniques de restauration écologique et la gestion temporelle des parcelles en restauration, projet de recherche mis en place durant la thèse d'Aure Durbecq, sur une surface d'environ 1 ha ;
- 2) Un dispositif de déploiement avec transfert de foin et semis visant à recoloniser les milieux ouverts remaniés, projet de restauration mis en œuvre par le bureau d'étude ECO-MED, sur une surface d'environ 10 ha.

Le présent rapport présente l'état d'avancement de la mesure et les résultats de la première année de suivi.

OBJECTIFS DE LA RESTAURATION ÉCOLOGIQUE

- Restauration écologique des milieux ouverts de Haute-Durance remaniés par des travaux de terrassement (revégétalisation des plateformes de travail et des pistes d'accès transitoires) par transfert de foin et de fleur de foin (dispositif de déploiement _ ECO-MED).
- Expérimentation ponctuelle avec différentes modalités de restauration des trajectoires de communautés végétales (dispositif expérimental _ Thèse Avignon Université).

La restauration écologique est définie comme 'le processus qui assiste le rétablissement d'un écosystème qui a été dégradé, endommagé ou détruit '(SER Primer 2004 ; McDonald *et al.* 2016 ; Gann *et al.* 2019). Son but est donc de rétablir l'écosystème dégradé en tâchant de retrouver sa structure, sa composition, ses fonctions et sa dynamique d'origine. Le but de la thèse (dispositif expérimental de la mesure de compensation MC2 : 'Restauration écologique des zones ouvertes remaniées ') est d'améliorer nos connaissances sur les mécanismes de succession végétale, de comparer les différentes méthodes utilisées pour la revégétalisation de zones ouvertes remaniées par les travaux de construction (prairies et pelouses), de développer des solutions techniques pour la restauration de prairies sèches à mésophiles en milieu montagnard, et donc de fournir des recommandations techniques en fonction de la gestion et des conditions pédoclimatiques du milieu pour mener à bien la restauration écologique (dispositif de déploiement de la mesure MC2). **L'objectif de la phase de déploiement est donc de restaurer les milieux ouverts de Haute-Durance remaniés par des travaux de construction de la ligne électrique en accélérant la recolonisation par les espèces végétales.** La restauration des prairies semi-naturelles de montagne est cruciale car on estime que la dynamique de recolonisation végétale naturelle peut être supérieure à 20 ans (Dupin *et al.* 2019), et un milieu mis à nu est d'autant plus exposé aux risques d'érosion du sol. La revégétalisation par des espèces locales améliore ainsi le contrôle des processus érosifs des sols et limite la progression de plantes invasives. Elle participe également au maintien de la continuité des milieux favorisant ainsi le retour et la circulation d'espèces patrimoniales.

AVANCEMENT DES ACTIONS DE RESTAURATION

Sur la base des premiers résultats de thèse menée par Aure DURBECQ, le déploiement de la mesure de compensation MC2 'Restauration des zones ouvertes remaniées' a débuté au printemps 2020.

En juillet 2019, **1 hectare de foin a été récolté en botte carré** sur la commune de Puy-Sanières et **2,8 ha de fleur de foin ont été récoltés** sur la commune de Ristolas.

Il s'est avéré difficile de trouver d'importante surface à récolter dans la vallée de la Haute-Durance. En effet, la surface de prairie de fauche est relativement faible vis-à-vis de la surface de prairie pâturée en Haute-Durance et le cheptel de bétail élevé amène les éleveurs à conserver l'intégralité de leur foin. Le Queyras, qui présente une richesse floristique similaire à la vallée de la Haute-Durance, s'est avéré propice à la récolte du foin.

La récolte de foin a rapidement été abandonnée au profit de la récolte de graines. En effet, les premiers résultats de thèse et la recherche bibliographie laissent à penser une bonne efficacité avec le semi de graines (aussi appelé semi de fleur de foin). De plus, l'épandage de foin s'avère inadapté aux petites surfaces difficiles d'accès car il nécessite une logistique lourde (apport de foin, épandage...); à l'inverse, la fleur de foin est facilement transportable et épandable sur tout type de surface; seule la récolte engendre un surcoût, cependant elle permet à l'agriculteur de conserver un fourrage quasiment indemne.

A l'automne 2019, l'ensemencement a été effectué sur **29 pistes** et plateformes associées, 16 sur la ligne Grisolles – MontDauphin (P6) et 13 sur la ligne Argentièrre – Serre Ponçon. **Au total, 4,1 km de pistes soit 2,9 ha ont été ensemencés.**

En juillet 2020, la récolte de fleur de foin s'est déroulée sur **1 ha à Château-Ville-Vieille** et sur **6,6 ha à Ristolas.**

A l'automne 2020, l'ensemencement a concerné **64 pistes** et plateformes associées, 11 sur la ligne Grisolles – MontDauphin (P6), 48 sur la ligne Argentièrre – Serre Ponçon et 1 sur la ligne Argentièrre – Briançon. **Au total 6,3 km de pistes soit 4,5 ha ont été ensemencés.**

En plus de ces lignes, 4 tronçons d'enfouissement de ligne souterraine ont été ensemencés sur une longueur de 521 mètres soit 0,25 ha.

Au total, 2,9 ha ont été restaurés en 2019 et 4,6 ha en 2020, soit un total de 7,5 ha sur la période 2019-2020.

Bilan des surfaces ensemencées en 2019-2020 (en m²)

		Ligne électrique				Projet RTE HD
		P3	P5	P4	P6	
Année	2019	0	0	15093	14401	29494
	2020	1678	1120	36307	8471	45898
Total		1678	1120	51400	22872	75392

Au total, 248 pistes temporaires ont été tracées pour le projet RTE-HD, dont 131 pistes tout ou en partie en milieu ouvert. En incluant les cultures et semis agricoles, 118 pistes ont été revégétalisées. Les 13 pistes restantes seront ensemencées en 2021. La mesure MC2 est donc achevée à 90%.

Le tableau suivant présente le nombre, le linéaire et la surface total de pistes restaurées par commune sur la période 2019-2020 (classement par ordre décroissant des surfaces restaurées).

Restauration des pistes par commune sur la période en 2019-2020 (en m²)

Communes	Nombre de pistes restaurées	Longueur de piste restaurée (m)	Surface restaurée (m ²)
Chorges	25	4830	30034
Châteauroux-les-Alpes	15	1443	11326
Réotier	11	1004	9832
Embrun	5	280	3770
La Bâtie-Neuve	9	775	3465
Savines-le-Lac	6	536	3082
Saint-Martin-de-Queyrières	3 tronçons souterrains	0	2640
Champcella	2	102	2620
Saint-Crepin	4	286	2490
La Roche-de-Rame	2	196	1886
Risoul	1 tronçon souterrain	0	1120
Prunières	2	318	1060
Puy-Saint-Eusèbe	2	243	810
La Rochette	1	100	700
Villar-Saint-Pancrace	1	100	540
L'Argentière	3	236	385
Espinasses	1	35	190
Total général	93	10484	75950

Certaines pistes en milieux ouverts n'ont pas fait l'objet d'un ensemencement pour les raisons suivantes :

- Terrain uniquement constitué d'éboulis ne permettant pas l'implantation d'une strate herbacée, même éparse.
- Revégétalisation naturelle de la piste très rapide, achevée avant notre passage.
- Piste en culture ou prairie temporaire, l'agriculteur préférant alors généralement labourer après la fermeture pour semer une culture céréalière ou fourragère de type luzerne.



Piste refermée en attente du labour
Y. SINDZINGRE, 20/07/2020, Chorges (05)



Prairie temporaire en lieu et place d'une ancienne piste
Y. SINDZINGRE, 16/08/2020, La Bâtie-Neuve (05)

Lors de la création des pistes, les entreprises ont systématiquement décapé et réservé la terre végétale. Sur certaine piste, un géotextile a été posé entre la terre réservée et la couche de gravât. Cette mise en œuvre a permis une refermeture des pistes plus soignée et a garantie un meilleur taux de reprise des graines en préservant la terre végétale.

Sur les ouvertures de tranchée, la terre végétale a là aussi été décapée et réservée. A noter que certaines pistes ont été exploité plusieurs années alors que l'enfouissement est une action ponctuelle, la terre végétale étant remis en place quelques semaines ou mois après l'ouverture. Ainsi le taux de reprise de la végétation sera probablement meilleur sur les pistes que sur les tranchées.



Piste en exploitation avant restauration
Y. SINDZINGRE, 01/06/2020, Saint-Clément-sur-Durance (05)



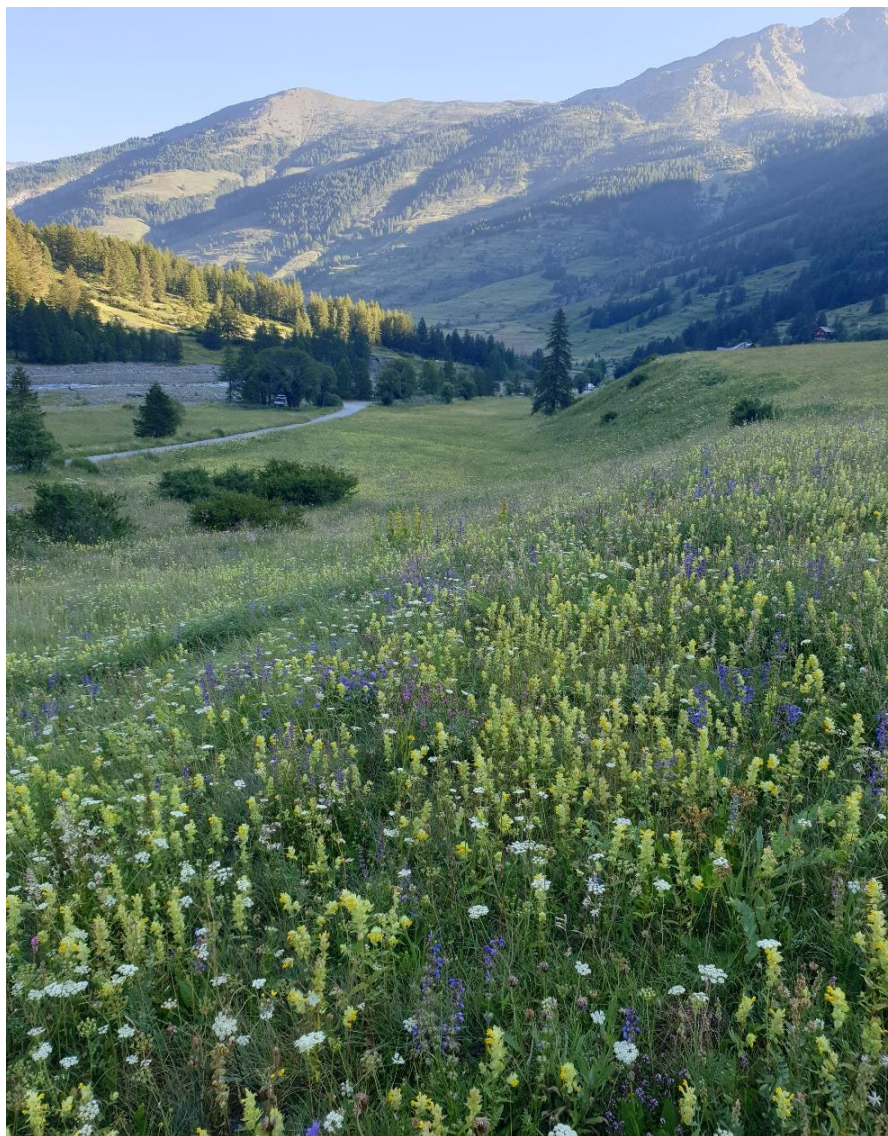
Enfouissement de ligne souterraine en prairie avant restauration
Y. SINDZINGRE, 01/06/2020, Saint-Martin-de-Queyrières (05)



Piste refermée prête à l'ensemencement
Y. SINDZINGRE, 10/10/2020, Villar-Saint-Pancrace (05)



Tranchée refermée prête à l'ensemencement
Y. SINDZINGRE, 01/06/2020, Saint-Martin-de-Queyrières (05)



Prairie source pour la récolte de fleur de foin
A. DURBECQ, 01/07/2020, Ristolas (05)



Prairie en cours de restauration (N+1)
A. DURBECQ, 01/07/2020, Chorges (05)

OBJECTIFS DU SUIVI

Le suivi se déroulera chaque année sur les 3 années après l'ensemencement, avec les indicateurs suivants :

- ✓ Suivi de la composition floristique (sites source, sites restaurés et sites de référence).
- ✓ Bon recouvrement végétal et similarité avec la référence.
- ✓ Diversité de composition des communautés végétales (en Poacées, Fabacées, et autres familles végétales).

METHODOLOGIE

Protocole de mise en œuvre de la restauration écologique

Récolte des graines et du foin

Plusieurs techniques complémentaires de revégétalisation sont couramment employées, en fonction des superficies à traiter, de leurs contraintes topographiques et des moyens techniques à disposition. Nous présentons ici les deux techniques qui ont été mises en œuvre pour la mesure MC2 : le transfert de foin sec et de graines récoltées par une brosseuse (nommé couramment et ci-après ‘fleur de foin’).

La prairie source pour la récolte en graine doit correspondre à la zone à restaurer en termes d’habitat, de composition floristique et de facteurs environnementaux. Le ratio surface d’ensemencement / surface de prélèvement est d’environ 1 / 1 pour des prairies de fauche jusqu’à 1300-1400 m d’altitude (Dupin *et al.* 2019).

Pour la récolte de graines (fleur de foin), différentes récoltes ont été effectuées en juillet 2019 et 2020 par Yvon Sindzingre et Aure Durbecq dans les communes de Ceillac, Puy-Sanières et Ristolas (05), à l’aide de la brosseuse avec aspirateur intégré (Fig. 2).



Fig. 1. Brosseuse tractée classique.

Il existe différent type de brosseuse pour récolter la fleur de foin. Si la surface est grande et peu pentue il est possible d’utiliser une brosseuse tractée par un tracteur (Fig. 1), mais cela demande d’effectuer un passage avec un aspirateur portatif (ex. : aspirateur-broyeur Stihl modèle SH86) pour ramasser les graines tombées au sol après le passage de la brosseuse.

Il existe une autre possibilité adaptable aux petites surfaces pentues : la brosseuse tractée avec aspirateur intégré de Mr. Mouchet Nicolas ; SARL [Mouchet Bois et Forêts](#) (Fig. 2).



Fig. 2. Brosseuse avec aspirateur

En 2019, 25,6 kg de fleur de foin sur 2,4 ha de prairies sources, soit environ 181 litres de graines ont été récoltés.

Pour la récolte du foin, une prairie d’une surface de 1 ha située à Puy-Saint-Eusèbe (44°6'67''N ; 6°41'66''E) a servi à récolter le foin utilisé pour ensemençer la piste refermée menant aux pylônes 38-39 de la ligne P6.

Le foin a été séché durant 72 heures après la récolte pour éviter l’échauffement et la pourriture pendant le stockage, puis mis en andain sous forme de 15 bottes rectangulaires de 45 x 30 x 75 cm, d’environ 7,5 kg chacune, soit un équivalent de 112 kg de foin, soit environ 1500 litres de foin.

Hormis la piste menant aux pylônes 38-39, toutes les pistes ont été restaurées par semi de fleur de foin, récolté dans le Queyras à l’aide d’une brosseuse avec aspirateur intégré.

Stockage

Les graines et le foin doivent être correctement séchées avant d'être stockées. Pour préserver la capacité germinative des semences, le stockage doit être effectué à l'abri de la lumière et de l'humidité, protégé des animaux et insectes susceptibles de manger les graines. Le temps de stockage doit être le plus court possible. Temps maximum : 1 à 2 ans (Scotton *et al.* 2012).

Préparation de la zone à restaurer avant semis

Avant le semis : Le substrat doit être meuble en surface au moment du semis. Si la terre est très compacte, il est possible de la décompacter juste avant le semis avec un passage de herse ou le godet d'une pelle mécanique.

En pratique : Si la remise en état après les travaux de terrassement est récente, le sol est encore meuble et donc opérationnel pour recevoir un semis de graine et/ou un transfert de foin. Il n'est donc pas nécessaire d'effectuer un décompactage. Cependant, un léger griffage (hersage) du sol est conseillé pour favoriser l'adhésion des graines au sol.

Par ailleurs, s'il y a suffisamment de terre végétale, il n'y a pas besoin d'enrichir le sol avec du fumier, du compost ou de l'engrais. De manière générale, plus le milieu est en altitude, plus la végétation est adaptée à des substrats pauvres en matières organiques et nutriments.

Epandage du foin et Semis de fleur de foin

Le foin est réparti de manière homogène sur environ 4 cm d'épaisseur (environ 500 grammes de foin/m²) à l'aide des outils disponibles. Exemples : pailleuse auto-chargeuse, épandeur à fumier, rouleau doseur, fourche à foin. Le passage de rouleau est recommandé mais pas indispensable.

En restauration écologique de prairies de montagne, il est conseillé d'effectuer le semis des graines récoltées en automne (entre octobre et décembre), juste avant les premières neiges, ceci afin de favoriser la levée de dormance rompue par l'effet du gel nécessaire aux espèces de graminées d'altitude.

En 2019, ECO-MED a effectué un épandage de 1500 litres de foin sur 2276 m² de piste de manière manuelle à l'aide d'une fourche à foin (ligne P6 pylônes 38-39).

Sur le reste des pistes refermées en 2019, un semis par fleur de foin a été réalisé dans les quantités suivantes : 143 litres semés au total (semis de 1 g/m²) sur 26150 m², revégétalisant ainsi la totalité des pistes refermées par RTE (c'est-à-dire disponibles pour la mise en œuvre de la phase de compensation) soit 2,6 ha de milieux ouverts compensés en 2019.

L'usage d'un semoir mécanique portatif a été essayé. La fleur de foin ne s'avère pas adaptée à ce type d'outil, du fait de la légèreté des graines, de leur taille très variable et de la présence d'épis et brins dans le mélange qui bloque le mécanisme. Le semis à la main, dit à *la volée*, s'avère être le plus efficace.



Fig. 3. Epandage du foin sur une piste à restaurer.

Protocole de suivi de la restauration écologique

Localisation des zones suivies

La zone d'étude se situe dans la vallée de la Haute-Durance, dans le département des Hautes-Alpes, en France. Elle s'étend sur plus de 100 km, du Briançonnais (commune de L'Argentière-la-Bessée : 44°79'29''N ; 6°55'90''E) au Gapençais (commune de La-Bâtie-Neuve : 44°56'53''N ; 6°19'61''E) en passant par l'Embrunais (**Figure 4**).

Les pistes et plateformes à restaurer se situent entre 1000 et 1400 mètres d'altitude, sur l'étage montagnard atteignant localement l'étage subalpin inférieur, sur pentes modérées bien exposées et bien drainées, sur sol principalement carbonaté et caillouteux.

Les zones ouvertes remaniées par les travaux concernent des pelouses et prairies de type semi-naturelles riches en espèces, sèches à mésophiles, et sont associées aux habitats suivants : Pelouses sèches (E1) et Prairies mésiques (E2), typologie EUNIS (Louvel *et al.* 2013) ; ou Habitat 6210 : Alliance Mésobromion des pelouses sèches semi-naturelles et faciès d'emboussissement sur calcaires (*Festuco-brometalia*) [*Sites d'orchidées remarquables], Cahiers d'Habitat Natura 2000.

Pour le suivi, 9 anciennes pistes ont été sélectionnées, il s'agit des pistes menant aux pylônes 15, 38, 83 et 108 de la ligne Argentière Serre Ponçon (P6) et 42, 43, 47, 53 de la ligne P6. Les pistes suivies ont été répartie de manière spatialement 'équitable' sur la zone d'étude ainsi qu'en faisant varier les facteurs environnementaux (orientation, raideur, quantité de terre végétale présentes...).

LOCALISATION DES SITES DE SUIVI DE LA RESTAURATION

Mesure compensatoire MC2 - Restauration écologique des zones ouvertes remaniées

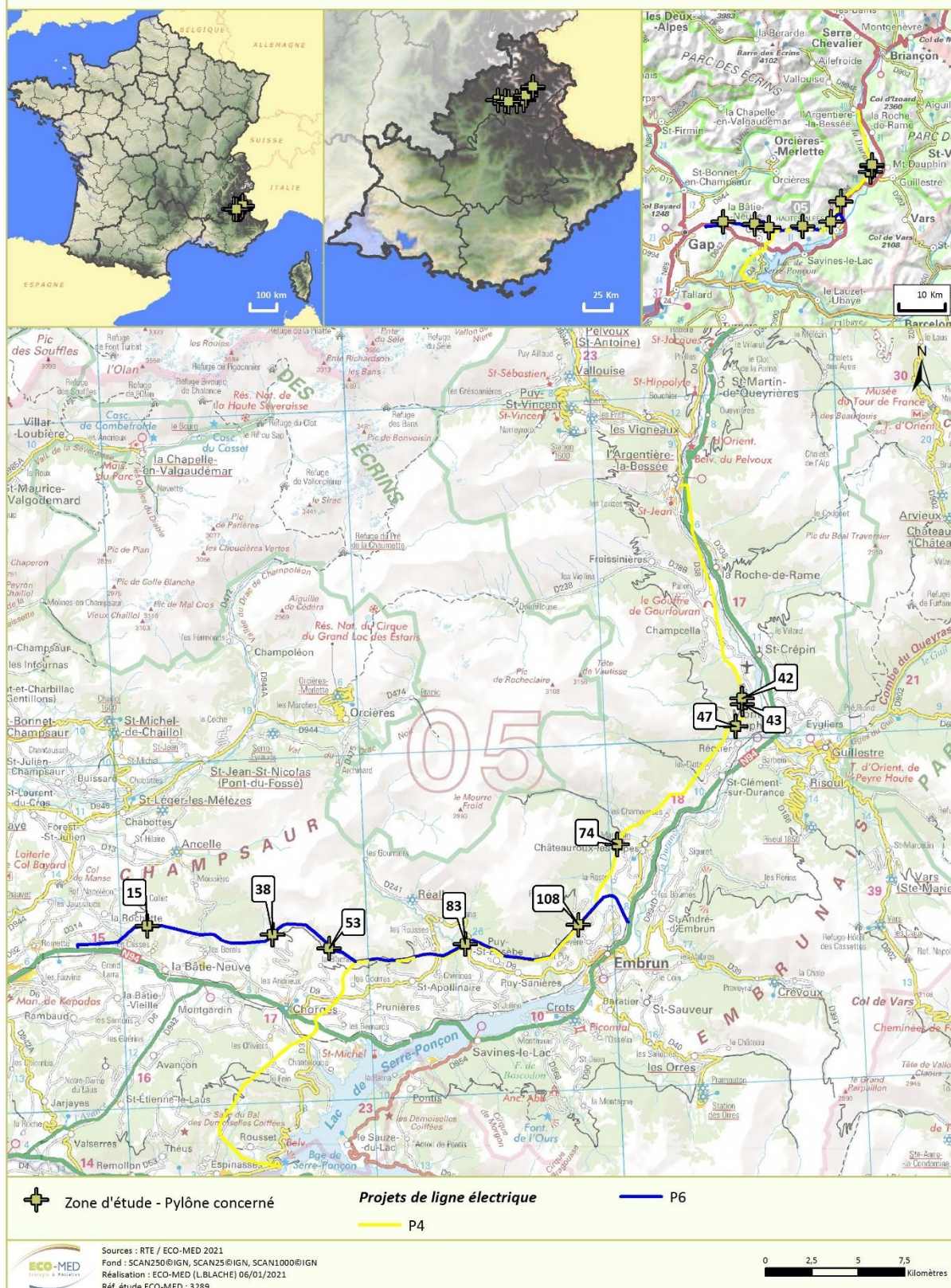


Figure 4. Carte de localisation du projet et des sites de suivi de la restauration écologique.

Protocole scientifique

En Juillet 2020, les suivis floristiques indicateurs de la restauration écologique, ont été effectués par Aure Durbecq et Bertrand Teuf (ECO-MED) au niveau des 9 pylônes électriques indiqués sur la **Figure 4**, selon le protocole suivant, et chaque placette de suivi a été marquée à l'aide d'une borne géomètre dans son coin inférieur gauche (face à la pente) :

6.2.1. Sur les pistes restaurées avec semis de fleur de foin (graines récoltées à la brosseuse) :

(a) Dans la zone restaurée : 1 quadrat de 2 x 2 mètres dans la zone restaurée et relever tous les recouvrements/espèces ; et 1 quadrat supplémentaire de 1 x 1 mètre tous les 25 mètres et y mesurer le recouvrement selon 3 classes : Fabaceae, Poaceae, Autres.

(b) Dans la zone à proximité non-impactée, dite de référence : 1 quadrat de 2 x 2 mètres et relever tous les recouvrements/espèces (*uniquement la première année de suivi*).

6.2.2. Sur la piste restaurée par transfert de foin (ligne P6 pylônes 38-39)

(a) Dans la zone restaurée avec foin : 8 quadrats de 2 x 2 mètres et relever tous les recouvrements/espèces

(b) Dans la zone à proximité non-impactée, dite de référence : 1 quadrat de 2 x 2 mètres et relever tous les recouvrements/espèces (*ici à mutualiser avec le relevé de fleur de foin en continue du foin ; uniquement la première année de suivi*).

6.2.3. Sur les prairies sources de fleur de foin (3 sites de récolte des graines : Puy-Sanières, Ceillac, Ristolas)

Suivi à réaliser uniquement la première année (objectif : connaître l'état initial des prairies sources)

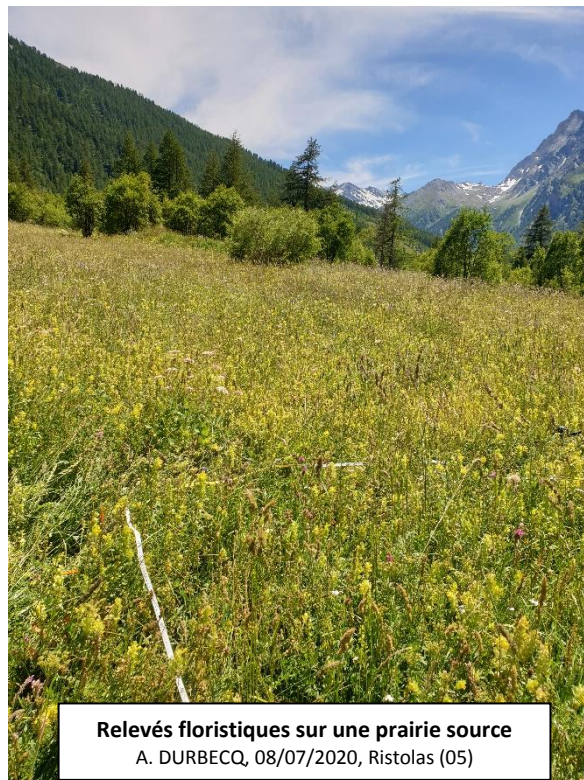
(a) 8 quadrats de 2 x 2 mètres dans les zones de récolte.

(b) 50 quadrats supplémentaires de 1 x 1 m pour mesurer le recouvrement de 3 classes : Fabaceae, Poaceae, Autres. Résultats du suivi

Les relevés du suivi floristiques ont été réalisé en Juillet 2020.



Relevés floristiques sur une piste restaurée
A. DURBECQ, 01/07/2020, Puy-St-Eusèbe (05)



Relevés floristiques sur une prairie source
A. DURBECQ, 08/07/2020, Ristolas (05)

RESULTATS

Les premiers sites restaurés ont été revégétalisés par semis de graines (fleur de foin) récoltées localement en juillet 2019 et semées en octobre 2019. Les suivis floristiques ont été effectués en juillet 2020 par ECO-MED sur les prairies sources, 9 sites restaurés et 9 sites non-dégradés (ci-après 'sites de référence') situés à proximité des sites dégradés (**Figure 4**).

En seulement 9 mois, les premiers résultats ont permis de mettre en évidence une bonne reprise de la végétation sur les sites restaurés. La revégétalisation par le semi de graines présentent d'ores et déjà un recouvrement végétal moyen supérieur à 30% sur les sites restaurés en 2019 (**Figure 6**).

Plusieurs études ont mis en évidence l'importance de la restauration des prairies dans les processus de stabilisation des sols et du contrôle de l'érosion, spécialement en milieu montagnard. Dans ces milieux, les conditions topographiques et climatiques rendent ces processus extrêmement lents, et la présence d'un couvert végétal est un facteur essentiel, d'où la recommandation d'une revégétalisation immédiate à partir de semences prélevées localement. Avec un recouvrement de 30% la première année après la revégétalisation (**Fig. 6**), nos premiers résultats indiquent un potentiel de réduction de l'érosion des sols de 60% par rapport à 100% de sol nu (Gu *et al.* 2019 : **Annexe 1 ; Figure. 9**).

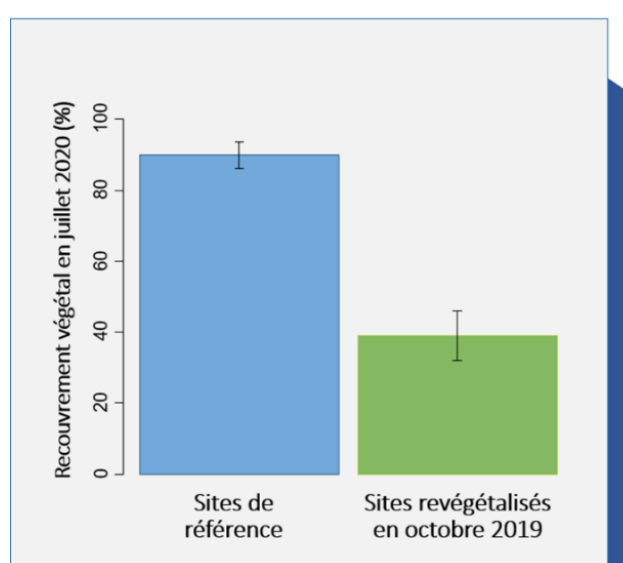


Fig. 6. Pourcentage de recouvrement végétal moyen en juillet 2020 sur les **sites de référence** (sites non-dégradés) et sur les **sites revégétalisés**.

Par ailleurs, l'analyse des compositions des communautés végétales a permis de comparer les recouvrements végétaux entre les prairies sources et les prairies restaurées. A travers la distinction en trois groupes de familles végétales (les fabacées, les poacées, et les autres familles ; **Figure 7**), nos résultats mettent en évidence la capacité plus lente des fabacées et des poacées à s'installer dans un milieu dégradé. Cependant, les sites restaurés en 2019 présentent un recouvrement végétal d'au moins 5% dans chacune de ces deux familles de plantes. Ces résultats indiquent une dynamique en faveur d'une bonne recolonisation de ces familles végétales dont les espèces annuelles pionnières sont souvent peu représentées sans action de restauration.

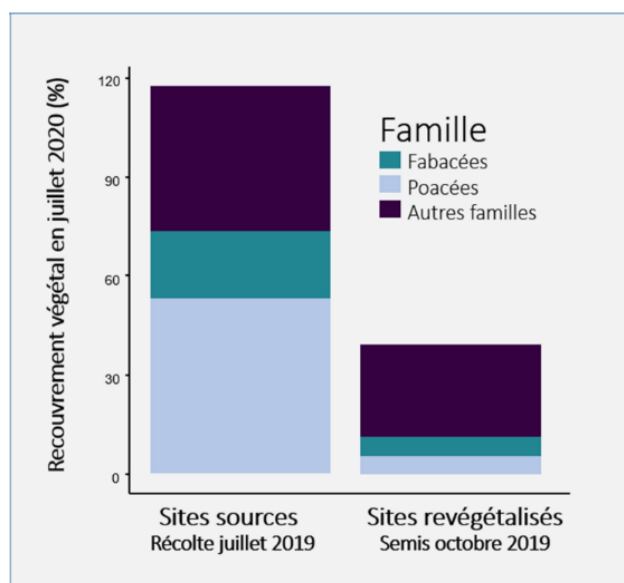


Figure 7. Pourcentage de recouvrement végétal moyen distingué en trois groupes : **fabacées**, **poacées** et **autres familles**, relevés en juillet 2019 sur les sites sources (pour la récolte en graines) et sur les sites revégétalisés.

Les sites restaurés et les sites non-dégradés (sites de référence) montrent des richesses floristiques équivalentes (Fig. 8). En effet, les sites restaurés contiennent en moyenne 27 espèces végétales différentes (contre 26 dans les sites de références), avec au minimum 18 espèces, et pouvant aller jusqu'à 40 espèces végétales. Ces résultats ne nous permettent pas de dire si ce sont les mêmes espèces qui sont représentées dans les sites restaurés et sites de référence. En effet, les sites de références étant composés de communautés végétales matures, il est impossible d'obtenir une communauté semblable sur des sites récemment perturbés qui favorisent l'installation d'espèces rudérales, non-représentatives de communauté végétale mature. Cependant, ces résultats indiquent une richesse en espèces non négligeable sur les sites restaurés, certainement favorisée par le semi qui a permis l'installation d'espèces non-pionnières et limité l'installation par les espèces adventives.

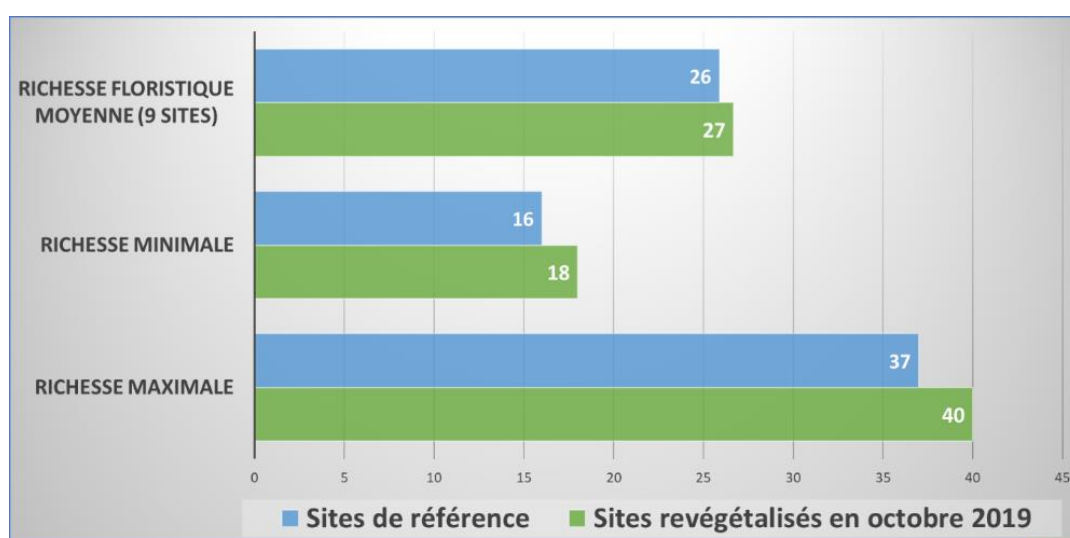


Figure 8. Richesses floristiques moyenne, minimal et maximale, en juillet 2019 sur les **sites de référence** (sites non-dégradés) et sur les **sites revégétalisés**.

CONCLUSION

Les mesures compensatoires mises en place afin de restaurer les prairies de Haute-Durance montrent, dès la première année de suivi, des résultats favorables à une reprise de la végétation par l'apport de graines via le foin ou graines récoltés localement. Les sites dégradés qui ont été restaurés en octobre 2019 présentaient un couvert végétal supérieur à 30% en juillet 2020, dont la présence d'espèces prairiales d'intérêt telles que des poacées ou des fabacées autochtones d'origine local. Même si l'analyse de ces deux groupes ne permet qu'une analyse approximative de la réussite des mesures de restauration, ce résultat est encourageant et représente une solution alternative au semis des mélanges commerciaux de fabacées et graminées qui sont souvent peu diversifiés et mal adaptés aux conditions locales. Le nombre d'espèces recensées dans les sites restaurés variait entre 18 et 40 espèces, représentant une richesse floristique moyenne équivalente à celle rencontrée sur les sites de référence. Bien que le suivis à n+1 présentent des résultats positifs, évaluer de façon certaine les effets écologiques des opérations de restauration par ensemencement nécessite un suivi à plus long terme (Klimbowska *et al.* 2007 ; Sengl *et al.* 2017 ; Bischoff *et al.* 2018 ; Albert *et al.* 2019 ; Shaw *et al.* 2020).

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Relation entre le couvert végétal et l'érosion du sol

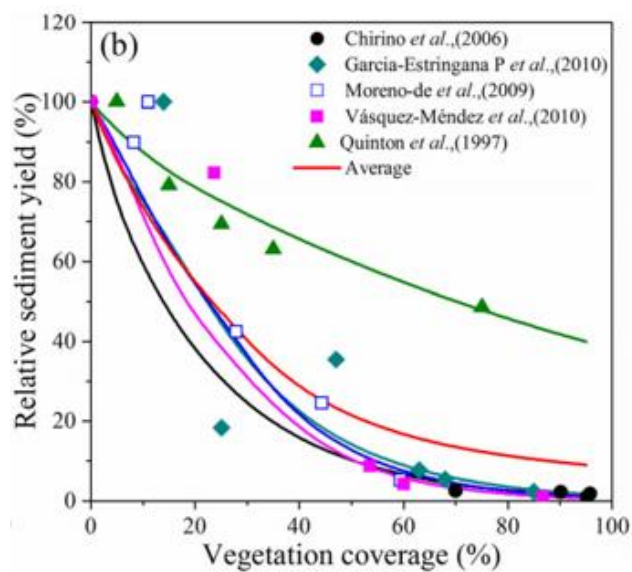


Figure 9. Relation entre le pourcentage de recouvrement végétal et l'érosion relative du sol (comparé à la perte de sol sur un sol nu), d'après Gu *et al.* (2020).

Tableau récapitulatif des pistes restaurées

Projet	Pylône	Commune	Etat	Période	Longueur (m)	Surface (m ²)	
P3	42	aero	Villar-Saint-Pancrace	Réensemencé	nov-20	100	540
P3	souterrain	Saint-Martin-de-Queyrières	Réensemencé	nov-20		600	
P3	souterrain	Saint-Martin-de-Queyrières	Réensemencé	nov-20		1020	
P3	souterrain	Saint-Martin-de-Queyrières	Réensemencé	nov-20		1020	
P4	3	L'Argentière	Thèse		31		
P4	4	L'Argentière	Réensemencé	nov-19	188	150	
P4	5	L'Argentière	Réensemencé	nov-19	17	235	
P4	21	Champcella	Revégétalisé naturellement	nov-19	52	0	
P4	30	Champcella	Revégétalisé naturellement	nov-20		0	
P4	31	Champcella	Réensemencé	nov-20		1600	
P4	41	Saint-Crepin	Réensemencé	nov-19	16	80	
P4	45	Réotier	Réensemencé	nov-19	150	1300	
P4	47	Réotier	Réensemencé	nov-19	86	1204	
P4	47	Réotier	Réensemencé	nov-20	32	360	
P4	52	Réotier	Réensemencé	nov-20		792	
P4	61	Châteauroux-les-Alpes	Réensemencé	avr-20	91	2000	
P4	74	Châteauroux-les-Alpes	Réensemencé	nov-19	95	575	
P4	75	Châteauroux-les-Alpes	Réensemencé	nov-19	117	685	
P4	76	Châteauroux-les-Alpes	Réensemencé	nov-19	154	2240	
P4	77	Châteauroux-les-Alpes	Réensemencé	nov-19	32	310	
P4	78	Châteauroux-les-Alpes	Réensemencé	nov-19	103	768	
P4	79	Châteauroux-les-Alpes	Réensemencé	nov-19	170	1020	
P4	90	Embrun	Revégétalisé naturellement	nov-19	11	0	
P4	163	Chorges	Culture	nov-19	180	0	
P4	166	Chorges	Revégétalisé naturellement	nov-19	122	0	
P4	100	Embrun	Revégétalisé naturellement	nov-20	12	0	
P4	107	Puy-Sanières	Revégétalisé naturellement	nov-20	36	0	
P4	11	La Roche-de-Rame	Réensemencé	nov-20	143	1430	
P4	116	Puy-Saint-Eusèbe	Réensemencé	nov-20	150	345	
P4	119	Savines-le-Lac	Réensemencé	nov-20	48	315	
P4	12	La Roche-de-Rame	Réensemencé	nov-20	53	456	
P4	120	Savines-le-Lac	Réensemencé	nov-20	77	462	
P4	122	Savines-le-Lac	Réensemencé	nov-20	46	480	
P4	123	Savines-le-Lac	Réensemencé	nov-20	233	1165	
P4	124	Savines-le-Lac	Réensemencé	nov-20	92	460	
P4	125	Savines-le-Lac	Réensemencé	nov-20	40	200	
P4	126	Savines-le-Lac	Revégétalisé naturellement	nov-20	32	0	
P4	139	Prunières	Réensemencé	nov-20	282	760	
P4	140	Prunières	Réensemencé	nov-20	36	300	
P4	141	Chorges	Revégétalisé naturellement	nov-20	58	0	
P4	142	Chorges	Réensemencé	nov-20	248	1275	
P4	143	Chorges	A faire en 2021	nov-20	21	0	
P4	144	Chorges	Réensemencé	nov-20	150	1375	
P4	145	Chorges	Réensemencé	nov-20	152	1272	
P4	146	Chorges	Réensemencé	nov-20	43	768	
P4	147	Chorges	Réensemencé	nov-20	196	1240	
P4	148	Chorges	Réensemencé	nov-20	206	1892	
P4	149	Chorges	Revégétalisé naturellement	nov-20	41	0	
P4	150	Chorges	Réensemencé	nov-20	215	1290	
P4	151	Chorges	Réensemencé	nov-20	116	150	
P4	152	Chorges	Réensemencé	nov-20	66	660	
P4	153	Chorges	Réensemencé	nov-20	49	490	
P4	154	Chorges	Réensemencé	nov-20	366	3660	
P4	155	Chorges	Revégétalisé naturellement	nov-20	147	0	
P4	156	Chorges	Réensemencé	nov-20	53	636	
P4	157	Chorges	Réensemencé	nov-20	123	200	
P4	158	Chorges	Réensemencé	nov-20	247	50	
P4	159	Chorges	Réensemencé	nov-20	205	1425	
P4	160	Chorges	A faire en 2021	nov-20	88	0	
P4	170	Espinasses	Réensemencé	nov-20	35	190	
P4	171	Espinasses	A faire en 2021	nov-20	134	0	

Projet	Pylône	Commune	Etat	Période	Longueur (m)	Surface (m²)
P4	175	Rousset	A faire en 2021	nov-20	46	0
P4	176	Rousset	A faire en 2021	nov-20	217	0
P4	177	Rousset	A faire en 2021	nov-20	89	0
P4	178	Rousset	A faire en 2021	nov-20	87	0
P4	179	Rousset	Revégétalisé naturellement	nov-20	58	0
P4	181	Rousset	Revégétalisé naturellement	nov-20	22	0
P4	24	La Bâtie-Neuve	A faire en 2021	nov-20	20	
P4	27	La Bâtie-Neuve	Réensemencé	nov-20	71	710
P4	28	La Bâtie-Neuve	Réensemencé	nov-20	10	100
P4	29	Champcella	Réensemencé	nov-20	102	1020
P4	32	La Bâtie-Neuve	Revégétalisé naturellement	nov-20	73	0
P4	33	Saint-Crepin	Réensemencé	nov-20	145	1050
P4	34	Saint-Crepin	Réensemencé	nov-20	39	1000
P4	35	Saint-Crepin	Réensemencé	nov-20	86	360
P4	43-44	Réotier	Réensemencé	nov-19	296	2568
P4	46	Réotier	Réensemencé	nov-20	135	384
P4	48	Réotier	Réensemencé	nov-20	96	1678
P4	49	Réotier	Réensemencé	nov-20	30	840
P4	51	Réotier	Réensemencé	nov-20	96	350
P4	53	Réotier	Réensemencé	nov-20	47	212
P4	54	Réotier	Revégétalisé naturellement	nov-20	48	0
P4	59	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20	313	0
P4	60	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20	184	0
P4	61	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20	67	0
P4	64	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20	24	0
P4	68	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20	80	0
P4	72	Châteauroux-les-Alpes	A faire en 2021	nov-20	28	0
P4	80	Châteauroux-les-Alpes	Réensemencé	nov-20	94	330
P4	81	Châteauroux-les-Alpes	Réensemencé	nov-20	10	120
P4	82	Châteauroux-les-Alpes	Réensemencé	nov-20	103	410
P4	84	Embrun	Réensemencé	nov-20	134	605
P4	92	Embrun	Réensemencé	nov-20	35	1620
P4	93	Embrun	Réensemencé	nov-20	42	1245
P4	94	Embrun	A faire en 2021	nov-20	33	
P4	95	Embrun	A faire en 2021	nov-20	17	
P5	souterrain	Risoul	Réensemencé	nov-20		1120
P6	13	La Bâtie-Neuve	Réensemencé	nov-19	73	665
P6	21	La Bâtie-Neuve	Thèse	nov-19	61	0
P6	22	La Bâtie-Neuve	Réensemencé	nov-19	22	210
P6	36	Chorges	Réensemencé	nov-19	126	630
P6	37	Chorges	Réensemencé	nov-19	112	660
P6	46	Chorges	Réensemencé	nov-19	16	850
P6	51	Chorges	Réensemencé	nov-19	39	156
P6	52	Chorges	Réensemencé	nov-19	109	436
P6	53	Chorges	Réensemencé	nov-19	200	800
P6	54	Chorges	Réensemencé	nov-19	92	368
P6	83	Puy-Saint-Eusèbe	Réensemencé	nov-19	93	465
P6	108	Embrun	Réensemencé	nov-19		300
P6	110	Embrun	Thèse	nov-19	69	0
P6	113	Embrun	Culture	nov-19	158	0
P6	114	Embrun	Culture	nov-19	33	0
P6	115	Embrun	Revégétalisé naturellement	nov-19	129	0
P6	14-15-16	Chorges	Réensemencé	nov-19	570	3550
P6	2	La Rochette	Revégétalisé naturellement	nov-20		0
P6	23	La Bâtie-Neuve	Réensemencé	nov-20	44	264
P6	24	La Bâtie-Neuve	A faire en 2021	nov-20		
P6	25	La Bâtie-Neuve	Réensemencé	nov-20	108	540
P6	26	La Bâtie-Neuve	A faire en 2021	nov-20		
P6	29	La Bâtie-Neuve	Revégétalisé naturellement	nov-20		0
P6	3	La Rochette	Culture	nov-20		0
P6	30	La Bâtie-Neuve	Réensemencé	nov-20	217	100
P6	31	La Bâtie-Neuve	Revégétalisé naturellement	nov-20		0
P6	32	La Bâtie-Neuve	Revégétalisé naturellement	nov-20		0
P6	33	La Bâtie-Neuve	Réensemencé	nov-20	169	876
P6	38-39	Chorges	Réensemencé	nov-19	346	2276
P6	4	La Rochette	Réensemencé	nov-20	100	700

Projet	Pylône	Commune	Etat	Période	Longueur (m)	Surface (m ²)
P6	43-44-45	Chorges	Réensemencé	nov-19	785	3925
P6	5	Réotier	Réensemencé	nov-20	36	144
P6	73	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20		0
P6	74	Châteauroux-les-Alpes	Réensemencé	nov-20	238	1190
P6	75	Châteauroux-les-Alpes	Réensemencé	nov-20	89	445
P6	76	Châteauroux-les-Alpes	Réensemencé	nov-20	50	300
P6	77	Châteauroux-les-Alpes	Réensemencé	nov-20	63	465
P6	8	Châteauroux-les-Alpes	Réensemencé	nov-20	34	468
P6	80	Châteauroux-les-Alpes	Revégétalisé naturellement	nov-20		0

Abstract

Large-scale ecological degradation of grasslands is increasing worldwide mainly due to land use changes and habitat destruction. The construction of high-voltage transmission lines in my study area, the Haute-Durance valley in the Southern French Alps, involves a degradation of grassland soil and plant communities along transitory access tracks and construction platforms. In these degraded ecosystems of the montane altitudinal belt, restoration is limited by a lack of available propagules and low seedling recruitment on stony and shallow soils characterised by summer drought. I focus on the restoration of species-rich dry to mesophilic grasslands covering a large part of the construction zone between 1000 and 1400 m. Most of these grasslands are Natura 2000 priority habitats, such as N6210 "Semi-natural dry grasslands of *Festuco-Brometalia* and scrubland facies on calcareous substrates" (EU directive habitat 92/43/EEC).

My thesis aims to better understand the factors limiting the restoration of mountain grasslands after soil disturbance, and to identify successful restoration techniques to compensate the degradation. Chapter 1 is focused on the identification of appropriate reference communities, which is a crucial step in ecological restoration. In order to understand the mechanisms favouring target species establishment, five degraded semi-natural grasslands were used to set up two different experiments, using a filter-based community assembly approach of community dynamics to analyse the restoration outcome. In the first one (Chapter 2), I examined whether soil preparation is required to improve soil conditions and to reduce competition of pre-existing vegetation, and whether traditional extensive grazing should be excluded in initial stages of grassland restoration. In the second experiment (Chapter 3), I focused on different techniques of propagule transfer increasingly used in mountain grasslands (brush-harvested propagules and hay transfers), and I analysed the potentially facilitative effect of dead (hay) and living (wheat) plant cover on the recruitment of transferred brush-harvested propagules, and on soil erosion. Finally, as community assembly is also highly influenced by dispersal that may lead to priority effects, I tested sequential sowing of different combinations of plant species (dominants vs. subordinates) (Chapter 4) and I analysed how they affect community assembly.

The results demonstrated that the analysis of regional plant-environment interactions provides a useful tool to identify reference communities or donor sites for seed transfer, particularly if donor sites are not available adjacent to degraded habitats. The restoration techniques applied indicated that the reduction of competition by soil preparation before seed addition of brush material had a positive effect on the transferred species recruitment and can thus clearly be recommended in my study system. Furthermore, I found that the weak negative effect of grazing may not always justify fencing costs.

Hay mulch favoured seedling recruitment of target species, but propagule transfer without mulch layer needed to be compensated by additional temporary plant cover, suggesting that restoration of mountain grasslands with shallow and stony soils clearly benefits from a facilitative effect of dead (hay) or living (wheat) vegetation cover. The chapter 4 provided evidence that plant community assembly is influenced by the order of arrival but highlighted strong response variations between species suggesting high species-specific priority effects. The strength of priority effects probably varies according to the niche characteristics of different species and may influence (negatively or positively) the late-arriving species establishment.

Keywords: ecological restoration, restoration ecology, mountain grassland, soil disturbance, plant community assembly, plant succession, priority effects

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