



UNIVERSITÀ
DEGLI STUDI
DI PADOVA

UNIVERSITY OF PADOVA

Department of Agronomy, Food, Natural Resources, Animals and Environment
(DAFNAE)

DOCTORAL SCHOOL OF CROP SCIENCES

CYCLE XXXII

***Seeds from species-rich grasslands:
Production patterns and use for ecological restoration***

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Date of thesis submission

May 3rd 2020

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Legnaro, May 3rd 2020
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Summary

Biodiversity loss has become an impelling global concern and the restoration of degraded or damaged habitats, including agricultural semi-natural vegetation, is a vital aspect for its conservation.

The species-rich grasslands are defined as one of the most important habitats with a high level of biodiversity connected with rural areas and their conservation is a goal of the “European Union biodiversity strategy to 2020” (EU, 2011).

A big limitation of their recover is the lack of available native species seeds in the conventional market and locally harvesting of native ecotypes of grassland species is often necessary. This approach is in line with the conservation principles of the ecological restoration.

The mixture of seed which can be collected from semi-natural meadows can have different characteristics in relation to the type of grassland management. The knowledge on the availability and characteristics of the seed of the main meadow species can facilitate the seed use for different agricultural and urban environments.

A study of the fertilisation effects on the seed production was therefore performed with particular attention to the seed production of forbs, which are an important biodiversity component, often threatened by management intensification. Compared to grasses forbs increased less the viable seed production at higher level of fertilisation. The higher increase of seed production for light-seeded grasses was an important reason for higher grass competitiveness in more fertilised grasslands. Grasses showed also higher dormancy which is an important feature affecting their annual presence in the grassland and their behavior in restoration.

To study the success of grassland restoration with seed of native species in real situations, multi-years monitoring were carried out at two high-extent alpine ecological restoration. The study of the development of vegetation during the following years has shown that the use of the green grass technique allows to transfer a large number of species, even if the percentage of transfer efficiency tends to decrease with the increase of the number of species in donor meadows. The restored grasslands showed a botanical composition comparable to the standard vegetation of the meadow in the same geographical region, which is not necessarily the same as the donor's grassland.

Riassunto

La perdita di biodiversità è diventata una impellente preoccupazione globale e il ripristino di habitat degradati o danneggiati, incluse le vegetazioni semi-naturali di origine agricola, è un aspetto vitale per la sua conservazione.

Le praterie ricche di specie sono definite come uno degli habitat più importanti con un alto livello di biodiversità e la loro conservazione è un obiettivo della "Strategia dell'Unione europea per la biodiversità fino al 2020" (UE, 2011).

Un importante limite al loro recupero è la mancanza di materiale di propagazione di specie autoctone disponibili nel mercato convenzionale e per rendere possibile l'utilizzo di ecotipi locali è spesso necessaria la raccolta del seme in situ. Questo approccio è in linea con i principi di conservazione del restauro ecologico.

La miscela di semi che può essere raccolta dai prati semi-naturali può avere caratteristiche diverse in relazione al tipo di gestione e la conoscenza della reale disponibilità del seme delle principali specie di prati può facilitarne l'uso in diversi ambienti agricoli e urbane.

Uno studio degli effetti della fertilizzazione sulla produzione di sementi venne pertanto realizzato con particolare attenzione alle specie non graminacee, che sono una componente importante della biodiversità, spesso minacciata dall'intensificazione della gestione. Queste specie sono risultate meno competitive rispetto alle graminacee per quanto riguarda la produzione di seme vitale ad alto livello di fertilizzazione. Il maggiore incremento della produzione di seme delle graminacee a seme leggero si è evidenziato come un importante ragione della maggiore competitività di queste specie nelle cotiche più fertilizzate. Inoltre, le graminacee studiate hanno mostrato maggiori livelli di dormienza del seme. Questo aspetto costituisce un importante elemento a favore di queste specie che influenza la loro presenza nella prateria e il loro comportamento nei restauri.

Per studiare l'esito di restauri di praterie con seme di specie native in situazioni reali, per più anni vennero condotti monitoraggi su due restauri ecologici eseguiti su ampie superfici in ambiente alpino. Lo studio dello sviluppo della vegetazione nel corso degli anni successivi al restauro ha mostrato che l'utilizzo della tecnica dell'erba verde permette di trasferire un numero elevato di specie, anche se l'efficienza percentuale di trasferimento tende a diminuire con l'aumento del numero di specie nei prati donatori. Le praterie restaurate hanno mostrato una composizione botanica comparabili a quella delle vegetazioni standard del prato della regione geografica, che non necessariamente risulta la stessa della prateria del donatore.

Chapter 1

Introduction

The species-rich grasslands are the result of a long and stable use of the plain and mountain areas such as meadows or pastures. These plant communities are made of species with long-lasting adaptations to the local environmental features (Keller et al., 1999 and Vander et al., 2010) which are originated from the interactions of historical, environmental and geographical factors.

During the past decades they have been in constant decline in many parts of Europe. In plain areas the causes are mainly the conversion into arable land and the intensification of management (Poschlod & Wallis De Vries, 2002). Instead, in the Alpine space the areas covered with herbaceous vegetation are increasingly affected by processes of natural succession and forest recolonization due to the abandonment or reduction of the activity of mowing and grazing. This alteration occurs also in the areas beyond the edge of the forest, where the dwarf tree stands are by the time widespread to the detriment to the natural grasslands (Chemini & Rizzoli, 2003).

The conservation and recovery of the species-rich grassland is a currently topic present into the territorial programs, not only Italian, but also at the European level. Europe have done important steps towards the biodiversity protection. The biggest network of protected areas in the world was created through the Nature 2000 program (Birds and Habitats Directives, 79/409/CEE and 92/43/CEE), with about 25 000 areas spread across the member states (17% of the European Union surface) (European Environmental Agency Data).

In the current planning and in particular the “European Union biodiversity strategy to 2020” the maximization of the species-rich grasslands areas is included into the strategy point three. The goal is to conserve species and habitats that depend on or are affected by agriculture and the to guarantee the provision of the connected ecosystem services (EU, 2011).

At the national level, the Rural Development Program shows in one of the five priorities in the 2014-2020 programming the "preserve, restore and enhance the ecosystems connected to agriculture". This has resulted in the adoption of some measures, such as the agro-climatic environmental and investment measures to improve the performance and global sustainability of the farm. In detail, the measure 10.1.4 (Sustainable management of meadows, semi-natural meadows, pastures and meadows-pastures) and 4.4.1 (Naturalistic-environmental recovery and landscape redevelopment of open mountain and hilly areas abandoned and degraded), while for Trentino the measure 10.1.1 (Management of grassland areas: improvement of biodiversity linked to permanent meadows).

In Trentino Province an important financial measure is the “Landscape’s fund”, adopted in the 2015. Through this the landscape areas, such as the rural mountain areas, when they are marked by abandoned agricultural and zootechnical practices and present the risk of loss of cultural values, landscape and biodiversity, can be preserved, arranged or restored.

Many studies have identified lack of viable seeds in the soil seed bank and the limited dispersion of the native species due do to fragmentation and isolation of the habitats as the main obstacles to the restoration of species-rich grasslands (Bakker, Poschlod, Strykstra, Bekker & Thompson, 1996; Bossuyt & Honnay, 2008; Bullock et al., 2002). Therefore, the

active introduction seed is essential when the target species are absent in the restoration sites or nearby sites (Kiehl et al., 2010).

In Italy as in many European countries, there is no market for herbaceous species that can respond to the request of native species seed (van Hulst et al. 1987) and this force the use the commercial mixture of seed for the recovery of the grassland (Poschlod et al., 1998; Bullock et al., 2002).

This contains homogeneous and optimized seed for the agriculture and gardening (Kirmer & Tischew, 2006) and often is made of foreign ecotypes, subspecies and even foreign species (Frank & John, 2007; Marzini, 2004). The hybridization between local and non-native genotypes can dilute native gene pools and reduce the suitability of subsequent hybrid populations (Keller & Kollmann, 2000; McKay, Christian, Harrison & Rice, 2005). The Ecological restoration, through several techniques, can provide to this lack bypassing the commercial chain of seed production and sales.

The objective of the restoration is the return to a habitat with particular conditions such as species composition, community structure and ecosystem functions (Noss, 1990). Its conservation principles require that the species are collected and used within the same region and that therefore local ecotypes are used (Sackville & Hamilton, 2001; Walker et al., 2004).

To evaluate the efficiency of the seed harvesting techniques the study of the biology of the species that form the communities and the potential seed production of the grasslands is necessary.

The general objective of this thesis is to widen the knowledge on the real availability of the seed of the main species of the meadows in relation to the type of management, facilitate the use of this in different urban and extra-urban environments and understand the development of vegetation through the monitoring of ecological restorations projects.

The Thesis is divided into three parts:

Chapter 2 Estimation of quantity and quality of the seed produced by seminatural grasslands in extensive and intensive management conditions and study of the influence of the fertilization on these factors. A total of thirty-two species were collected for six years and analysed for several reproductive features.

Chapter 3 Study of forb seed ecology, in order to facilitate forb conservation and seed-based restoration in grassland ecosystems. Six forb species that are important components of European grasslands were collected from different sites in the Italian Pre-Alps and analysed for the main traits of seed production.

Chapter 4 Monitoring of ecological restoration projects of species-rich grassland in the Alps to analyse the results of the green grass technique. Twenty-six restored sites were monitored for two-four years to study the vegetation development along the years and in comparison with the donor sites.

References

- Bakker, J.P., Poschlod, P., Strykstra, R.J., Bekker, R.M., Thompson, K. 1996. Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* 45, 461–490.
- Bossuyt, B. & Honnay, O. 2008. Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. *Journal of Vegetation Science*, 19(6), 875-884.
- Bullock, J. M., Moy, I. L., Pywell, R. F., Coulson, S. J., Nolan, A. M., & Caswell, H. 2002. Plant dispersal and colonization processes at local and landscape scales. In J. M. Bullock, R. E. Kenward & R. S. Hails (Eds.), *Dispersal ecology* (pp. 297–302). Oxford: Blackwell.
- Chemini, C. & Rizzoli, A. 2003. Land use change and biodiversity conservation in the Alps. *Journal of Mountain Ecology*, 7 (Supplement): 1-7.
- EU. 2011. Communication from the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions Our life insurance, our natural capital: an EU biodiversity strategy to 2020. COM 2011/0244.
- Frank, D., & John, H. 2007. Bunte Blumenwiesen – Erhöhung der Biodiversität oder Verstoß gegen Naturschutzrecht? *Mitteilungen zur floristischen Kartierung in Sachsen-Anhalt*, 12, 31–45.
- Keller, M., & Kollmann, J. 1999. Effects of seed provenance on germination of herbs for agricultural compensation sites. *Agriculture, ecosystems & environment*, 72(1), 87-99.
- Keller, M., Kollmann, J., & Edwards, P. J. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology*, 37, 647 – 659.
- Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L. & Holzel, N. 2010. Species introduction in restoration projects – evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology* 11: 285–299.
- Kirmer, A., & Tischew, S. (Eds.) 2006. *Handbuch naturnahe Begrünung von Rohböden*. Wiesbaden: Teubner Verlag.
- Marzini, K. 2004. Naturschutzgesetz contra Saatgutverkehrsgesetz. *Rasen-Turf-Gazon*, 4, 63–67.
- McKay, J. K., Christian, C. E., Harrison, S., & Rice, K. J. 2005. “How local is local?” A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology*, 13, 432–440.
- Noss RF. 1990. Indicators for monitoring biodiversity: A hierarchical approach. *Conservation Biology* 4: 355–364.
- Poschlod, P., WallisDe Vries, M.F. 2002. The historical and socioeconomical perspective of calcareous grasslands - lessons from the distant and the recent past. *Biol. Conserv.* 104, 361–376.

- Sackville Hamilton, N.R. 2001. Is local provenance important in habitat creation? A reply. *Journal of Applied Ecology* 38, 1374– 1376.
- Van Hulst R, Shipley B, Theriault A. 1987. Why is *Rhynanthus minor* (*Scrophulariaceae*) such a good invader? *Can J Bot* 65: 2373 – 2379.
- Vander Mijnsbrugge, K., Bischoff, A., & Smith, B. 2010. A question of origin: where and how to collect seed for ecological restoration. *Basic and Applied Ecology*, 11(4), 300-311.
- Walker KJ, Stevens PA, Stevens DP, Mountford JO, Manchester SJ, Pywell RF. 2004. The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* 119:1–18.

Chapter 2

Effects of fertilisation on grass and forb gamic reproduction in semi-natural grasslands

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Abstract

Studying the fertilisation effects on the seed production of grassland species can help understand the vegetation changes due to different soil nutrient levels and, for practical uses, the characteristics of the seed mixture which can be collected from differently fertilised meadows. Such a study was however never faced at the whole community level. On a temperate hay-meadow cut twice per year and located in the Italian pre-Alps, the seed production of thirty-two species (fifteen grasses and seventeen forbs) was studied in three fertilisation treatments: 0-0-0, 0-54-108 and 192-108-216 kg N, P₂O₅ and K₂O respectively per year. Samples of fertile shoots were collected at the seed maturation stage during the first and second growth periods of years 2012-2017 and analysed for all main traits of their gametic reproduction, including size of the reproduction system, ovule site utilization, viability, germinability, 1000 seeds weight and fertile shoot density. On average, forbs produced more ovules and viable seeds per shoot (199 and 65 respectively) than grasses (112 and 35 respectively). Among size traits, fertilisation increased especially the number of inflorescences per shoot in both grasses and forbs. Fertilisation affected germinability and viability less than the size traits and the effect was different in the two species groups. Viability increased in grasses but often decreased in forbs. This pattern resulted in a +55% and +11% viable seed production in grasses and forbs respectively. At the higher fertilisation level shoot density in the vegetation was positively related to the number of viable seeds per shoot (i.e. low seed size) in grasses and to the seed size in forbs. Higher grass seed dormancy allowing for a later germination in a fertilised meadow where the vegetation cover is low in autumn, probably contributes to the spread of light-seeded grasses in fertilised meadows. The effects of the soil nutrient enrichment on the reproductive traits of grasslands species give probably an important contribution to the ongoing reduction of the forb biodiversity of these ecosystems.

Keywords: fertile shoot density, flower, forbs, gametic reproduction, grasses, seed, seed set, semi-natural grasslands, viability.

Introduction

Extensively managed semi-natural grasslands, with their high plant and animal richness, play a central role in the biodiversity conservation, so that the strong decrease of their extent in the last decades is of great concern for European environmental policy (EEA, 2004).

One issue important for their conservation and restoration is investigating the seed production of the many species which compose them. In-depth studying the gametic reproduction can help understand why grassland species react differently to the cultivation practices and the grassland vegetation evolves following management changes (Schütz, 2000). Further, the current increase of nutrient concentration in terrestrial soils due to impacting human activities (Galloway et al., 2004) makes this topic important to better understand the possible effect of soil eutrophication on grassland functioning and biodiversity. This is particularly important as regards the persistence of forbs which reproduce mainly by seed whereas most grasses can

rely on both agamic and gametic reproduction and propagate through tillering (Klotz et al., 2002). Knowing the patterns of the grassland species seed production is also crucial for an efficient seed harvesting aimed at restoring species-rich grasslands (Scotton et al., 2009).

At the fertile shoot level, the output of the gametic reproduction is the amount of viable seeds produced during its life. This amount is the result of a process where several reproductive traits are involved (Pearson & Ison, 1997): numbers of inflorescences per shoot, flowers per inflorescence and ovules per flower determining the number of ovules per shoot (i.e. the seed yield potential); the proportions of ovules transformed to filled seed (seed set) and filled seeds which are viable (utilisation of the yield potential).

All mentioned traits are primarily under genetic control but can also be influenced by environmental factors (Hampton & Fairey, 1997). The influence of soil fertility has received particular attention due to its importance for seed propagation and, more recently, its current increase in terrestrial soils as a consequence of human activities (Galloway et al., 2004). Past studies highlighted how the soil fertility can differently affect the individual traits of the seed yield. For example, in grasses high nitrogen (N) supply can enlarge the inflorescence size by increasing the number of flowers per spikelet or the number of spikelets per shoot (Hampton & Fairey, 1997). The high phosphorous (P) and potassium (K) availability was found to enhance flower formation indirectly through increasing the cytokinin plant concentration (Engels et al., 2012). Stephenson (1981) reported seed set being generally increased by higher availability of macronutrients which are necessary for fertilised ovules to develop to seeds. However Wiens (1984) found seed set being more dependent on genetic factors. N and NP fertilization were also reported to indirectly influence seed viability in outcrossing species as it decreased the nectar attractivity for pollinators (Nye et al., 1973), therefore increasing self-pollination and the consequent higher number of unviable seeds (Arista and Talavera, 1996). Fenner (1986) reported that seed size and mineral concentration are usually not affected by soil fertility, so that even in unfertile soils, the seedling recruitment is not negatively affected. Much information on the gametic reproduction of temperate grassland species is available from the field of the seed propagation, where fertilisation is the main agronomic practice applied to increase the seed yield (Rolston et al., 1997). The fertilisation rate is usually not very high, to avoid excess of vegetative growth competing with reproductive development (Hebblethwaite & Ivins, 1977) and, depending on the propagated species and soil fertility, only one, two or all three macronutrients are added. In semi-natural grasslands, too, fertilisation is the management factor with the strongest influence on the grassland traits (Klapp, 1971). Contrasting fertilisation levels have very different effects on the seed yield which can vary greatly by species, amount and time distribution and also affect the strategy of gametic reproduction (Scotton et al., 2009). However, these effects can be significantly different from those found in the forage seed propagation, because:

- + grasslands are composed of many species with contrasting phenologies and different reaction to the same fertilisation;
- + the different aim of the grassland cultivation (forage vs. seed yield) entails different management practices (e.g. earlier harvesting to obtain higher-quality forage);
- + in grasslands, fertilisation is usually organic, therefore applying all macronutrients together, often at very high rate.

Despite the importance of the knowledge of the gamic reproduction in grasslands, no comprehensive studies were performed in the past at the community level addressing the fertilisation effect on all main species composing these plant ecosystems in temperate climates. A study was therefore conducted on the gamic reproduction of an important type of temperate grassland with three main specific aims in mind:

- + analysing the fertilisation effect on the reproductive traits of the main grassland species and the two functional groups grasses and forbs;
- + identifying group of species with similar gamic reproduction behaviour and response to fertilisation;
- + identifying multispecies correlations among reproductive traits and characterising the fertilisation effect on their pattern.

Materials and methods

Site, meadow and fertilisation

The studied grassland is located in Sedico (BL: 420 m a.s.l., Italian Eastern Pre-alps) where the mean yearly temperature is 10.6°C and rainfall 1366 mm per year (389, 326, 401 and 250 mm in spring, summer, autumn and winter respectively). The site is level and has alluvial, calcareous substratum. The soil has 12.2% gravel content, sandy loam texture, 14.6% total carbonates content and a 7.5 pH. Since 1977, a meadow sector was used for a fertilisation trial organised as a three completely randomized blocks with 24 m² plots and twenty-seven treatments obtained from combining three levels of yearly N, P and K application per ha: 0, 96 and 192 kg N as ammonium nitrate; 0, 54 e 108 kg P₂O₅ as triple superphosphate; and 0, 108 e 216 kg K₂O as K sulphate. Since 2010 the trial was cut twice per year and surveyed for seed production in three fertilisation treatments: no fertilisation (000); fertilization with no N and intermediate P and K rate (011); fertilisation with the highest rate of all nutrients (222). The vegetation of the three treatments (Annex 1) corresponded to the following meadow types:

- + in 000, a vegetation intermediate between a poor-soil form of the *Arrhenatherum elatius* meadow (Ar0) and a *Bromus erectus* meadow (Br) (phytosociological alliances *Arrhenatherion elatioris* and *Mesobromion*, respectively) with high species richness and low legume abundance;
- + in 011 an Ar0 meadow with high species richness and legume abundance;
- + in 222, a grass-rich form of the *Arrhenatherum elatius* meadow with low species richness and legume abundance.

Plant sampling and laboratory analysis

During the two growth periods of years 2012-2017, fertile shoots were sampled from each fertilization treatments at the plot and sub-plot levels. From each plot, 5-10 shoots of each flowering species were randomly collected at the stage of optimal seed maturation (most fruits/inflorescences still intact, i.e. no seed shedding). Due to the different species

phenologies, collection dates varied accordingly. From one 1-m² subplot per plot, all shoots of all present species were collected at the time of maximum standing seed yield placed at the end of each growth period and estimated with phenological surveys. In the six trial years, the dates of collection from the subplots varied in the ranges 30/6-12/7 (first mowing) and 29/9-15/10 (second mowing). Collected shoots were put into porous paper bags, dried and conserved in a refrigerator until laboratory analysis.

During autumn and winter after collection, the 15-30 shoots of each species collected at the plot level were analysed for the size of the reproductive system (number of compound and/or simple inflorescences and flowers per simple inflorescence or shoot). For species with high number of flowers or inflorescences, an inflorescence length or diameter which could be related to the flower number was also measured (e.g. the panicle length in grasses). In sample flowers, intact fruits or intact simple inflorescences, the number of ovules per flower and the number of ovules transformed to seed were observed under binocular. Mature seeds were weighted and tested for germinability and viability according to ISTA (2003). Germination trials were performed with three seed samples per species put on filter paper in petri-dishes and moved to a germinator for 4 weeks (8 hr light / 25°C and 16 hr darkness / 15°C) with weekly observation and extraction of germinated seeds. At the end of the germination test, ungerminated seeds were checked for viability with the tetrazolium test. Total viability was calculated as sum of germinability and viability of ungerminated seeds.

All shoots collected on the sub-plots were counted and measured for number of inflorescences and flowers. When inflorescences and flowers were too numerous to be counted rapidly (e.g. in all grasses), only the same length/diameter measured on the three 5-10 shoot samples collected at the plot level was recorded.

A more detailed description of the laboratory analyses is available in Scotton (2018).

Data analysis

The value of each reproductive trait of each species, was calculated for each year and growth period at the plot level. The values of the traits describing the size of the reproductive system were mainly obtained from the shoots collected on the sub-plots. However, for species with too many flowers per shoot, a relation was calculated between this last trait and the length/diameter of the inflorescences measured on the 15-30 shoot samples. The relation was then used to calculate the flower number of each shoot. The number of ovules per flower, portion of ovules transformed to seed (ovule site utilisation; OSU), 1000-seeds weight, germinability and viability were calculated from the results of the lab analyses of the 15-30 shoot samples.

As not all species collected were always present in the six study years, only the thirty-two species (fifteen grasses and seventeen forbs: Table 1) found in at least three years were considered to obtain enough reliable results.

The statistical analyses were performed at the levels of individual species and the two grassland functional groups of grasses and forbs. Nine main reproductive traits describing the whole process of the gamic reproduction were considered: number of simple inflorescences per shoot, flowers per simple inflorescence, ovules per flower, ovules and viable seeds per shoot, OSU, percent viability and germinability and seed weight. Percent dormancy

(difference between percent viability and germinability) and the shoot density recorded in the subplots were also considered in some analyses.

Only sixteen species being present in all fertilisation treatments was a problem in the tests including all species together, as a balanced among-treatments comparison was possible only by discarding the many data from species not present in all treatments. To overcome it, it was considered that in the same geographical region the symbiotic N-fixation the high legumes presence in the PK treatment is equivalent to a N fertilisation of about 3.5 kg per percent legume abundance (Scotton et al., 2002; Dietl & Lehmann, 2004). Therefore, treatment 011 (30% more legumes than in treatment 222: Annex 1) was regarded as a N addition of 105 kg per ha and year. The values of the reproductive traits were then calculated for two fertilisation levels, low (LowFert) and high (HighFert). For species present in only two fertilisation treatments (000-011 or 011-222), LowFert and HighFert were matched to the two treatments. For species present in three fertilisation treatments LowFert was 000 and HighFert was the average between 011 and 222. Statistical analysis considering only species present in all fertilisation treatments showed a similar pattern of the fertilisation effect to that found by considering the two fertilisation levels. Therefore, only the latter set of analysis was considered as it is representative of a larger species number.

GRASSES	Code	Fertilisation treatment (NPK level)			FORBS	Code	Fertilisation treatment (NPK level)		
		000	011	222			000	011	222
<i>Anthoxanthum odoratum</i>	AnOd	X	X	X	<i>Achillea roseo-alba</i>	AcRo	X	X	X
<i>Cynosurus cristatus</i>	CyCr	X	X	X	<i>Clinopodium vulgare</i>	CIVu	X	X	X
<i>Dactylis glomerata</i>	DaGl	X	X	X	<i>Trifolium pratense</i>	TrPr	X	X	X
<i>Festuca pratensis</i>	FePr	X	X	X	<i>Centaurea nigrescens</i>	CeNi	X	X	X
<i>Holcus lanatus</i>	HoLa	X	X	X	<i>Rhinanthus freynii</i>	RhFr	X	X	X
<i>Trisetum flavescens</i>	TrFl	X	X	X	<i>Salvia pratensis</i>	SaPr	X	X	X
<i>Briza media</i>	BrMe	X	.	.	<i>Silene vulgaris</i>	SiVu	X	X	X
<i>Brachypodium rupestre</i>	BrPi	X	X	.	<i>Cerastium fontanum</i>	CeFo	X	X	X
<i>Avenula pubescens</i>	AvPu	X	X	.	<i>Medicago lupulina</i>	MeLu	X	X	X
<i>Festuca rupicola</i>	FeRu	X	X	.	<i>Ranunculus acris</i>	RaAc	X	X	X
<i>Arrhenatherum elatius</i>	ArEl	.	X	X	<i>Plantago media</i>	PIMe	X	X	.
<i>Bromus hordeaceus</i>	BrHo	.	X	X	<i>Primula veris</i>	PrVe	X	X	.
<i>Carex contigua</i>	CaCo	.	X	X	<i>Stachys officinalis</i>	StOf	X	X	.
<i>Lolium perenne</i>	LoPe	.	X	X	<i>Knautia drymeia</i>	KnDr	X	X	.
<i>Poa trivialis</i>	PoTr	.	X	X	<i>Leontodon hispidus</i>	LeHi	X	X	.
-	-	-	-	-	<i>Leucanthemum vulgare</i>	LeVu	X	X	.
-	-	-	-	-	<i>Rumex acetosa</i>	RuAc	.	X	X

Table 1 Species studied for reproductive traits in a grassland fertilisation trial in the Italian eastern Alps.

Statistical analyses aimed at: 1. studying the fertilisation effect on the reproductive behaviour of individual species and the two species groups of grasses and forbs; 2. finding and characterising subgroups of grasses and forbs with similar behaviour and similarly affected by fertilisation and; 3. identifying multispecies correlations among reproductive traits and the possible fertilisation effect on their pattern.

For the first aim, the fertilisation effect was tested for the reproductive traits of each individual species by applying a linear mixed model (LMM) under a repeated measure approach. In the model, fertilisation treatment and block were input as fixed factors, year as random factor, and a plot identifier as repeated measure subject. In case of significant fertilisation effect, the among-treatments differences were tested using the Tukey multiple comparison adjustment. Prior to performing the mixed model, data were checked for homoscedasticity and normality and, if necessary, log-transformed.

From the individual species LMM's, a table was calculated containing for each reproductive trait and species group the frequency of cases with significant fertilisation effect (three levels; no, positive or negative effect). To check if grasses and forbs differed for the obtained frequencies, for each trait a chi-square test on the frequency table "fertilisation effect x species group" was performed.

In a following set of analysis, the effect of the grassland functional group (grasses and forbs) on the across-years means of each reproductive trait was tested with general linear models (GLM). Prior to the analysis, the data were log-transformed in case of homoscedasticity or normality problems. In these analyses, the species was considered as a replicate within the species group (therefore not included as class factor) and also the fertilisation level was input as class factor. The effect of the fertilisation level on each reproductive trait was tested separately for each species groups. In this case, the GLM included both fertilisation level and species as class factors.

For the second aim, subgroups of species with similar reproductive behaviour were searched for with cluster and principal component analysis (respectively CA and PCA) performed on the table "species x reproductive traits averaged across fertilisation treatments". This type of analysis was done separately for the two species groups. In CA, the standardised reproductive traits and species were clustered using the similarity ratio and the minimum variance method (Wildi & Orłóci, 1996). In PCA the standardised reproductive traits were log-transformed in order to reduce the weight of the traits with the highest values and the data were centred by species (Leps & Šmilauer, 2003).

CA and PCA were also carried out separately for grasses and forbs to find species groups with similar response to fertilisation. In this case, for each reproductive trait the data used were the percent value of HighFert compared to LowFert. The data were clustered and ordered with the same methods as above but without any prior transformation.

Possible determinants of the individual species response to fertilisation were investigated by relating the percent values of two important reproductive traits (the ovule number per shoot and the viable seed number per shoot) found in HighFert compared to LowFert (response variables, Y) to some possible explanatory variables (X). The explanatory variables were the average values of each reproductive trait, the Ellenberg bioindicator values (Pignatti, 2005) and the percent variation of shoot density. The relationships were fitted according to a linear regression approach, for grasses and forbs together or separately and checked for the parametric assumptions of residual normality and homoscedasticity.

For the individual species HighFert-minus-LowFert differences of OSU, seed germinability, seed viability and seed weight, one-way variance analyses were performed with three traits of the species reproductive biology (type of reproduction, breeding system and pollen vector

(Annex 1: retrieved from Klotz et al., 2002) used as categorical factor. A GLM approach was used in this case, too.

Multispecies correlations were analysed by in-pairs relating the reproductive trait values of individual species averaged across fertilisation treatments and years. A supplementary characteristic used in these analyses was the shoot density recorded in the 1-m² subplots. Nonlinear relationships were linearized using a log-transformation. As the purpose of the analysis was not to predict one trait from the other but to summarise in the best way the relationship, the standardised major axis (SMA) approach was used instead of the linear regression method (Warton et al., 2006). The analyses were performed for grasses and forbs together and separately. In order to verify if fertilisation could affect the characteristics of the found relationships, a second set of SMA analyses was performed by separating the two fertilisation levels. The possible difference among the two lines was checked by testing them for common slope and elevation according to Warton et al. (2006).

Even if taken into account in statistical analyses, the year effect is not discussed here as it will be reported in a next paper.

Software used were: SAS (1985) with procedures MIXED (LMM), GLM (GLM), REG (linear regressions) and UNIVARIATE (chi-square tests); CANOCO (PCA: Ter Braak & Smilauer, 2002); Mulva-5 (CA: Wildi & Orloci, 1996); R 3.0.0 (Core Team R 2013) with package SMATR (SMA analysis).

Results

Frequency, amount, and determinants of the fertilisation effect in grass and forb functional groups

The frequency of a significant fertilisation effect was highly variable depending on the reproductive trait (Fig. 1 calculated from Annex 2). For the size traits of the reproductive system a significant fertilisation effect was more frequent than for biological traits (OSU, seed size, viability and germinability) and generally positive. Instead, for biological traits, the effect was often both positive and negative.

Between grasses and forbs, individual traits which determine the viable seed production per shoot did not differ for the frequency of types of response to fertilisation (Fig. 1) but the resulting viable seed production per shoot increased more frequently in grasses, especially due to a more frequent increase of the number of ovules per shoot and to seed viability more often increasing vs. more often decreasing in forbs.

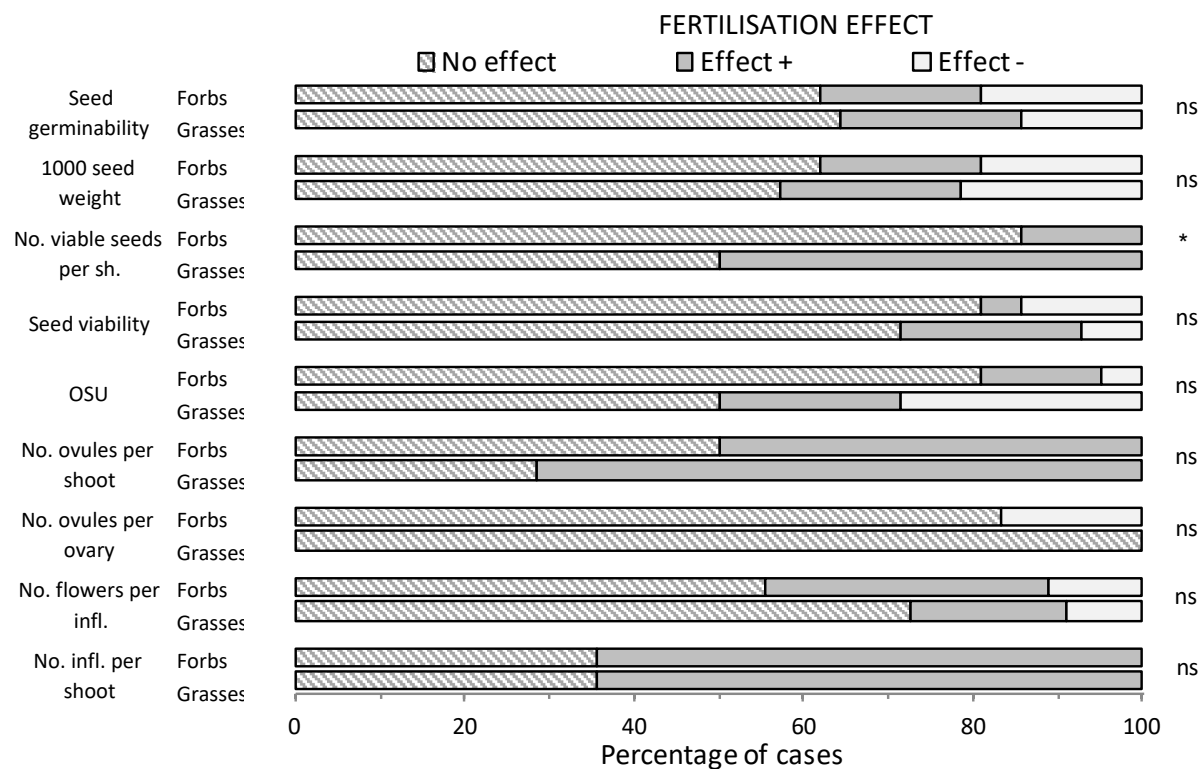


Figure 1 Frequency of the different fertilisation effects on the reproductive traits of fifteen grasses and seventeen forbs in a grassland fertilisation trial in the Italian eastern Alps. No effect, Effect +, and Effect - mean statistically not significant, significant positive or negative fertilisation effect, respectively. ns and * indicate no significance or significance at $p \leq .05$ of the chi-square test for the association between species group (grasses vs. forbs) and type of fertilisation effect (no, positive or negative effect).

In grasses, a higher number of simple inflorescences per shoot was found than in forbs (47 vs. 4; Fig. 2A) but a lower number of flowers per inflorescence (3.4 vs. 43) and ovules per flower (1 vs. 10). Overall, the number of ovules per shoot was significantly higher in forbs (193 vs. 112). OSU and seed viability did not differ in the two species groups. The combined effect of these results was that forbs produced on average more viable seeds per shoot than grasses (65 vs. 35). Percent dormancy (difference between percent viability and germinability) resulted significantly higher (26 vs. 18%) in grasses than in forbs (data not shown). In grasses, fertilisation strongly increased the size of the reproductive system (Fig. 2.B). The increase was exclusively due to a higher number of spikelets per shoot which elicited a 37% increase of the number of ovules per shoot. Fertilisation increased seed viability, too, but not OSU. The resulting increase of the viable seed production per shoot was +54% (43 vs. 28 seeds). Fertilisation affected the seed quality traits positively even if less strongly than the size traits, increasing by only 2% the seeds size and by 10% the seed germinability.

In forbs, fertilisation increased the number of ovules per shoot by 30% (Fig. 2.B). However, seed viability decreased significantly by 5%, so that the number of viable seeds per shoot increased only by 11% (68 vs. 61 seeds), much less than in grasses. Seed biological traits were also increased by fertilisation but less than in grasses.

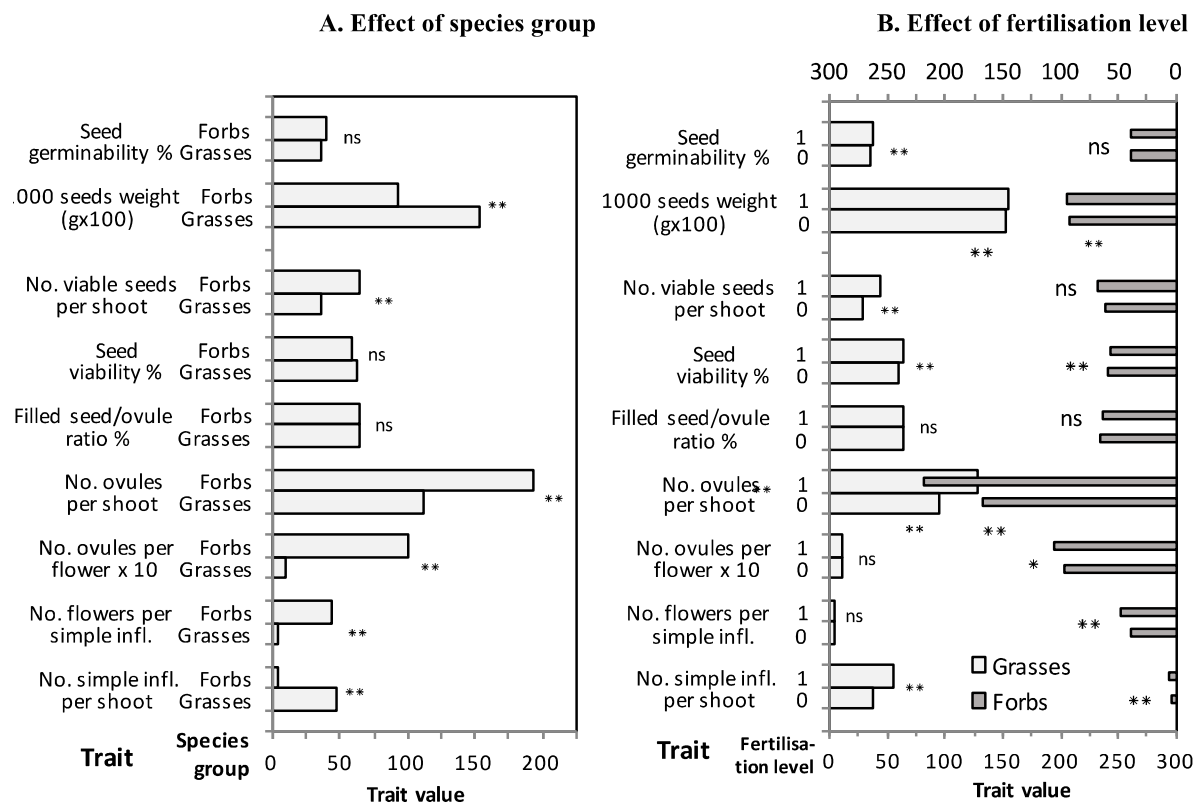


Figure 2 Mean values of nine reproductive traits in fifteen grasses and seventeen forbs. The left graph shows the effect of the species group (grasses versus forbs). The right graph shows the effect of the fertilisation level (0 and 1, low and high fertilisation level, respectively) within each species group. ns, * and ** indicate the Anova results being not significant and significant at $p \leq .05$ and $p \leq .01$ respectively. Individual species values are shown in Annex 2.

Grass and forb groups with similar reproductive behaviour and response to fertilisation

In grasses PCA and CA (Annex 3) identified two main groups. The first group (positive PC1 values in Fig. 3A) included species with high number of viable seeds per shoot but low seed weight while the second group (negative PC1 values) was composed of species with an opposite behaviour. In the first group, species with positive PC2 values had low number of spikelets per shoot but high OSU and seed viability, while species with negative PC2 values had high number of spikelets per shoot but low OSU and seed viability. In the second group, species with negative PC2 values produced the lowest number of viable seeds per shoot due to a particularly low OSU.

As for response to fertilisation, PCA and CA created three grass groups. The viable seed production per shoot increased especially due to a much higher number of spikelets in species in the low-left part of the PCA graph (Fig. 3B) and especially due to the higher seed viability in species at the right side the PCA graph. The third group (high-left part in Fig. 3B) had a less pronounced increase of the viable seed yield.

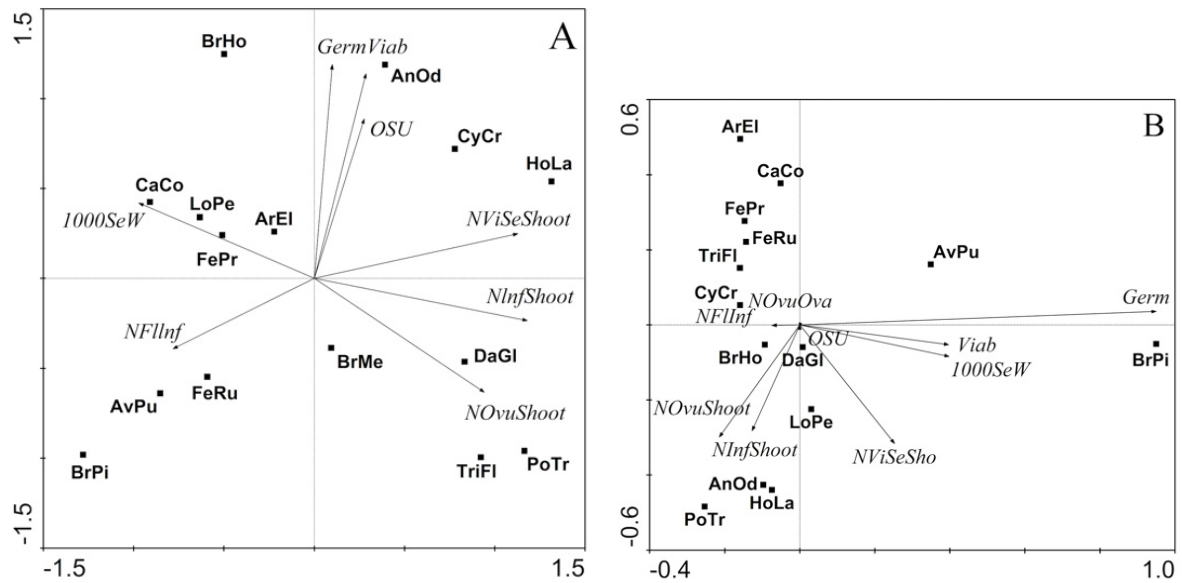


Figure 3 Principal component analysis (PCA) of the reproductive traits of fifteen grassland *Poaceae*. Data from a fertilisation trial in the Italian eastern Alps. PCA was performed on the log-transformed trait values averaged across fertilisation treatments in graph A and on the percent values of the reproductive traits found in the high fertilisation level compared to the low fertilisation level (i.e. response to fertilisation) in graph B. The variability explained by the principal components 1 and 2 was respectively 43.6 and 29.5% in graph A and 73.1 and 15.2% in graph B. In forbs, PCA and CA (Annex 4) identified the same main pattern of variation found for grasses (i.e. high seed size and low viable seed number vs. the opposite) (Fig. 4A). A first species group showed high seed size but low viable seed number (right graph side). In a second group (upper graph part) the seed size was on average low and the viable seed number was high due to high number of ovules per ovary. In a third, more numerous group (central-left low side of the graph), the seed size was also relatively low, but the high ovule and viable seed numbers were due especially to the presence of flower-rich inflorescences.

As for forb response to fertilisation, the most influencing traits were the inflorescence number per shoot, the flower number per inflorescence and seed viability (Fig. 4B). Mainly due to higher seed viability, the effect was strongly positive for the species group at the right side of the PCA graph. Species with intermediate PC1 values had a less strong production increase. In a third group (more negative PC1 values) the viable seed production decreased as a consequence of lower seed viability.

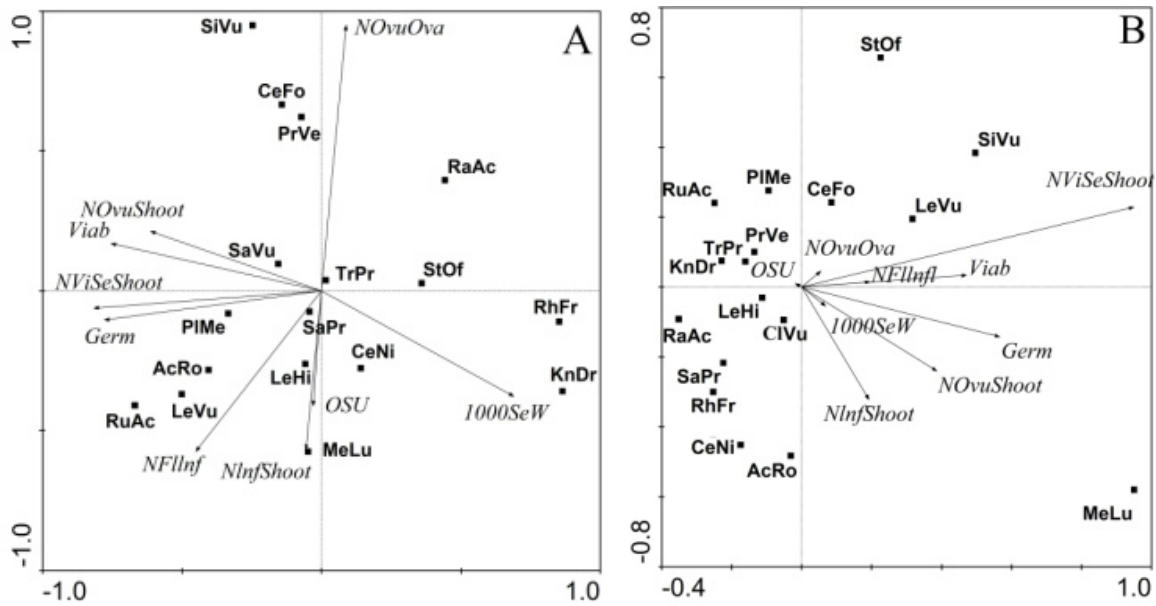


Figure 4 Principal component analysis of the reproductive traits of seventeen grassland forbs. Data from a fertilisation trial in the Italian eastern Alps. In graphs A and B, PCA was performed as explained in the title of Fig. 3. The variability explained by PC1 and PC2 was respectively 32.9 and 22.1 % in graph A and 72.6 and 10.4 % in graph B.

Both in grasses and in forbs, no relation was found between the percent value recorded in HighFert compared to LowFert for the number of ovules per shoot and the number of viable seeds per shoot (Y) and the average values of the reproductive traits or the Ellenberg's bioindicators (X). Only in forbs, a positive relationship was found between the percent value of the number of viable seeds per shoot (Y) and the percent shoot density in HighFert compared to LowFert (Y) ($\log_{10}(Y) = 0.174 \log_{10}(X) + 1.79$; $R^2 = 0.30$, $p = 0.026$).

The breeding system and the species group significantly influenced the response of seed viability to fertilisation (Fig. 5). Compared to LowFert, viability in HighFert was 9% higher for obligate or facultative autogamous species but 4% lower for obligate or mainly outcrossing species ($p = 0.0032$); viability was also 4.2 % higher for grasses and 3.1% lower for forbs ($p = 0.05$).

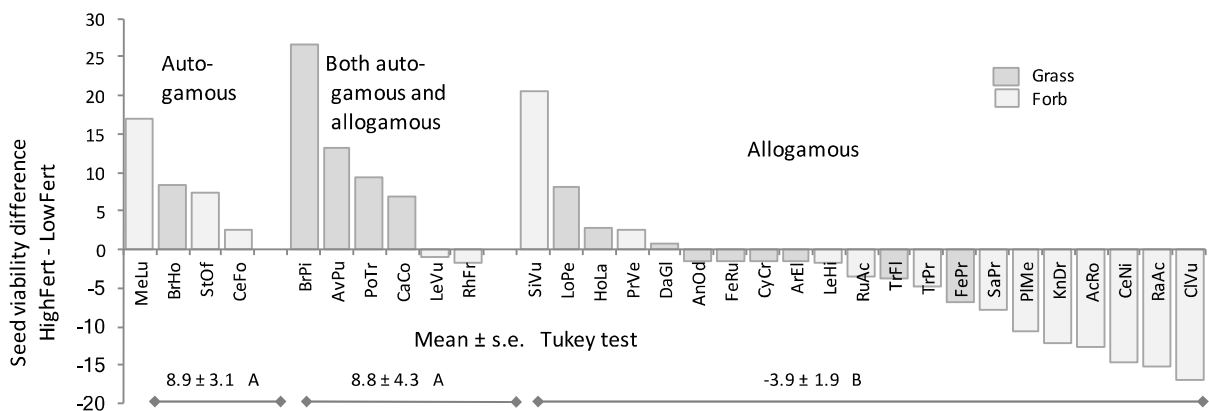


Figure 5 Relationship between variation of seed viability at higher compared to lower fertilisation level and breeding system of thirty-one grassland species. Data from a fertilisation trial in the Italian eastern Alps. For species codes see Table 1.

Multispecies correlations among reproductive traits

Reproductive traits were often linked to each other according to negative relationships:

- the number of simple inflorescences was related with the number of flowers per simple inflorescence similarly in grasses and forbs (Fig. 6A);
- the number of ovules per flower was related with the number of flowers per shoot in forbs (Fig. 6B);
- the number of flowers per spikelet was related with OSU in grasses (Fig. 6C);
- the number of viable seeds per shoot was related with the seed size in a similar way in grasses and forbs (Fig. 6D).

Fitting lines were power functions with negative exponent in 6A, 6B and 6D and a straight line in 6C. When relationships found for the two fertilisation levels were both significant, in no case they also differed one from the other, therefore, only one common line was calculated. However, when fitting was done separately, fertilisation had the effect to move the fitted line to the direction of the main variation of the involved traits. This was particularly evident for the seed size - seed number relationship (Fig. 6D).

In grasses the shoot density was positively related with the number of viable seeds per shoot (Fig. 6E) and, as viable seed number and seed size were inversely related (Fig. 6D), negatively with the seed size (not shown). In forbs, the shoot density was positively related with the ratio between seed size and number of viable seeds per shoot (Fig. 6F).

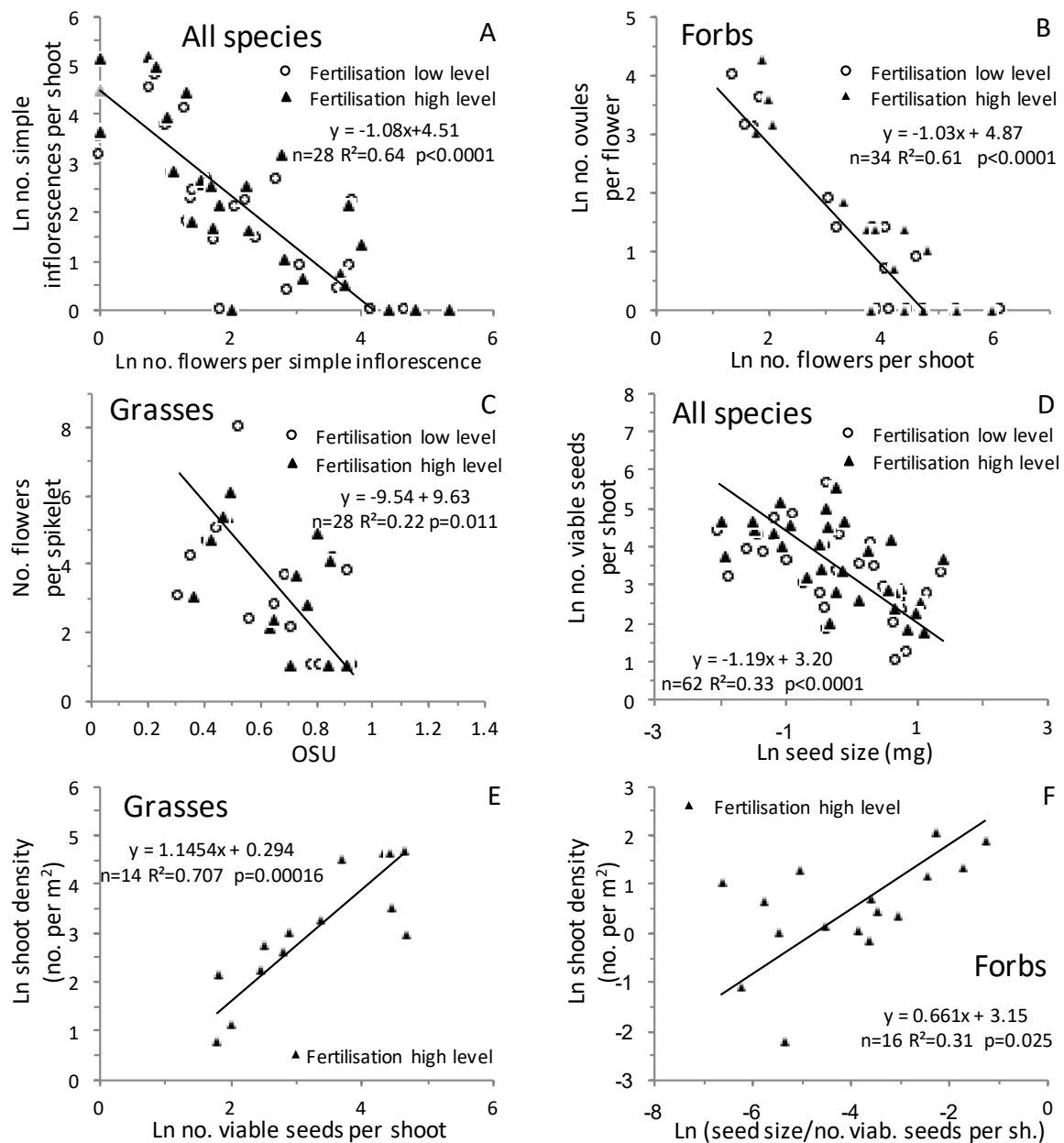


Figure 6 Multispecies relationships between reproductive traits of thirty-two grassland species based on data from a fertilisation trial in the Italian eastern Alps. Lines obtained with the standardized major axis method. In F, the line was calculated without the outlier value of *Cerastium fontanum*.

Discussion

Average values of grass and forb reproductive traits

Results of the current study are hardly comparable with most studies on the seed production in temperate herbaceous species, which never took all main reproductive traits and grassland species into account. A comparison can be done with a study performed with similar methods at a grassland of the same region, with similar altitude, temperature and rainfall but earlier time of seed maturation (May instead of June) (Scotton, 2018). This comparison showed a high multispecies correlation (Pearson $r > 0.9$) for most size traits of the reproductive system. Only the number of forb inflorescences per shoot was less strictly correlated ($r = 0.61$),

probably due to forbs having usually indeterminate growth of the flowering shoot which is strongly affected by site-specific weather and climate conditions, as found, e.g., for most temperate legumes by Puri & Laidlow (1984).

Among the biological traits, seed size and OSU from the two studies were rather well correlated in both species groups (on average $r=0.88$ and 0.64 respectively). Germinability and viability were well correlated in forbs ($r=0.91$ and 0.82 respectively) but uncorrelated in grasses even if the across-species average was similar. The latter finding can indicate a high effect of the main factor differentiating the two study sites, i.e. the photoperiod length during seed maturation (June vs. May), which can elicit both positive and negative effects depending on the species (Baskin and Baskin, 2014) and confirms the particularly strong dependence of grass seed germinability and viability on the climatic conditions during seed maturation reported by Aamlid (1992).

Response of grass and forb reproductive traits to fertilisation

The overall size of the reproductive system (viable seed number per shoot) was significantly affected by fertilisation more often in grasses than in forbs (38 and 10% of species, respectively). However, in both species groups, the traits more frequently and strongly affected were the inflorescence number per shoot and the seed viability.

As for the grass inflorescence size, a much higher fertilisation effect on the spikelet number per shoot than on the flower number per spikelet was found in the current study. To this regard, past studies from the seed propagation field showed contrasting results with cases of stronger response for the former and the latter trait (e.g. in *Festuca pratensis* and *Lolium perenne*: Hill and Watkin, 1975). Probably, the relative fertilisation effect on the two traits is species-specific but the higher effect on spikelet number per shoot found here may depend on the intrinsic characteristics of permanent grasslands. Temperate perennial grasses are dual induction species, and the contribution to the seed production of the following year is highest for shoots which attained the maximum development (i.e. maximum number of buds at the shoot apex) during late summer and autumn of the previous year (Aamlid et al., 1997). The higher spikelet number per shoot observed here in the fertilized treatments was therefore probably due to the faster shoot development (i.e. more buds) induced by the fertilisation during the longer period (several months of the second part of the growing season) available for grass shoot growth in permanent grasslands than under seed propagation conditions.

In forbs, a positive fertilisation effect was frequent on the inflorescence number per shoot (as in grasses) but also on the flower number per inflorescence. As regards inflorescence number, the positive fertilisation effect can be due both to enhanced vegetative growth (i.e. high number of axillary buds) and increased floral differentiation (higher proportion of branches with an inflorescence) and was found in both legume and not-legume forbs (Durner, 2017; Zhang et al., 2014). Also higher flower numbers per inflorescence in more fertile soils were often found, and at both taxonomic and ecological levels. For example, the head size of different *Helianthus* species was shown to be directly related to the fertility of the typical species habitat (Mason et al., 2017) and the flower number per inflorescence in plants from the same population was higher in *Ipomopsis aggregata* (Burkle & Irwin, 2009), *Leucanthemum vulgare* and *Trifolium pratense* (Korevaar, 2013) growing on more fertile soils.

Compared to inflorescence size traits, biological traits were affected by fertilisation less frequently and to a lesser extent. OSU was in most cases independent of fertilisation. This result is not uncommon in the seed propagation studies of grassland species (e.g. see results for *Lolium perenne* in Hampton & Fairey (1997) and Marshall & Ludlam (1989)). However, it is not consistent with the Stephenson's (1981) general view of seed set being normally limited by resource availability. Instead, it is in agreement with the results of the Wiens' study (1984) that resource limitations being more critical for flower production while seed set being largely determined genetically. Indeed, for the species studied here, in HighFert the flower number increased but OSU remained the same.

Seed viability was much more clearly affected by fertilisation than OSU. Discussing this result is difficult as to our knowledge, no past studies reported about fertilisation effect on viability, due to most researchers considering this trait and germinability one and the same and investigating the first trait through germinability tests (Copeland and McDonald, 1995). However, as viability and germinability found here were strictly correlated ($r^2=0.83$), some considerations are possible. Past studies reported about cases of positive, no, or negative fertilisation effect on germinability (see the review by Gray & Thomas, 1982), as obtained here. In the current study, the different fertilisation effect was found to be largely related to the breeding system, as in most cases viability increased in obligate or facultative autogamous species and particularly in the wind-pollinated grasses whereas it decreased in allogamous species and particularly in insect-pollinated forbs. Past studies showed that fertilisation can improve seed germination by increasing the nutrient concentration in seeds (e.g. Cheplick & Sung, 1998). This finding could explain the behaviour of most grasses for which actually viability increased but does not fit most forbs' behaviour of reduced viability under HigFert. Two not incompatible hypotheses are possible to explain the result for forbs. The first refers directly to the allogamous character of the involved species. Some studies found that fertilisation can change the pollen and nectar quality reducing their attractivity for pollinators (Nye et al., 1973; Ramos et al., 2018) and that less pollinator visits can result in an increased selfing with a consequent decrease of seed viability in allogamous species (Arista & Talavera, 1996). The second hypothesis is that a lower forb seed viability may be due to higher competition from other species (Allison, 2002), in our study from grasses which highly increased in the fertilised treatments.

Multispecies correlations among reproductive traits

All negative relationships found between reproductive traits can be interpreted as trade-off behaviour due to resources allocated to one function becoming unavailable for another (Fenner & Thompson, 2005). PCA showed that for both grasses and forbs the main strategy differentiation is that among species yielding many light seeds with low probability to produce seedlings able to win the competition of the established plants and species producing a few heavy seeds where the seedling generated have higher probability to survive (Fig. 6D) (Jakobsson and Eriksson, 2000). Figures 6A and 6B show other trade-off behaviours found at the shoot level. Instead, relationship of Fig. 6C, found only for grasses, represents a trade-off example within the same reproductive structure. Species with high flower number per spikelet showed on average lower OSU. This was due both to the highest flower in the spikelet being often sterile and more fertile flowers not developing to filled seed (data not shown), probably due to an intra-spikelet competition for resources. Significant relationships

very similar to those described above were found in the Scotton (2018) study, which confirms their strength and consistency.

A certain effect of fertilisation on the described trade-offs is visible from the point distribution in Figure 6, but was never so high to reach a level of statistical significance. However, Figure 6D shows a result that is of particular interest: among the traits defining the main trade-off strategy, i.e. seed number and seed size, the seed number underwent the highest positive changes due to fertilisation. This could imply a competitive advantage for grasses which following fertilisation increased more than forbs the seed number per shoot.

No relationship was found between any reproductive trait and the shoot density in any species group at LowFert. This result confirms that species typical for poor soils rely more on vegetative than sexual reproduction (Fujita et al., 2014). Instead, two significant, positive relationships were found only in HighFert between reproductive traits measured at different levels. The shoot density (population level) was positively related to the seed size in forbs (see also Scotton, 2018) and to the number of viable seeds per shoot in grasses (shoot level). As seed production per shoot and seed size were strongly negatively correlated, the two relationships showed that in HighFert the shoot density of the two species groups was regulated in two opposite ways by the seed number/seed size trade-off.

Forbs which could better maintain in HighFert had a high seed size, even if their seed production per shoot was lower. The main species behaving in this way were *Centaurea nigrescens*, *Knautia drymeia*, and *Rhinanthus freynii*. As reminded before, the seed size can favour the species recruitment by increasing the probability of seedling survival in a closed vegetation (Jakobsson and Eriksson, 2000). The result obtained here underlines that this is especially true in a fertilised meadow and for forbs, probably because in HighFert forb seedlings from large seeds can better overcome the recruitment obstacle represented by the strong competitive ability especially of grass species in high-N environments (Shaver and Chapin, 1986). This does not mean that the seed production per shoot is unimportant, as it in any case increases the baseline number of seeds which are going to face the establishment stage. This would explain why for forbs the shoot density was found to be significantly related to the increase due to fertilisation of the number of viable seeds per shoot. Contrary to forbs, in HighFert grasses with the highest shoot density were characterised by high viable seed number per shoot. The main species showing this behaviour were the light-seed *Holcus lanatus*, *Poa trivialis*, and *Trisetum flavescens*. A possible explanation, supported by the results of this and Scotton's (2018) studies, could be that the higher percentage of dormant seeds allows grasses to germinate and establish in autumn, which is the time of predominant grass seed germination (Stampfli & Zeiter, 2008). The following strong tillering occurring under the fresh autumn climate (Gillet, 1980) would allow many grass tillers being vernalised during winter and, therefore, able to enter the reproductive stage in the subsequent spring (Aamlid et al., 1997). In autumn, when the growth of the established vegetation is low, many, even if little, grass seeds could produce seedlings able to establish, whereas the competitive advantage of large seeds would be less important. This effect could be reinforced by the fact that the percentage of soil surface not covered by vegetation (=space free for seedling establishment) in the last part of the growing season is particularly high in fertilised meadows (e.g. D'Ottavio et al., 2002).

The above discussed relationships between reproductive traits and shoot density were not found for the low fertilisation level. This result probably means that on unfertile soils where

the amount of above-ground biomass is low, competition from the established plants is less of a problem and the stress-tolerance against the soil nutrient shortage becomes more important.

Conclusion

Although with a considerable among-species variability, in both grasses and forbs fertilisation strongly modified the inflorescence size traits but had a lower effect on the seed biological traits.

The inflorescence size became generally larger due to fertilisation and this was true especially for the number of inflorescences per shoot in both species groups and the number of flowers per inflorescence in forbs.

The efficiency of the ovule-to-viable-seed transformation generally increased in grasses but more often decreased in forbs. The seed viability was the trait which made the difference among the two species groups, as it was positively or not affected by fertilisation in wind-pollinated grasses but often decreased in allogamous insect-pollinated forbs. Instead seed set was generally not influenced by fertilisation.

The overall effect was that on average fertilisation determined a significant increase of the viable seed production per shoot in grasses (+ 55%) but not in forbs.

Fertilisation did not change the trade-off relationships between individual reproduction traits. However, the results obtained showed that reproductive traits had important effects on the species shoot composition of the grassland and that these effects changed depending on the level of the soil fertility. In the no or less fertilised treatments, no one of the considered traits was found to affect the shoot density in either species group, therefore confirming the relatively low importance of the reproduction by seed at low levels of soil fertility. At higher soil fertility significant but opposite relationships were found for the two species groups. The species with higher shoot density were those with the largest number of viable seeds produced per shoot in the case of grasses and those with higher seed size in the case of forbs. The higher dormancy of grass seeds and the lower soil vegetation cover of the fertilised meadows during the last part of the growing season can probably explain the first result. The higher forb seedling probability to overcome the grass competition was probably the reason for the second result.

In front of the increasing nutrient enrichment of world soils due to increasing atmospheric deposition and of grassland soils as a consequence of higher fertilisation inputs, results from this study highlighted that reproductive traits and their response to a changing soil nutrient status can play an important role in shaping the species composition of the future grasslands and contribute to explain why grasslands of nutrient-rich soils get poor in many insect-pollinated allogamous forbs.

Acknowledgments

This study received funding from the Padova University, ex 60% projects. The authors thanks Antonio Timoni for data collection and laboratory analyses, and the Italy State Forestry Corps (Candaten station) for making the study meadow and meteorological data available.

Data availability statement

The raw data of the study can be found online within the supporting information of this paper (Annex 2).

Supporting information

Additional Supporting Information (Annexes 1-6) may be found on the paper online version.

References

- Aamlid T.S., 1992. Dormancy and germination of temperate grass seed as affected by environmental conditions - A literature review. *Norwegian Journal of Agricultural Sciences* 6, 217-240.
- Aamlid T.S., Heide O.M., Christie B.R. & McGraw R.L., 1997. Reproductive development and the establishment of potential seed yield in grasses and legumes. In D. T. Fahey, & J. G. Hampton (Eds.), *Forage seed production volume 1: Temperate species* (pp. 9–44). Wallingford, UK: CAB International.
- Allison V.J., 2002. Nutrients, arbuscular mycorrhizas and competition interact to influence seed production and germination success in *Achillea millefolium*. *Functional Ecology* 16, 742–749.
- Arista M. & Talavera S., 1996. Density Effect on the Fruit-set, Seed Crop Viability and Seedling Vigour of *Abies pinsapo*. *Annals of Botany* 77, 187-192.
- Baskin C.C. & Baskin J.M., 2014. *Seeds—ecology, biogeography, and evolution of dormancy and germination*, 2nd ed. San Diego, London, Waltham: Academic Press.
- Burkle L.A., Irwin R.E. (2009) The effects of nutrient addition on floral characters and pollination in two subalpine plants, *Ipomopsis aggregata* and *Linum lewisii*. *Plant Ecology* 203: 83–98.
- Cheplick G.P. & Sung L.Y., 1998. Effects of maternal nutrient environment and maturation position on seed heteromorphism, germination, and seedling growth in *Triplasis purpurea* (*Poaceae*). *International Journal of Plant Science* 159, 338–350.
- Copeland L.O. & McDonald M.B., 1995. *Seed science and technology*. Boston/Dordrecht/London: Kluwer academic publishers.
- Core Team R, 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria Available at: <http://www.R-project.org/>, Accessed date: 17 July 2019.
- Dietl W. & Lehmann J., 2004. *Ökologische Wiesenbau*. Österreichischer Agrarverlag, Leopoldsdorf.
- D'Ottavio P., Scotton M. and Ziliotto U., 2002. Effects of different management on vegetation cover in mountain meadows (Asiago, NE Italy). *Grassland Science in Europe* 7, 780-781.

- Durner E.F., 2017. Fall nitrogen enhances spring nitrogen enhanced flowering in the long day strawberry cultivar ‘Elan’. *AIMS Agriculture and Food*, 2(2): 149-164. DOI: 10.3934/agrfood.2017.2.149
- EEA, European Environment Agency, 2004. EEA Report No 1/2004. High Nature Value Farmland. Characteristics, Trends and Policy Challenges. Available from: http://www.eea.europa.eu/publications/report_2004_1 (Accessed 01.02.17).
- Engels C., Kirkby E. & White P., 2012. Mineral Nutrition, Yield and Source–Sink Relationships. In Marschner P., (Ed.) *Marschner’s Mineral nutrition of higher plants*. Third edition. Pp. 85-133. London: Academic press.
- Fenner, M. 1986b. A bioassay to determine the limiting minerals for seeds from nutrient-deprived *Senecio vulgaris* plants. *Journal of Ecology* 74, 497-505.
- Fenner M. & Thompson K., 2005. *The ecology of seeds*. New York and Cambridge: Cambridge University Press. <https://doi.org/10.1017/CBO9780511614101>
- Fujita Y., Venterink H.O., van Bodegom P.M., Douma J.C., Heil G.W., Hölzel N., Jabłońska E., Kotowski W., Okruszko T., Pawlikowski P., de Ruiter P.C. and Wassen M.J., 2014 Low investment in sexual reproduction threatens plants adapted to phosphorus limitation. *Nature* 505, 82-86.
- Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth R.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Green P.A., Holland E.A., et al. 2004. -Nitrogen cycles: past, present, and future. *Biogeo-chemistry* 70,153–226.
- Gillet M., 1980. *Les graminées fourragères*. Ordas, Paris: Gauthier-Villars.
- Gray D. & Thomas T. H., 1982. Seed germination and seedling emergence as influenced by the position of development of the seed on, and chemical applications to, the parent plant. In Khan A. A. (Ed.), *The Physiology and Biochemistry of Seed Development, Dormancy and Germination*. Elsevier biomedical press. Pp. 81-110.
- Hampton J. G. & Fairey D. T., 1997. Components of Seed yield in Grasses and Legumes. In D. T. Fairey, & J. G. Hampton (Eds.), *Forage seed production volume 1: Temperate species* (pp. 45–70). Wallingford UK and New York: CAB International.
- Hebblethwaite P.D. & Ivins J.D., 1977. Nitrogen studies in *Lolium perenne* grown for seed I. Level of application. *Journal of the British Grassland Society* 32 (4), 195-204.
- Hill M.J. & Watkin R.R., 1975. Seed production studies on perennial ryegrass, timothy and prairie grass. 1. Effect of tiller age on tiller survival and, hear emergence and seed head components *Journal of the British grassland society*30, 63-71.
- ISTA, 2003. *International rules for seed testing*. Zürich, Switzerland: International Seed Testing Association.
- Jakobsson A. & Eriksson O., 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos*, 88, 494–502. <https://doi.org/10.1034/j.1600-0706.2000.880304.x>
- Klapp, E., 1971. *Wiesen Und Weiden*. 4. Auflage. Verlag Paul Parey, Berlin und Hamburg.

- Klotz S., Kühn I. & Durka W., 2002. Biolflor: eine Datenbank mit biologisch- ökologischen Merkmalen zur Flora von Deutschland [Biolflor: search and information system on vascular plants in Germany]. Bonn: Bundesamt für Naturschutz. Germany.
- Korevaar H., 2013. Effects of fertilization on flower size in species-rich grasslands. In Helgadóttir Á. and A. Hopkins (Eds.), *The Role of Grasslands in a Green Future. Threats and Perspectives in Less Favoured Areas*. Grassland Science in Europe. Vol. 18, pp. 445-447.
- Leps J. & Šmilauer P., 2003. *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge: Cambridge University Press.
- Nye W.P., Shasha'a N.S., Campbell W.F. & Hamson A.R.. 1973. Insect Pollination and Seed Set of Onions (*Allium cepa* L.). Utah State Univ. Agr. Exp. Sta. Res. Rpt. 6, 1-15.
- Marshall C. & Ludlam D., 1989. The pattern of abortion of developing seeds in *Lolium perenne*. *Ann Bot* 63, 19–27.
- Mason C.M., Patel H.S., Davis K.E. & Donovan L.A., 2017. Beyond pollinators: Evolution of floral architecture with environment across the wild sunflowers (*Helianthus*, *Asteraceae*). *Plant Ecology and Evolution* 150, 139-150.
- Nye W.P., Shasha'a N.S., Campbell W.F. & Hamson A.R.. 1973. Insect Pollination and Seed Set of Onions (*Allium cepa* L.). Utah State Univ. Agr. Exp. Sta. Res. Rpt. 6, 1-15.
- Pearson C.J. & Ison R.L., 1997. *Agronomy of Grassland Systems Second Edition*. Cambridge: Cambridge University Press.
- Pignatti S., 2005. Valori di bioindicazione delle piante vascolari della flora d'Italia. Bioindicator values of vascular plants of the Flora of Italy. *Braun-Blanquetia* 39, 1-97.
- Puri K.P. & Laidlaw A.S., 1984. The effects of temperatures on components of yield and hard seededness in three cultivars of red clover (*Trifolium pratense* L.). *Journal of Applied Seed Production* 2, 18-23.
- Ramos D., Bustamante M.M.C., Silva F., Carvalheiro L.G., 2018. Crop fertilization affects pollination service provision – Common bean as a case study. *PLOS ONE*. <https://doi.org/10.1371/journal.pone.0204460>.
- Rolston M.P., Rowarth J.S., Young III W.C. & Mueller-Warrant G.W., 1997. Grass seed crop management. In D. T. Fahey, & J. G. Hampton (Eds.), *Forage seed production volume 1: Temperate species* (pp. 105–126). Wallingford UK and New York: CAB International.
- SAS, 1985. *Users guide: Statistics*. Cary: SAS Institute.
- Shaver G.R. & Chapin F.S., 1986. Effect of fertilizer on production and biomass of tussock tundra, Alaska, USA. *Arctic and Alpine Research* 18, 261–268.
- Schütz W., 2000. The importance of seed regeneration strategies for the persistence of species in the changing landscape of central Europe. *Zeitschrift für Ökologie und Naturschutz* 9, 73–83.
- Scotton M., 2018. Seed production in grassland species: Morpho-biological determinants in a species-rich semi-natural grassland. *Grass and Forage Science* 73, 764–776.

- Scotton M., D'Ottavio P. & Ziliotto U., 2002. Nitrogen fixation of mountain meadows (Asiago, NE Italy). *Grassland Science in Europe* 7, 730-731.
- Scotton M., Piccinin L., Dainese M. & Sancin F., 2009. Seed production of an *Arrhenatherion elatioris* hay-meadow in the eastern Italian Alps. *Grass and Forage Science*, 64, 208–218.
- Stampfli A. & Zeiter M., 2008. Mechanisms of structural change derived from patterns of seedling emergence and mortality in a semi-natural meadow. *Journal of Vegetation Science* 19, 563-574.
- Stephenson A.G., 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* 12, 253–279.
- Ter Braak C.J.F. & Smilauer P., 2002. CANOCO reference manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination Version 4.5. Ithaca, NY: Microcomputer Power.
- Warton D.I., Wright I.J., Falster D.S. & Westoby M., 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* 81, 259-291.
- Wiens D., 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia* 64, 47–53. <https://doi.org/10.1007/BF00377542>
- Wildi O. & Orłóci L., 1996. Numerical exploration of community patterns. A guide to the use of MULVA-5 (2nd ed.). Amsterdam: SPB Academic Publishing.
- Zhang T., Kang J., Zhao Z., Guo W. & Yang Q., 2014. Frequency, depth and rate of phosphorus fertilizer application effects on alfalfa seed yields. *Canadian Journal of Plant Science* 94, 1149–1156.

Annexes

Annex 1 2012-2017 mean species composition (columns 3-5) of the three fertilisation treatments surveyed for seed production at the end of the first growth period in the Sedico fertilization trial (Italian eastern Pre-Alps). The values represent visual estimations of the species percent abundance. Estimations smaller than 1% are indicated with +. Species identification and names according to Pignatti (1982) (*). Traits of reproductive biology (columns 4-6) from BIOLFLOR.

Species group (2)	Fertilisation treatment	SPECIE COMPOSITION			TRAITS OF REPRODUCTIVE BIOLOGY		
		000	011	222	Type of reproduction (3)	Breeding system (4)	Pollen vector (5)
	Grasses abundance (%)	33	40	77			
	Legume abundance (%)	8	33	3			
	Forb abundance (%)	63	30	23			
	Mean yearly no. of species on 24 m ²	30.3	29.6	20.5			
	Total no. of species surveyed in six years on 96 m ²	61.0	53.0	48.0			
3	<i>Achillea roseoalba</i> Ehrend.	1	1	1	SV	X	IN
1	<i>Anthoxanthum odoratum</i> L.	3	3	1	SV	X	WI
3	<i>Centaurea nigrescens</i> Willd.	1	1	0	S	X	IN
3	<i>Cerastium fontanum</i> subsp. <i>vulgare</i> (Hartm.) Greuter & Burdet	1	1	0	SV	A	IN
1	<i>Cynosurus cristatus</i> L.	1	2	+	S	X	WI
1	<i>Festuca pratensis</i> subsp. <i>pratensis</i> Huds.	1	1	+	S	X	WI
2	<i>Trifolium pratense</i> subsp. <i>pratense</i> L.	3	15	1	S	X	IN
1	<i>Holcus lanatus</i> L.	1	3	15	SV	X	WI
3	<i>Salvia pratensis</i> subsp. <i>pratensis</i> L.	5	+	+	S	X	IN
1	<i>Trisetum flavescens</i> subsp. <i>flavescens</i> (L.) P. Beauv.	1	10	16	S	X	WI
3	<i>Clinopodium vulgare</i> subsp. <i>vulgare</i> L.	+	1	+	SV	X	IN
3	<i>Silene vulgaris</i> subsp. <i>vulgaris</i> (Moench) Garcke	+	+	+	S	X	IN
3	<i>Rhinanthus freynii</i> (Sterneck) Fiori	+	+	+	S	AX	IN
3	<i>Galium album</i> subsp. <i>album</i> Mill.	1	3	11	.	.	.
1	<i>Festuca rubra</i> subsp. <i>rubra</i> L.	5	2	2	.	.	.
3	<i>Ranunculus acris</i> subsp. <i>acris</i> L.	1	2	1	S	X	IN
3	<i>Plantago lanceolata</i> L.	1	1	+	.	.	.
2	<i>Lotus corniculatus</i> L.	3	2	+	.	.	.
3	<i>Leucanthemum vulgare</i> aggr.	7	10	+	SV	AX	IN
3	<i>Daucus carota</i> L.	+	+	+	.	.	.

Species group (2)	Fertilisation treatment	SPECIE COMPOSITION			TRAITS OF REPRODUCTIVE BIOLOGY		
		000	011	222	Type of reproduction (3)	Breeding system (4)	Pollen vector (5)
	Grasses abundance (%)	33	40	77			
	Legume abundance (%)	8	33	3			
	Forb abundance (%)	63	30	23			
	Mean yearly no. of species on 24 m ²	30.3	29.6	20.5			
	Total no. of species surveyed in six years on 96 m ²	61.0	53.0	48.0			
3	<i>Prunella vulgaris L.</i>	+	+	+	.	.	.
1	<i>Poa pratensis L.</i>	+	+	+	.	.	.
3	<i>Campanula glomerata L.</i>	+	+	+	.	.	.
3	<i>Arabis hirsuta (L.) Scop.</i>	+	+	+	.	.	.
3	<i>Ranunculus tuberosus Lapeyr.</i>	+	.	+	.	.	.
1	<i>Brachypodium pinnatum (L.) P. Beauv.</i>	7	.	.	SV	AX	WI
1	<i>Briza media L.</i>	5	+	.	SV	X	WI
3	<i>Plantago media L.</i>	3	+	+	SV	X	WI
3	<i>Thymus pulegioides subsp. pulegioides L.</i>	1
3	<i>Polygala vulgaris subsp. vulgaris L.</i>	1
1	<i>Carex caryophyllea Latourr.</i>	1
3	<i>Orobanche gracilis Sm.</i>	1
3	<i>Bellis perennis L.</i>	+
1	<i>Bromus erectus subsp. erectus Huds.</i>	+
1	<i>Carex montana L.</i>	+
3	<i>Euphrasia rostkoviana subsp. rostkoviana Hayne</i>	+
3	<i>Potentilla erecta L. Raeusch.</i>	+
3	<i>Scabiosa columbaria subsp. columbaria L.</i>	+
3	<i>Tragopogon pratensis subsp. orientalis (L.) Celak</i>	+
1	<i>Helictotrichon pubescens subsp. pubescens (Huds.) Pilg.</i>	1	2	+	SV	AX	WI
3	<i>Knautia drymeia subsp. drymeia Heuff.</i>	16	5	+	S	X	IN
3	<i>Leontodon hispidus L.</i>	24	1	+	SV	X	IN
3	<i>Primula veris subsp. veris L.</i>	1	+	.	SV	X	IN
1	<i>Festuca rupicola Heuff.</i>	2	+	.	S	X	WI
3	<i>Stachys officinalis subsp. officinalis (L.) Trevis.</i>	+	+	.	SV	A	IN
2	<i>Medicago lupulina L.</i>	2	1	+	SV	A	IN

Species group (2)	Fertilisation treatment	SPECIE COMPOSITION			TRAITS OF REPRODUCTIVE BIOLOGY		
		000	011	222	Type of reproduction (3)	Breeding system (4)	Pollen vector (5)
	Grasses abundance (%)	33	40	77			
	Legume abundance (%)	8	33	3			
	Forb abundance (%)	63	30	23			
	Mean yearly no. of species on 24 m ²	30.3	29.6	20.5			
	Total no. of species surveyed in six years on 96 m ²	61.0	53.0	48.0			
3	<i>Crepis biennis</i> L.	+	+
1	<i>Koeleria pyramidata</i> (Lam.) P. Beauv.	+	+
1	<i>Luzula campestris</i> (L.) DC. in Lam & DC.	+	+
3	<i>Alchemilla xanthochlora</i> aggr.	+	+
3	<i>Silene nutans</i> subsp. <i>nutans</i> L.	+	+
3	<i>Ajuga reptans</i> L.	+	+
1	<i>Dactylis glomerata</i> subsp. <i>glomerata</i> L.	+	3	18	S	X	WI
1	<i>Arrhenatherum elatius</i> (L.) J. Presl & C. Presl	+	8	10	S	X	WI
1	<i>Lolium perenne</i> L.	+	3	3	S	X	WI
3	<i>Rumex acetosa</i> L.	+	+	2	SV	X	WI
1	<i>Poa trivialis</i> subsp. <i>trivialis</i> L.	+	2	9	SV	AX	WI
1	<i>Carex spicata</i> Huds.	.	+	+	S	AX	WI
1	<i>Bromus hordeaceus</i> subsp. <i>hordeaceus</i> L.	.	+	2	S	A	WI
2	<i>Trifolium repens</i> subsp. <i>repens</i> L.	+	15	1	.	.	.
3	<i>Pimpinella major</i> (L.) Huds.	+	1	1	.	.	.
3	<i>Taraxacum officinale</i> Weber	+	1	2	.	.	.
3	<i>Veronica chamaedrys</i> subsp. <i>chamaedrys</i> L.	+	+	1	.	.	.
3	<i>Myosotis sylvatica</i> Hoffm.	.	+	2	.	.	.
3	<i>Silene flos-cuculi</i> (L.) Clairv.	.	+	+	.	.	.
2	<i>Trifolium dubium</i> Sibth.	.	+
3	<i>Rumex obtusifolius</i> subsp. <i>obtusifolius</i> L.	.	.	+	.	.	.
3	<i>Veronica arvensis</i> L.	.	.	+	.	.	.
2	<i>Vicia cracca</i> subsp. <i>cracca</i> L.	.	.	+	.	.	.
2	<i>Lathyrus pratensis</i> L.	.	.	+	.	.	.
3	<i>Myosotis arvensis</i> Hill	.	.	+	.	.	.

Legend. (1) Pignatti S., 1982. Flora d'Italia. Edagricole, Bologna. (2) Species group: 1 graminoids; 2 legume forbs, 3 other forbs. (3) Type of reproduction: S by seed or mostly by seed, SV by seed and vegetatively. (4) Breeding system: X obligate or mainly outcrossing, AX both outcrossing and autogamous, A obligate autogamous (5) Pollen vector: WI wind, IN insect.

Annex 2 Mean values of the reproductive traits of thirty-two grassland species studied in a fertilisation trial in the Italian eastern Alps. The effects of fertilisation, year and their interaction are also shown in the table.

Species group	Species (1)	Growth period	Mean fertilis. Treatment (2)	Differenc e among means (3)	No. compound inflorescenc e per shoot	No. simple inflorescence per compound inflorescence	No. simple inflorescenc es per shoot	No. flowers per simple inflorescenc e	No. flowers per shoot	No. ovules per ovary	No. ovules per shoot	No. seeds per shoot	OSU	1000 seeds weigth	Germinabili ty %	Viability %	No. viable seeds per shoot	
Grasses	AnOd	1	000	.	1 -	23.7 c	23.7 c	1 -	23.7 c	1 -	23.7 c	19.0 c	0.8 b	0.6 a	64.0 a	88.5 a	15.2 c	
		1	011	.	1 -	33.0 b	33.0 b	1 -	33.0 b	1 -	33.0 b	29.2 b	0.9 a	0.6 a	68.7 a	88.2 a	23.9 b	
		1	222	.	1 -	46.6 a	46.6 a	1 -	46.6 a	1 -	46.6 a	41.7 a	0.9 a	0.6 a	67.6 a	85.8 a	34.6 a	
		1	Plevel (4)	>, =, < (5)	- -	<0.0001 >	<0.0001 >	- -	<0.0001 >	- -	<0.0001 >	<0.0001 >	0.0 >	ns =	ns =	ns =	ns =	<0.0001 >
	ArEI	1	000	.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
		1	011	.	1 -	34.0 a	34.0 a	1 -	34.0 a	1 -	34.0 a	31.7 a	0.9 a	3.2 a	18.1 a	43.8 a	15.3 a	
		1	222	.	1 -	38.0 a	38.0 a	1 -	38.0 a	1 -	38.0 a	32.1 a	0.8 b	2.8 b	17.7 a	42.3 a	12.3 a	
		1	Plevel (4)	>, =, < (5)	- -	ns =	ns =	- -	ns =	- -	ns =	ns =	0.0 <	0.0 <	ns =	ns =	ns =	ns =
	AvPu	1	000	.	1 -	16.2 a	16.2 a	3.0 a	49.4 a	1 -	49.4 a	15.4 b	0.3 a	2.3 a	8.5 b	38.7 b	3.4 b	
		1	011	.	1 -	16.6 a	16.6 a	3.0 a	50.7 a	1 -	50.7 a	18.2 a	0.4 a	2.3 a	20.4 a	51.8 a	6.2 a	
		1	222	.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	
		1	Plevel (4)	>, =, < (5)	- -	ns =	ns =	ns =	ns =	- -	ns =	0.0 >	ns =	ns =	0.0 >	0.0 >	0.0 >	0.0 >
	BrPi	1	000	.	1 -	8.1 a	8.1 a	8.0 a	64.2 a	1 -	64.2 a	34.2 a	0.5 a	1.3 b	8.0 b	20.6 b	2.8 a	
		1	011	.	1 -	8.3 a	8.3 a	6.1 b	51.0 a	1 -	51.0 a	25.9 b	0.5 a	3.0 a	28.3 a	47.2 a	5.9 a	
		1	222	.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	
		1	Plevel (4)	>, =, < (5)	- -	ns =	ns =	0.0 <	ns =	- -	ns =	0.0 <	ns =	0.0 >	0.0 >	0.0 >	ns =	
	BrMe	1	000	.	1 -	26.0 -	26.0 -	3.8 -	99.1 -	1 -	99.1 -	46.1 -	0.4 -	0.5 -	19.0 -	66.9 -	20.8 -	
		1	011	.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	
		1	222	.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	
		1	Plevel (4)	>, =, < (5)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	
	BrHo	1	000	.	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	
		1	011	.	1 -	9.6 b	9.6 b	4.1 b	41.2 b	1 -	41.2 b	34.6 b	0.9 a	4.0 a	73.0 a	84.5 b	26.7 b	
		1	222	.	1 -	12.8 a	12.8 a	4.9 a	62.0 a	1 -	62.0 a	49.4 a	0.8 b	4.0 a	78.3 a	92.8 a	40.0 a	
		1	Plevel (4)	>, =, < (5)	- -	<0.0001 >	<0.0001 >	0.0 >	0.0 >	- -	0.0 >	<0.0001 >	0.0 <	ns =	ns =	ns =	0.0 >	<0.0001 >

CaCo	1	000	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	1	011	.	1	-	6.1	a	6.1	a	3.8	a	22.7	a	1	-	22.7	a	20.7	a	0.9	a	2.1	a	19.2	a	52.4	a	9.5	a
	1	222	.	1	-	6.1	a	6.1	a	4.1	a	24.4	a	1	-	24.4	a	20.9	a	0.8	b	1.9	b	22.7	a	59.2	a	11.5	a
	1	Plevel (4)	>, =, < (5)	-	-	ns	=	ns	=	ns	=	ns	=	-	-	ns	=	ns	=	0.0	<	<0.0001	<	ns	=	ns	=	ns	=
CyCr	1	000	.	1	-	42.1	b	42.1	b	2.8	a	117.2	b	1	-	117.2	b	76.9	b	0.7	b	0.7	a	76.0	a	91.6	a	55.0	b
	1	011	.	1	-	44.9	b	44.9	b	2.8	a	125.9	b	1	-	125.9	b	94.6	b	0.8	a	0.7	a	65.4	a	93.0	a	77.0	a
	1	222	.	1	-	57.2	a	57.2	a	2.9	a	165.0	a	1	-	165.0	a	127.6	a	0.8	a	0.7	a	48.0	b	87.1	a	97.1	a
	1	Plevel (4)	>, =, < (5)	-	-	<0.0001	>	<0.0001	>	ns	=	<0.0001	>	-	-	<0.0001	>	<0.0001	>	0.0	>	ns	=	0.0	<	ns	=	<0.0001	>
DaGl	1	000	.	1	-	59.9	c	59.9	c	3.7	a	226.2	c	1	-	226.2	c	151.8	b	0.7	b	0.9	b	11.1	b	50.4	a	69.9	a
	1	011	.	1	-	75.6	b	75.6	b	3.6	a	275.0	b	1	-	275.0	b	209.2	a	0.8	a	0.9	b	23.9	a	47.7	a	98.8	a
	1	222	.	1	-	97.6	a	97.6	a	3.8	a	372.8	a	1	-	372.8	a	253.2	a	0.7	b	1.0	a	33.5	a	54.5	a	114.2	a
	1	Plevel (4)	>, =, < (5)	-	-	<0.0001	>	<0.0001	>	ns	=	<0.0001	>	-	-	<0.0001	>	<0.0001	>	0.0	=	0.0	>	0.0	>	ns	=	ns	=
FePr	1	000	.	1	-	14.5	b	14.5	a	5.0	a	69.1	b	1	-	69.1	b	28.6	b	0.4	a	2.1	a	52.7	a	74.7	a	13.4	a
	1	011	.	1	-	13.8	b	13.8	a	5.2	a	72.3	b	1	-	72.3	b	33.0	b	0.5	a	2.3	a	53.4	a	72.7	b	16.2	a
	1	222	.	1	-	15.8	a	15.8	a	5.5	a	87.9	a	1	-	87.9	a	43.7	a	0.5	a	2.0	a	37.1	b	63.2	b	19.4	a
	1	Plevel (4)	>, =, < (5)	-	-	0.0	>	0.0	=	ns	=	0.0	>	-	-	0.0	>	0.0	>	ns	=	ns	=	0.0	<	0.0	<	ns	=
FeRu	1	000	.	1	-	11.7	b	11.7	b	4.6	a	53.5	b	1	-	53.5	b	21.9	a	0.4	a	0.7	a	20.3	a	51.8	a	6.0	a
	1	011	.	1	-	14.0	a	14.0	a	4.7	a	66.3	a	1	-	66.3	a	25.7	a	0.4	a	0.7	a	18.3	a	50.3	a	7.3	a
	1	222	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	1	Plevel (4)	>, =, < (5)	-	-	0.0	>	0.0	>	ns	=	0.0	>	-	-	0.0	>	ns	=	ns	=	ns	=	ns	=	ns	=	ns	=
HoLa	1	000	.	1	-	94.2	c	94.2	b	1	-	89.8	b	1	-	89.8	b	72.3	b	0.8	a	0.3	a	46.7	a	76.0	a	46.4	b
	1	011	.	1	-	159.3	b	159.3	a	1	-	159.3	a	1	-	159.3	a	109.2	a	0.7	a	0.3	a	53.3	a	77.3	a	70.4	a
	1	222	.	1	-	180.0	a	180.0	a	1	-	180.0	a	1	-	180.0	a	120.8	a	0.7	b	0.3	a	58.7	a	80.3	a	79.8	a
	1	Plevel (4)	>, =, < (5)	-	-	<0.0001	>	<0.0001	>	-	-	<0.0001	>	-	-	<0.0001	>	0.0	>	0.0	<	ns	=	ns	=	ns	=	0.0	>
LoPe	1	000	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	1	011	.	1	-	11.5	b	11.5	b	4.2	b	48.0	b	1	-	48.0	b	17.1	b	0.4	b	1.9	a	46.3	a	73.9	a	7.2	b
	1	222	.	1	-	12.5	a	12.5	a	5.4	a	67.4	a	1	-	67.4	a	31.3	a	0.5	a	1.9	a	59.6	a	82.1	a	16.1	a
	1	Plevel (4)	>, =, < (5)	-	-	0.0	>	0.0	>	<0.0001	>	<0.0001	>	-	-	<0.0001	>	<0.0001	>	<0.0001	>	ns	=	ns	=	ns	=	<0.0001	>

PoTr	1	000	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	1	011	.	1	-	97.5	b	97.5	b	2.1	a	197.0	b	1	-	197.0	b	141.3	b	0.7	a	0.2	b	11.3	a	39.3	a	48.3	b
	1	222	.	1	-	173.0	a	173.0	a	2.1	a	359.9	a	1	-	359.9	a	229.7	a	0.6	a	0.2	a	4.9	a	48.6	a	82.7	a
	1	Plevel (4)	>, =, < (5)	-	-	<0.0001	>	<0.0001	>	ns	=	1	>	-	-	<0.0001	>	0.0	>	ns	=	0.0	>	ns	=	ns	=	0.0	>
TrFl	1	000	.	1	-	119.1	b	119.1	b	2.4	a	280.0	b	1	-	280.0	b	156.4	b	0.6	a	0.2	a	31.1	a	60.1	a	70.0	a
	1	011	.	1	-	120.1	b	120.1	b	2.5	a	295.5	b	1	-	295.5	b	192.0	b	0.7	a	0.2	b	21.1	a	53.7	a	84.9	a
	1	222	.	1	-	159.6	a	159.6	a	2.3	a	362.6	a	1	-	362.6	a	232.6	a	0.6	a	0.2	b	29.7	a	59.1	a	124.0	a
	1	Plevel (4)	>, =, < (5)	-	-	0.0	>	0.0	>	ns	=	0.0	>	-	-	0.0	>	0.0	>	ns	=	0.0	<	ns	=	ns	=	ns	=

Legend. (1) Species 1) Grasses: AnOd, *Anthoxanthum odoratum*; ArEl, *Arrhenatherum elatius*; AvPu, *Avenula pubescens*; BrPi, *Brachypodium rupestre*; BrMe, *Briza media*; BrHo, *Bromus hordeaceus*; CaCo, *Carex contigua*; CyCr, *Cynosurus cristatus*; DaGl, *Dactylis glomerata*; FePr, *Festuca pratensis*; FeRu, *Festuca rupicola*; HoLa, *Holcus lanatus*; LoPe, *Lolium perenne*; PoTr, *Poa trivialis*; TrFl, *Trisetum flavescens*; Forbs; AcRo, *Achillea roseo-alba*; CeNi, *Centaurea nigrescens*; CeFo, *Cerastium fontanum*; ClVu, *Clinopodium vulgare*; KnDr, *Knautia drymeia*; LeHi, *Leontodon hispidus*; LeVu, *Leucanthemum vulgare*; MeLu, *Medicago lupulina*; PlMe, *Plantago media*; PrVe, *Primula veris*; RaAc, *Ranunculus acris*; RhFr, *Rhinanthus freynii*; RuAc, *Rumex acetosa*; SaPr, *Salvia pratensis*; SiVu, *Silene vulgaris*; StOf, *Stachys officinalis*; TrPr, *Trifolium pratense*. (2) Mean fertilisation treatment: see main text. (3) Difference among means: means with no litters in common are different at least at $p \leq 0.05$. (4) P level: level of the among treatments comparison. (5) >, =, <; means of the treatments with higher fertilisation significantly higher, not significantly different and significantly lower than the means with lower fertilisation, respectively.

Annex 3 Mean values of the reproductive traits of fifteen grasses. Results from a fertilisation trial in the Italian eastern Alps. Species and traits are grouped according to the cluster analysis performed after trait values standardisation. The similarity ratio as similarity function and the minimum variance method were used for clustering.

Group	Species (1)	No. viable seeds per shoot	No. ovules per shoot	No. spikelets per shoot	Viability %	Germinability %	OSU	1000 seeds weight (g)	No. flowers per spikelet
2	FeRu	6.7	59.9	12.9	51.1	19.3	0.42	0.71	4.66
	BrMe	20.8	99.1	26.0	66.9	19.0	0.4	0.45	3.78
	LoPe	11.7	57.7	12.0	78.0	53.0	0.4	1.93	4.79
	FePr	16.3	76.4	14.7	70.2	47.7	0.47	2.15	5.23
	BrHo	33.3	51.6	11.2	88.7	75.6	0.83	3.99	4.50
	CaCo	10.5	23.6	6.1	55.8	20.9	0.88	1.97	3.94
	ArEl	13.8	36.0	36.0	43.0	17.9	0.89	2.99	1.00
	AvPu	4.8	50.0	16.4	45.2	14.4	0.34	2.33	3.03
	BrPi	3.7	60.4	8.2	28.2	13.8	0.52	2.28	7.47
1	TriFl	59.8	228.7	97.5	40.3	17.5	0.40	0.23	2.34
	PoTr	65.5	278.4	135.2	43.9	8.1	0.67	0.22	2.13
	DaGl	78.2	305.2	78.5	50.9	23.4	0.63	0.79	3.85
	HoLa	65.5	143.0	144.5	77.9	52.9	0.74	0.29	1.00
	CyCr	76.3	136.0	48.1	90.6	63.1	0.73	0.69	2.81
	AnOd	24.5	34.4	34.4	87.5	66.8	0.87	0.64	1.00
Mean		32.8	109.4	45.5	61.2	34.2	0.62	1.44	3.44

Legend. (1) For species code see legend of Annex 2.

Annex 4 Percent values of the reproductive traits of fifteen grasses in the high fertilisation level compared to the low fertilisation level. Results from a fertilisation trial in the Italian eastern Alps. Traits and species clustered as explained in the title of Annex 3.

Group	Species (1)	Viability %	Germinability %	OSU	No. ovules per shoot	No. viable seeds per shoot	No. flowers per infl.	1000 seeds weight (g)	No. Infl. per shoot	No. ovules per ovary
1	KnDr	31.8	0.3	0.6	48	9.7	18.9	2.43	2.62	1.00
	RhFr	13.4	0	0.9	162	12.8	5.7	1.91	4.85	6.39
	StOf	18.8	6.9	0.6	193	17.3	10.5	0.59	4.62	4.00
2	CeFo	68.0	54.2	0.5	160	35.5	7.2	0.15	1.00	22.93
	ClVu	60.1	49.0	0.6	140	44.7	19.8	0.35	1.78	4.00
	RaAc	37.2	2.5	0.8	116	26.3	5.6	1.00	1.00	21.03
	PrVe	87.4	0.8	0.8	249	140.2	6.9	0.67	1.00	36.17
	SiVu	85.1	83.1	0.3	348	44.3	5.7	0.57	1.00	64.18
3	MeLu	62.3	57.2	1.0	154	79.2	11.5	1.73	15.55	1.00
	AcRo	52.3	35.6	0.7	326	96.8	15.8	0.13	21.09	1.00
	RuAc	86.6	79.0	0.8	429	272.3	46.2	0.74	8.89	1.00
	LeVu	80.8	73.7	1.0	148	115.8	148.3	0.39	1.00	1.00
	PIMe	46.6	20.9	0.7	300	79.2	116.2	0.39	1.00	2.62
	TrPr	85.9	70.8	0.2	130	12.7	40.7	1.16	1.62	2.00
	LeHi	78.5	63.8	0.6	73	28.7	72.6	0.86	1.00	1.00
	CeNi	48.1	36.5	0.4	172	17.5	51.0	1.71	3.30	1.00
	SaPr	37.5	30.2	0.6	298	52.2	39.3	1.30	1.90	4.00
Mean		57.7	39.1	0.6	203	63.8	36.6	0.95	4.31	10.25

Legend. (1) For species code see legend of Annex 2.

Annex 5 Mean values of the reproductive traits of seventeen forbs. Results from a fertilisation trial in the Italian eastern Alps. Species and traits are grouped according to the cluster analysis performed after trait values standardisation. Traits and species clustered as explained in the title of Annex 3.

Group	Species (1)	Viability	1000 seeds weight	Germinability	No. ovules per ovary	No. flowers per infl.	OSU	No. ovules per shoot	No. Infl. per shoot	No. viable seeds per shoot
1	AvPu	134	99	240	100	101	117	103	103	183
	BrPi	229	238	354	100	76	93	79	103	216
2	DaGl	101	107	143	100	100	106	143	144	152
	BrHo	110	99	107	100	117	93	150	134	150
	HoLa	104	116	120	100	100	86	189	180	162
	AnOd	98	98	106	100	100	115	168	168	193
	PoTr	124	108	43	100	99	89	183	177	171
	LoPe	111	100	129	100	129	132	140	108	224
3	TriFl	94	90	82	100	101	114	118	117	149
	CyCr	98	102	75	100	101	117	124	121	158
	FeRu	97	103	90	100	102	100	124	119	122
	FePr	91	100	86	100	107	108	116	102	133
	CaCo	113	90	118	100	107	92	108	101	121
	ArEl	96	88	98	100	100	90	112	112	81
Mean		114	110	128	100	103	104	133	128	158

Legend. (1) For species code see legend of Annex 2.

Annex 6 Percent values of the reproductive traits of seventeen forbs in the high fertilisation level compared to the low fertilisation level. Results from a fertilisation trial in the Italian eastern Alps. Traits and species clustered as explained in the title of Annex 3.

Group	Species (1)	OSU	No. ovules per ovary	1000 seeds weight	No. ovules per shoot	No. Infl. per shoot	No. flowers per infl.	Viability	Germinability	No. viable seeds per shoot
1	LeVu	91	100	81	170	100	170	132	151	204
	CeFo	102	105	94	144	100	140	104	108	169
	SiVu	96	131	123	197	100	162	129	136	271
	StOf	104	100	75	102	117	88	149	100	233
	MeLu	95	100	125	217	203	135	219	276	296
2	ClVu	101	100	90	163	132	123	77	74	140
	AcRo	112	100	103	173	170	106	79	90	129
	CeNi	78	100	104	174	150	115	75	62	93
	TrPr	97	100	99	112	108	108	95	93	105
	PrVe	90	97	100	115	100	116	103	100	106
	LeHi	89	100	107	126	100	126	98	118	100
	PIMe	100	108	103	114	100	108	97	85	135
3	RuAc	101	100	113	85	89	93	96	90	90
	KnDr	102	100	120	89	110	78	79	100	97
	RaAc	112	87	68	107	100	118	63	96	51
	SaPr	93	100	94	135	128	105	81	81	83
	RhFr	109	96	102	119	130	98	88	100	64
Mean		98	101	100	138	120	117	104	109	139

Legend. (1) For species code see legend of Annex 2.

Chapter 3

Efficiency and allometry of seed production in grassland forbs: site and year comparative study in the Italian Eastern Pre-Alps

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Published on Plant Biosystems

DOI: 10.1080/11263504.2018.1508084

Abstract

Forbs are important biodiversity components of grasslands and are often threatened by management intensification. As most forbs propagate predominantly by seed, knowledge of their seed regenerative traits would improve the conservation and restoration of forb-rich ecosystems. The main seed production traits of six forbs that are common in European species-rich grasslands were studied by collecting fertile shoots from different sites and over several years. Among sites and years, variability was high, particularly in the number of inflorescences per shoot, which affected ovule production more than any other trait. Relationships between inflorescence size and the number of ovules were mainly negatively allometric or almost so, with lower flower densities in larger inflorescences. The average ovule-to-seed transformation efficiency was 58%. There was significant variation among collections of the same species, and even more between species. Species with a low ovule-to-seed transformation efficiency generally exhibited compensatory, high seed viability. Large inflorescences had high ovule to seed utilization values, probably because of better nutrient conditions. Seed germinability (average, 30%) was much lower than seed viability (average, 54%); therefore, seed dormancy was an important feature of the species studied.

Keywords: Allometry; forbs; germinability; inflorescence size; seeds; viability.

Introduction

Forbs are an important component of grassland biodiversity (Corbin et al., 2007). On European grasslands, forbs usually represent the highest proportion of plant species richness and are therefore strongly correlated with the total number of plant species (Bertke et al., 2005). The forb presence on grasslands is fundamental for many above-ground insects and other animal groups (Schmid et al. 2007). Forbs are also regarded as prime components when attempting to under-take ecosystem improvement. Native forbs included in the seed mixes for grassland restoration can increase the biodiversity of the vegetation obtained and enhance its agricultural production potential (Bullock et al., 2007). In urban environments, forbs can be introduced to improve the status and ornamental value of the plant and animal populations (Gilbert, 1989; Bretzel & Romano, 2013).

Forbs are grassland species that suffer most from management intensification. For example, in the mountain environments of North-Eastern Italy, forbs have diminished from 40 species on extensive meadows to 18 species on intensive grasslands (Scotton et al., 2014). At the same time, the forb percent abundance has decreased from 34 to 24%, while grasses have increased from 66 to 76%. In ecological restoration, the resulting vegetation is often found to be forb-poor and grass-rich compared to the reference vegetation (Gibson, 2009; Scotton, 2016).

Among the biological and ecological traits that can affect forbs' ability to persist in herbaceous covers, the regeneration strategy is of fundamental importance (Schutz, 2000). In this regard, a potential drawback of forbs is that most of them reproduce exclusively or

predominantly by seed whereas most grasses can reproduce both vegetative and by seed and can efficiently propagate through tillering (Klotz et al., 2002).

Therefore, increasing our knowledge of forb seed production and ecology is important to increase the success of conserving and restoring species-rich grasslands.

Past studies have identified the following traits as the most important for the seed reproductive strategy: the number of seeds produced (Westoby et al., 1997), seed mass (Westoby, 1998), germinability, viability, and dormancy (Baskin & Baskin, 2014).

The amount of ovules/seeds produced (reproductive output) is related to plant size. This last trait is measured by different authors as plant height, mass or stem diameter, depending on the study aim. The relationship between plant size and reproductive output is usually broadly allometric, that is, it changes with size, with longer-lived herbaceous plants usually having less output in larger plants (Weiner et al., 2009). However, it has not yet been investigated whether the same type of relationship also exists between inflorescence size and ovule/seed production.

The proportion of ovules that transform into seeds (ovule site utilization; OSU) is limited by many factors, such as egg cell nonviability, poor pollination, resource deficiencies, and particularly in outbreeders, a high frequency of deleterious allelic combinations (Lorenzetti, 1993). Weather and climate can also significantly affect OSU (Elgersma et al., 1989). As inflorescence size can be positively related to the nutritional status of the shoot (Tegegnetwork et al., 2015), it is possible that this trait also affects OSU.

A large number of seeds produced per shoot often leads to a smaller seed size, because variability in the total amount of resources allocated to reproduction is not high (Fenner & Thompson, 2005). Large-seeded species are able to establish in undisturbed vegetation, where seedlings have to overcome competition from plants that are already present in the herbaceous cover (Schutz, 2000). In contrast, numerous light seeds are effectively dispersed by wind (Fenner & Thompson, 2005) and can more easily colonize vegetation gaps that act as safe sites for seed germination and seedling establishment (Schutz, 2000).

While seed viability represents overall germination ability, germinability, and dormancy determine when germination will occur (Baskin & Baskin, 2014). Their interaction with temperature, moisture, and light determines the main season for seed germination and the associated probability of seedling establishment (Schutz, 2000).

The main objective of this study was to increase our knowledge of forb seed ecology, in order to facilitate forb conservation and seed-based restoration in grassland ecosystems. Six forb species that are important components of European grasslands were collected from different sites in the Italian Pre-Alps and analysed for the main traits of seed production. The specific aims of the study were:

- to characterize the amount and quality (size, germinability, viability, and dormancy) of seed production;
- to characterize among-year and among-site variability in reproductive traits;
- to investigate relationships between the size of the inflorescence structure and ovule/seed production and;
- to acquire useful information regarding the collection of forb seeds and their use in increasing the biodiversity of degraded ecosystems.

Materials and methods

Sites, meadows, and species

Fertile forb shoots were collected from three species-rich semi-natural grasslands located in the Italian Eastern Pre-Alps at Candaten, Pianari, and Rubbio (Veneto region). The geological substratum was calcareous at all sites and the soils were shallow with a pH of between 6.6 and 7.5. The mean annual rainfall was 1266–1440 mm and the mean annual temperature was 7.5–11.1 °C. Management was extensive with little or no fertilizer use and one to three mows per year, depending on the altitude (420–1030 m above mean sea level). Species richness was high and ranged between 44 and 50 vascular plants in 100 m² sampling areas. The reference vegetation was *Arrhenatherion elatioris* (Candaten and Pianari) and *Mesobromion* (Rubbio). More detailed information is available in the Appendix (Supporting Information Table S1).

Between 2009 and 2014, fertile shoots of *Achillea roseo-alba*, *Centaurea nigrescens*, *Tragopogon pratensis* subsp. *orientalis*, *Salvia pratensis* subsp. *pratensis*, *Scabiosa columbaria* subsp. *Columbaria*, and *Rhinanthus freynii* (species names according to Pignatti 1982) were collected from the above grasslands. These species are frequently found in North-Italian grasslands with extensive management (Chiappella Feoli & Poldini, 1993; Buffa et al., 1995) but their presence considerably decreases when grasslands are intensively managed (Scotton et al., 2014). With the exception of *A. roseo-alba*, which often reproduces vegetatively via runners, these species are characterized by amphimictic reproduction by seed (Klotz et al., 2002), exclusively so for the biennial *T. pratensis* and the annual *R. freynii*. In these two species breeding occurs both by autogamy and allogamy. Allogamy is the most common mating system in the other species (Klotz et al. 2002).

The collection sites and years are shown in Table 1. Ten fertile shoots per species were sampled in each collection year from three plots randomly distributed within each grassland (30 fertile shoots per site and year). The plot sizes were 4x6 m at Candaten and 10x10 m at Pianari and Rubbio. Sampling was conducted during the first growth period and at the time of optimal seed maturation of each species.

Collection site	Candaten		Pianari			Rubbio	
	2013	2014	2009	2010	2012	2009	2011
Collection code	C2013	C2014	P2009	P2010	P2012	R2009	R2011
<i>Achillea roseo-alba</i>	x	x		x			
<i>Centaurea nigrescens</i>					x	x	
<i>Tragopogon pratensis</i>			x		x	x	
<i>Scabiosa columbaria</i>						x	x
<i>Salvia pratensis</i>			x	x		x	
<i>Rhinanthus freynii</i>			x		x	x	

Table 1 Collection sites and years

Analysis of inflorescences and seeds

Each collected shoot was placed in a paper bag, dried and stored in a refrigerator. In the following 3–6 months, the shoots were analysed in the laboratory for the most important traits of their reproductive structures.

The numbers of inflorescences per shoot, and flowers per inflorescence were determined. In *S. pratensis* and *R. freynii*, lateral fertile branches of the shoot were considered independent racemes. Similarly, in *A. roseo-alba*, each group of heads carried at the same height on a lateral branch of the fertile shoot was considered as independent corymb.

On each inflorescence, flowers were sampled to be analysed for ovule and seed content. In *S. pratensis* and *R. freynii*, each raceme was divided into three sections (low, medium, and high), and two flowers per section were chosen. In *A. roseo-alba*, all flowers of four heads sampled from the centre to the periphery of each corymb were considered. In *T. pratensis*, *C. nigrescens*, and *S. columbaria*, all flowers of all heads were analysed. In all species, a necessary pre-condition for choosing sample flowers or heads was that they were intact (i.e. the fruit had not yet opened or the head still contained all flowers). The number of ovules/seeds per ovary/fruit or head was counted by binocular observation. Ovules/seeds were extracted and divided into five groups: no ovules or seeds, aborted ovule, normal ovule, growing seed, and filled seed (Scotton et al., 2009).

After seed removal, the inflorescence size was measured by recording corymb number per shoot in *A. roseo-alba*, diameter of the head receptacle in *C. nigrescens* and *T. pratensis*, raceme length in *S. pratensis* and *R. freynii*, and height of the head receptacle in *S. columbaria* (in this species, at seed maturity, the receptacle is more developed in height than in width).

The total number of ovules/seeds per ovary/fruit and OSU (corresponding to the seed/ovule ratio in Wiens, 1984) were calculated from the data obtained. Seeds extracted from the fruits were counted, weighed and analysed for germinability and viability according to the ISTA (2008) rules. Four replicates of 100 seeds were placed on filter paper in Petri dishes and moved into a growth chamber to germinate for four weeks under the following conditions: 8h light/25 C and 16h dark-ness/15 C. Ungerminated seeds were checked for viability using the tetrazolium test. Total viability was the germinability value plus the viability of the ungerminated seeds. The numbers of ovules and viable seeds per shoot was calculated based on the above reproductive characteristics.

Data analysis

The reproductive traits were subjected to statistical analysis in order to detect differences among the collections. After checking whether the data met parametric analysis assumptions using the Shapiro–Wilk and Bartlett tests, the mean values obtained for the collections were compared by analysis of variance (ANOVA). When the means were three, significant ANOVA results were followed by two orthogonal contrasts (Sokal & Rohlf, 2012), which checked for differences among sites and years within site. For each trait, the percent coefficient of variation (CV) among the collection means was calculated.

The allometric relationship between inflorescence size and ovule number was investigated using the statistical approach suggested by Warton et al. (2006). Bivariate lines were fitted with the standardized major axis (SMA) method after log₁₀ data transformation. The

functions obtained were checked for differences in their slopes from 1 (test for isometry) and differences in their elevations from 0. Among-collections comparisons were then performed of the slopes and elevations of the relationships by contrasting collections from different sites and in different years within the same site.

The relationship between the number of ovules per inflorescence and the number of filled seed produced was analysed using the same SMA approach as above.

The software used for all analyses was R 3.0.0 (Core Team R, 2013). The packages were STATS for the ANOVA, the Shapiro–Wilk test, Bartlett test and orthogonal contrasts among sites and years within site, and SMATR (Warton et al., 2012) for line-fitting and among-line slope and elevation comparison.

Results

Achillea roseo-alba

The Candaten collections had similar size characteristics of their reproductive structures, and several traits differed significantly to those in the Pianari collection (Table 2).

The individual size parameters (number of corymbs per shoot, number of heads per corymb, and number of flowers per head) were sometimes higher at Candaten and sometimes at Pianari. As a consequence, there was relatively little difference in the number of ovules per shoot (range 274–321). The trait was most influenced by the number of heads per corymb.

The ovule-to-seed transformation efficiency was significantly higher at Pianari than at Candaten (71 vs. 31–36%, respectively; Table 2). This result was counterbalanced by lower germinability and viability, so that the numbers of viable seeds produced were not significantly different.

Centaurea nigrescens

Morphological characteristics of the reproductive structures of the two collections never differed significantly (Table 2). The mean number of ovules per shoot was about 350.

High OSU and 1000-seeds weight values were recorded for the Pianari 2012 collection (Table 2), so the number of filled seeds per shoot was also high in P2012. However, the Rubbio collection had higher seed germinability and viability.

Tragopogon pratensis subsp. orientalis

The number of flowers per head was similar in all collections (average, 60.2) (Table 2). However, the number of heads per shoot differed significantly in both the site and year contrasts, as did the number of ovules per shoot (average, 189).

The Pianari collections had higher OSUs, seed masses and germinabilities than the Rubbio collections, but similar seed viability (Table 2). The greater head number per shoot and OSU values meant that the Pianari collections also had higher viable seed production (average, 79.2 vs. 35.4 viable seeds per shoot for Pianari and Rubbio, respectively).

Scabiosa columbaria subsp. columbaria

For all of the inflorescence-size traits, the Rubbio 2011 values were much higher than the Rubbio 2009 values (Table 2) with a wide range for the resulting number of ovules per shoot (184–489), that was mainly caused by a large difference in the number of heads per shoot.

With the exception of germinability, which was always very low, seed quality was higher in 2011 (significantly higher OSU and 1000-seed weight) than in 2009 (Table 2). The concordant higher values of inflorescence size, OSU, and viability in R2011 resulted in an almost fourfold higher viable seed production for this collection.

Salvia pratensis subsp. pratensis

The main reproductive characteristic influencing ovule production per shoot (range 225–453, Table 2) was the number of racemes per shoot (range 1.4–3). Both characteristics varied significantly among sites and years. However, the number of flowers per raceme differed little among the collections.

OSU was rather high (on average 75%) and varied little among the collections. All of the seed quality traits exhibited high between-years and between-sites variability, without any clear relationship with the site. Germinability and viability were very low in all collections; the number of viable seeds per shoot (average, 24.5, Table 2) was much lower than ovule number.

Rhinanthus freynii

Collection site had a significant effect on size characteristics. The number of racemes per shoot was higher at Rubbio, but the numbers of flowers per raceme and ovules per flower were higher at Pianari (Table 2). As the differences were not consistent, the number of ovules per shoot did not significantly differ between sites.

The OSU values were high for all collections (average, 84.5%). Therefore, the number of filled seeds per shoot did not decrease much compared to ovule number (Table 2). Seed germinability was very low or nil in all collections. However, viability increased to 24% on average, being very variable among years. Therefore, the number of viable seeds was much lower than the number of ovules, and highly variable among collections.

Species	<i>Achillea roseo-alba</i>					<i>Tragopogon pratensis</i> subsp. <i>orientalis</i>					<i>Centaurea nigrescens</i>		
Site / year of collection ¹	C2013	C2014	P2010	Site ²	Year ²	P2009	P2012	R2009	Site ₂	Year ₂	P2012	R2009	Site ²
No. corymbs per shoot	5.3	4.1	2.5	**	ns	-	-	-	-	-	-	-	-
No. heads per corymb	4.4	4.7	7.0	*	ns	-	-	-	-	-	-	-	-
No. heads per shoot	23.2	19.2	17.2	ns	ns	4.01	2.80	2.50	**	**	6.7	5.6	ns
No. flowers per head	12.1	14.4	17.5	*	ns	62.3	59.2	59.2	ns	ns	58.2	55.8	ns
No. ovules per shoot	290	274	321	*	ns	250	166	152	*	*	387	315	ns
Ovule Site Utilization (%)	31.1	36.1	71.0	**	ns	39.4	45.6	27.2	*	ns	38.2	16.0	**
1000-seed weight (g)	0.149	0.148	0.160	**	ns	8.94	7.95	5.62	**	**	2.4	1.7	**
Germinability (%)	28.5	25.3	9.8	ns	ns	88.3	89.5	77.5	*	ns	53.7	63.3	**
Viability (%)	62.5	55.5	33.8	ns	ns	93.5	89.5	90.8	ns	ns	68.4	81.3	**
No. viable seeds shoot ¹	56.7	59.8	72.7	ns	ns	91.1	67.3	35.4	**	*	102	39	**

Species	<i>Salvia pratensis</i> subsp. <i>pratensis</i>					<i>Rhinanthus freynii</i>					<i>Scabiosa columbaria</i> subsp. <i>col.</i>		
Site / year of collection ¹	P2009	P2012	R2009	Site ²	Year ²	P2009	P2012	R2009	Site ₂	Year ₂	R2009	R2011	Year ²
No. racemes per shoot	1.42	3.00	1.67	**	**	1.67	1.17	3.68	**	ns	3.95	8.06	**
No. flowers per raceme	33.4	37.8	34.8	ns	ns	12.4	16.4	9.6	**	**	45.90	62.6	**
No. ovules per ovary	4	4	4	ns	ns	13.7	11.9	9.7	**	**	1	1	ns
No. ovules per shoot	188	453	225	*	**	282	224	327	ns	ns	184	489	**
Ovule Site Utilization (%)	75.9	75.2	76.2	ns	ns	82.8	98.6	72.1	**	**	68.9	88.6	**
1000-seed weight (g)	0.89	1.27	1.01	ns	**	3.1	3.1	2.8	**	ns	0.73	0.99	**
Germinability (%)	5.10	1.00	11.9	**	**	0	0	5.16	**	ns	13.1	3.8	**
Viability (%)	16.4	6.5	26.5	**	**	39.6	9.3	23.0	ns	**	67.1	71.3	ns
No. viable seeds shoot ¹	17.8	18.7	37.0	*	ns	92.7	20.0	55.4	ns	**	85	305	**

Legend, 1) Site / year of collection; see Table I. 2) Significant site and year effects: ns not significant, * significant at $p \leq 0.05$, ** significant at $p \geq 0.01$.

Table 2 Reproductive characteristics of six grassland forbs collected from different sites and in different years in the Italian Eastern Pre-Alps.

Among-species comparison of reproductive characteristics

In most species, inflorescence number per shoot had the highest effect on ovule and seed production, and was the most variable morphological trait among the collections (average CV 34% vs. 31% for the flower number per inflorescence: Table 3).

The average OSU value was 58% for all species. Within-species variability due to site or year was often significant, but among-species variation was much higher: the trait values were relatively low in *Asteraceae* (27-46%) and high in the other species (76–85%).

The average germinability was generally low at 30% for all species. However, variability was high amongst species (range 2–85%) and collections of the same species (average CV, 68%). The average viability was 54%, which was considerably higher than germinability with the exception of *R. freynii*, which had particularly low values. The large difference between viable and germinable seeds resulted in a high proportion of dormant seeds (all-species average, 44.5% of viable seeds).

In most species the number of viable seeds per shoot was low compared to the ovule number (on average 79 viable seeds vs. 290 ovules). The low proportion of ovules that had formed viable seeds was either due to low OSU values with a concurrent higher viability (particularly *Asteraceae*) or low viability with a contemporaneously higher OSU (the other species). Only in *S. columbaria* were both OSU and viability high.

Allometric relationships

Relationships between inflorescence size and ovule number were most frequently negatively allometric (Table 4), meaning that larger inflorescences had fewer flowers (1/4ovules) per unit of inflorescence size (lower flower density on the inflorescence).

In almost all species, the slope of the linear relationship between the ovule and filled seed numbers was significantly higher than 1 (Figure 1), which resulted in a higher ovule-to-seed transformation efficiency in larger inflorescences. The values of the elevation were always negative, that is, no species was able to transform all of their ovules into seeds. More negative elevation values (e.g. in the three *Asteraceae*) corresponded to low OSU values (see Table 2), and values closer to 0 corresponded to high OSU values.

For both relationships inflorescence size and ovule number, and ovule number and filled-seed number, equations for the same species in different sites or years differed significantly in slope or elevation (Table 4 and Figure 1), indicating considerable environmental and/or genetic variability of the reproductive structure and OSU.

Species	<i>Achillea roseo-alba</i>	<i>Centaurea nigrescens</i>	<i>Tragopogon pratensis</i>	<i>Scabiosa columbaria</i>	<i>Salvia pratensis</i>	<i>Rhinanthus freynii</i>	Mean
No. corymbs per shoot	4.0 (36)	-	-	-	-	-	4.0 (36)
No. heads per corymb	5.3 (27)	-	-	-	-	-	5.3 (27)
No. simple infl. per shoot	20 (15)	6.1 (13)	3.1 (26)	6.0 (48)	2 (42)	2.2 (61)	7 (34)
No. flowers per simple infl.	15 (18)	57 (3)	60 (3)	54 (22)	35 (6)	13 (27)	39 (13)
No. ovules per shoot	295 (8)	351 (15)	189 (28)	337 (64)	289 (50)	277 (19)	290 (31)
Ovule Site Utilization (%)	46 (47)	27 (58)	37 (25)	79 (18)	76 (1)	85 (16)	58 (28)
1000-seed weight (g)	0.15 (4)	2.05 (21)	7.5 (23)	0.86 (21)	1.1 (18)	3.0 (6)	2.4 (16)
Germinability (%)	21 (47)	58 (12)	85 (8)	8.4 (78)	6.0 (92)	1.7 (173)	30 (68)
Viability (%)	51 (30)	75 (12)	91 (2)	69 (4)	16 (61)	24 (63)	54 (29)
No. viable seeds per shoot	63 (13)	70 (63)	65 (43)	195 (80)	24 (44)	56 (65)	79 (51)

Table 3 Comparison of the reproductive traits of six grassland forbs from the Italian Eastern Pre-Alps. Mean values with percent coefficient of variation among collections from different sites or years

Species	Collection	Slope	Elevation	Species	Collection	Slope	Elevation
<i>Achillea roseo-alba</i>	C2013	1.75*	1.18*	<i>Salvia pratensis</i> subsp. <i>pratensis</i>	P2009	1.06ns	-0.04ns
	C2014	1.01ns	1.81*		P2010	1.07ns	-0.13ns
	P2010	1.13ns	2.04*		R2009	0.68*	0.75*
	Site	ns	.		Site	.	.
	Year	.	.		Year	ns	.
<i>Centaurea nigrescens</i>	P2012	0.63*	0.80*	<i>Scabiosa columbaria</i> subsp. <i>columb.</i>	R2009	0.64*	1.04*
	R2009	0.78ns	0.52*	R2011	0.93ns	1.04*	
	Site	ns	.	Year	ns	.	
<i>Tragopogon pratensis</i> subsp. <i>orientalis</i>	P2009	0.6*	0.99*	<i>Rhinanthus freynii</i>	P2009	0.91ns	0.6*
	P2012	0.38*	1.36*		P2012	1.18*	-0.17ns
	R2009	0.77ns	0.72*		R2009	0.97ns	0.34*
	Site	.	..		Site	.	ns
	Year	.	.		Year	.	.

Next to the parameter value, ns and 0 indicate whether the slope significantly differed from 1 and the elevation significantly differed from 0: ns not significant, 0 different at $p \leq 0.05$. The significance of the site and year contrasts between lines is shown below the parameter values. A graphical representation of the relationships can be found in Supporting Information Figure S1.

Table 4 Characteristics of the allometric relationships between inflorescence size and number of ovules in collections of grassland forbs from the Italian Eastern Pre-Alps. Values shown are the line slopes and elevations obtained after log₁₀ data transformation.

Discussion

Efficiency and ecology of the seed production

Comparing the results obtained here with those from other studies shows that the size of the reproductive structures was highly variable. For example, the number of inflorescences per shoot found here versus that obtained by Steiner (2011) was 6 versus 3.4, respectively, in *S. columbaria* and 2.03 versus 2.53, respectively, in *S. pratensis*. The difference was even greater for the number of seeds per inflorescence (46 and 110 in *S. columbaria* and *S. pratensis*, respectively, vs. 13 and 28 in Steiner, 2011). The high variability of the first trait was probably due to shoot age at collection time, because the species studied exhibit indeterminate growth. The different results obtained for the second trait were probably caused by the measurement method. Counting the number of seeds on shoots collected at the end of the reproductive stage underestimates the total seed production, because it does not take into account the seeds that were shed before collection (Scotton et al., 2009).

The trait with the greatest effect on seed production was almost always inflorescence number per shoot. Clifford and Baird (1993) also found that ovule and seed production by *Trifolium repens* is mainly affected by the number of heads per shoot. Flower number per inflorescence, and in *R. freynii*, ovule number per ovary had much less influence due to poor trait variability. This result is very similar to that reported by Lorenzetti (1993), who found that ovule number per flower is the least variable of all reproductive traits.

OSU variability among collections of the same species was much lower than among species. It seems, therefore, that the OSU level is a characteristic trait of a species. Similarly, Thomas (1987) reported that different authors had recorded comparatively low OSU levels (about

50%) for *T. repens*. A multispecies analysis by Wiens (1984) suggested that the seeds/ovules ratio appears to be largely genetically determined. With regard to the *Asteraceae*, the low OSU could have been caused by increased competition for nutrients among many, close flowers on the same head. A similar hypothesis was suggested by Atwood (1943) to explain the abortion of developing seeds in heads of *T. repens*.

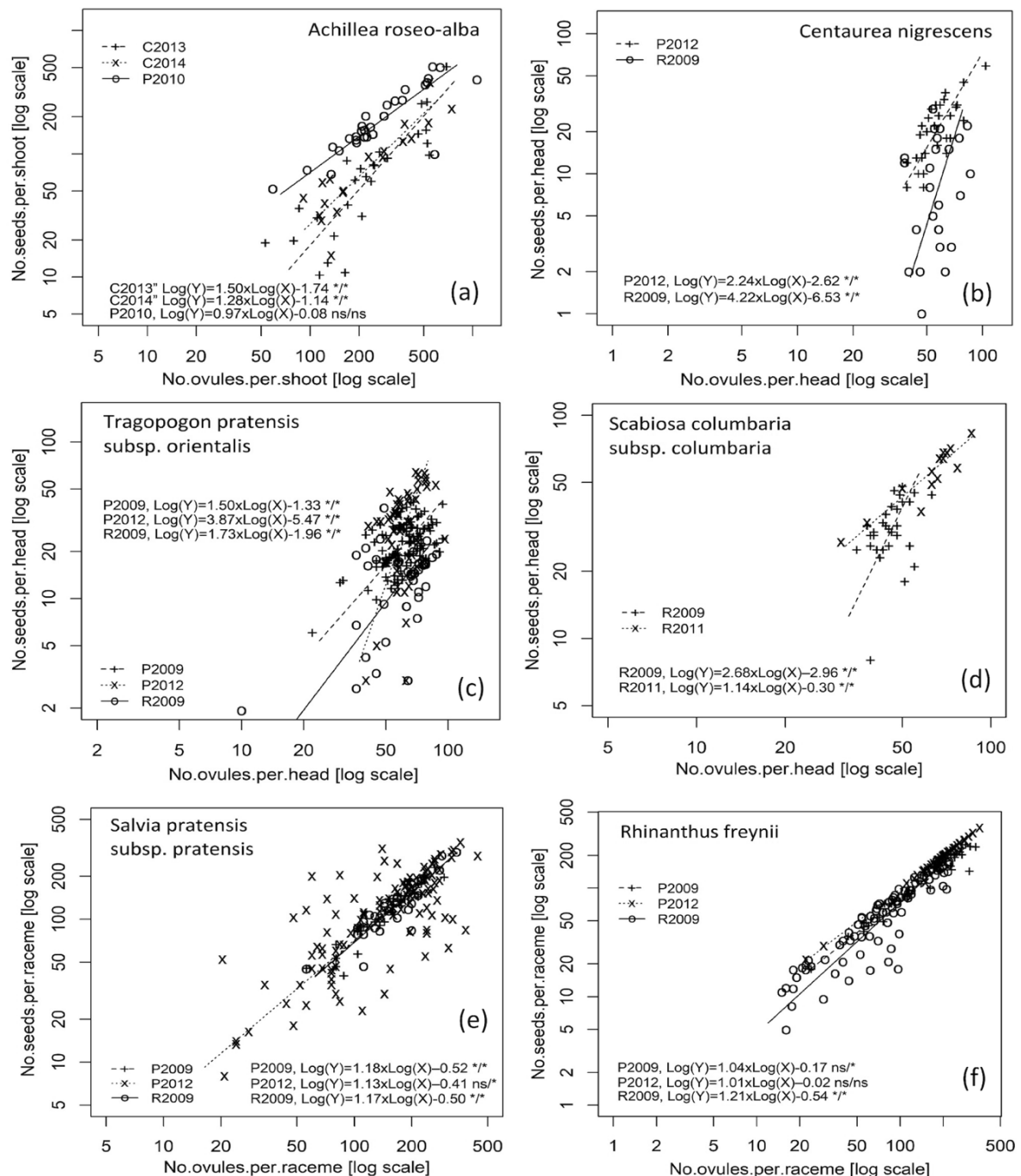


Figure 1 Relationships between ovule and filled seed number per shoot, head or raceme in collections of six grassland forbs from the Italian Eastern Pre-Alps. Collection codes: see legend in Table 1. All equations were significant at $p \leq .05$, except for R2009 in *C. nigrescens*. Symbol/symbol after an equation indicates that the slope and elevation significantly differed from 1 and 0, respectively, at $p \leq .05$ (0) or were not significantly different (ns) from 1 and 0, respectively. Significance of the orthogonal contrasts: *A. roseo-alba*: site (C2013/C2014 vs. P2010), slope 0,

elevation 0; year (C2016 vs. C2014), slope ns, elevation ns; *C. nigrescens*: site (P2012 vs. R2009), slope ns, elevation 0; *T. pratensis*: site (P2009/P2012 vs. R2009), slope ns, elevation 0; year (P2009 vs. P2012), slope 0, elevation 0; *S. columbaria*: year (R2009 vs. R2011), slope 0, elevation 0; *S. pratensis*: site (P2009/P2012 vs. R2009), slope ns, elevation ns; year (P2009 vs. P2012), slope ns, elevation ns; *R. freynii*: site (P2009/P2012 vs. R2009), slope 0, elevation 0; year (P2009 vs. P2012), slope ns, elevation 0.

We found an inverse relationship between OSU and percent viability. Therefore, there may be a compensatory effect between the two traits, as found by Scotton (2018) in a set of 20 grassland forbs. In the *Asteraceae*, the combination of high viability and low OSU is probably related to inflorescence structure. The closeness of many flowers in the head favours pollination (Ishii et al., 2008), because the head is much more easily seen by pollinators than individual, separate flowers. However, competition among close flowers means that the proportion of them filling with seeds is low. Larger inflorescences, being more efficient at transforming ovules to seeds may confirm this hypothesis, because in these inflorescences, the flowers are less dense (negative allometry), so competition between them is lower. In contrast, on racemes, flowers are more distant and less easy to see (lower pollination and seed viability), but there is less competition for seed formation (higher OSU). Germinability was generally low and highly variable within species. Similar low germinability percentages (about 25%) for seeds collected from grasslands of the same type as those studied here were found by Heilinger & Florineth (2003). High among-collections inconsistencies in the trait are often found in native species because germinability is highly influenced by environmental variability (McDonald, 2002). Viability was considerably higher than germinability, but still much lower than the minimum threshold needed for the seed marketing of selected varieties of forage forbs (in most cases, 80%) (EEC 1966). This difference is primarily due to the selection of cultivars that produce high-quality seeds (Schroder & Prasse, 2013) and to a different incidence of pathogens, which are kept under control with appropriate treatments during seed propagation (Hill et al., 1997).

Negative allometric relationships between plant size and reproductive output have been reported in several species by Weiner et al. (2009). Our results show that this type of relationship can also occur within the inflorescence, that is, between the size of the supporting inflorescence structures and ovule number. Tegegnetwork et al. (2015) also reported that flower (1/4ovule) density in cultivated *Helianthus annuus* was low on heads with large receptacles, probably because of the good nutritional status of large inflorescences that positively affects the vegetative more than the reproductive part of the inflorescence. Our results also suggest that when studying reproductive allometry, supporting structures should be included in the vegetative biomass rather than in the reproductive biomass (Bazzaz & Reekie, 1985).

Slope values significantly higher than 1 for the linear relationships between log-transformed ovule and seed numbers (i.e. high OSU in large inflorescences) were the most frequent results of this study. Similarly, Tegegnetwork et al. (2015) showed that heads produced by sunflower under high nutrient availability were more ovule-rich and more efficient at transforming ovules into seeds. In grass-seed propagation, high OSU values have been observed when nutrient availability is high (Hampton & Fairey, 1997). Therefore, it is probable that besides inducing the formation of more flower-rich but less flower-dense inflorescences (as seen before), good nutritional conditions also positively affect OSU, and a major reason for low

OSU is the abortion of developing seeds due to insufficient assimilate availability (Hampton, 1988).

Implications for a seed-based restoration

Significant relationships between inflorescence size and ovule/seed number useful to predict the amount of the seed production could be calculated for all studied species using acceptable time consumption. However, in most cases, the equations differed among the collections, so should, therefore, be created for each growth season and site if they are to be used to accurately estimate reproductive output. More reliable predictions could be made from the trait that frequently had the greatest effects on the ovule production per shoot, that is, the number of simple inflorescences. This is also a trait that can be simply and rapidly assessed.

The high percentage of dormant seeds found in this study means that the direct sowing of naturally collected seed may result in slow plant establishment, which would be particularly problematic for *R. freynii* as seed survival in the soil is usually short-lived for *Rhinanthus* spp. (Thompson et al., 1997). To overcome this problem, suitable dormancy breaking treatments could be applied. For example, *S. pratensis* seeds can be pre-chilled for up to seven days (ISTA 2008), *Rhinanthus* for several months (Ter Borg, 2005) and *S. columbaria* for four weeks (Plant World Seeds, 2017). Alternatively, an autumn sowing is advisable to obtain a spring germination after natural cold stratification (Benvenuti & Piotta, 2013).

The high seed weights recorded for *T. orientalis* and *R. freynii* show that these short-lived species have a reproductive strategy that is based on a high probability of seedling establishment. For the former species, this was confirmed by its high seed germinability and viability values. In the hemiparasitic *R. freynii*, low viability can be compensated for by a high rate of seedling survival, and the seedlings' ability to acquire water and nutrients from the host's roots after early haustoria formation (Westbury, 2004). *S. pratensis*, *A. roseo-alba*, and *C. nigrescens* had relatively low seed production efficiencies, which could hamper their establishment on restored grasslands. To overcome this, a relatively high sowing density is advisable. The problem can also be overcome for by the perennial growth character of the three species, and their ability to vegetatively propagate. In fact, *C. nigrescens* and *S. pratensis* are often abundant in the mesic hay meadows of Northern Italy (Buffa et al., 1995). *S. columbaria* had the most efficient seed production, which is probably why it is found at high frequency in the calcareous grasslands of North-Eastern Italy (Chiappella Feoli and Poldini, 1993).

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by: the European Union under the European Territorial Cooperation Programme CENTRAL EUROPE 2007–2013, project 1CE052P3 SALVERE; the Padova University, DOR funding.

Reference

- Atwood SS. 1943. 'Natural crossing' or white clover by bees. *J Am Soc Agron.* 35:862–870.
- Baskin CC, Baskin JM. 2014. *Seeds—ecology, biogeography, and evolution of dormancy and germination*. 2nd ed. Sand Diego, London, Waltham: Academic Press.
- Bazzaz FA, Reekie EG. 1985. The meaning and measurement of reproductive effort in plants. In: White J, editor. *Studies on Plant Demography*. London: Academic Press; p. 373–387.
- Benvenuti S, Piotta B. 2013. La biologia florale e la germinazione [Flower biology and germination]. In: Bretzel F, Romano D, editors. *Specie erbacee spontanee mediterranee per la riqualificazione di ambienti antropici* [Native Mediterranean herbaceous species for the improvement of anthropic environments]. Roma: ISPRA; p. 47–79. Italian.
- Bertke E, Margraaf R, Isselstein J. 2005. Species-rich grassland as an ecological good in an outcome-based payment scheme. In: O'Mara FP, Wilkins RJ, Mannelje L, Lovett DK, Rogers PAM, Boland T, editors. *XX International Grassland Congress: Offered papers. Conference Proceedings*. Wageningen: Wageningen Academic Publishers; p. 757.
- Bretzel F, Romano D. 2013. Gli ecosistemi antropici [Anthropic ecosystems]. In: Bretzel F, Romano D, editors. *Specie erbacee spontanee mediterranee per la riqualificazione di ambienti antropici* [Native Mediterranean herbaceous species for the improvement of anthropic environments]. Roma: ISPRA; p. 16–28. Italian.
- Buffa G, Marchiori S, Ghirelli L, Bracco F. 1995. I prati ad *Arrhenatherum elatius* (L.) Presl delle Prealpi Venete [The *Arrhenatherum elatius* grasslands in the Italian Eastern Pre-Alps]. *Fitosociologia*. 29: 33–47. Italian.
- Bullock JM, Pywell RF, Walker KJ. 2007. Long-term enhancement of agricultural production by restoration of biodiversity. *J Appl Ecol.* 44: 6–12.
- Chiappella Feoli L, Poldini L. 1993. Prati e pascoli del Friuli (NE Italia) su substrati basici [Grasslands of Friuli (NE Italy) on basic substrates]. *Studia Geobotanica*. 13:3–140. Italian.
- Clifford PTP, Baird IJ. 1993. Seed yield potential of white clover: characteristics, components and compromise. *Proceedings of the XVII International Grassland Congress*. Palmerston North: New Zealand Grassland Association; p. 1678–1679.
- Corbin JD, Andrew RD, Seabloom EW. 2007. Competitive interactions. In: Stromberg MR, Corbin JD, D'Antonio CM, editors. *California Grasslands: Ecology and Management*. Berkeley, Los Angeles, London: University of California Press; p. 169–179.
- Core Team R. 2013. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. <http://www.R-project.org/>.
- EEC. 1966. Council Directive 66/401/EEC of 14 June 1966 on the marketing of fodder plant seed.
- Elgersma A, Stephenson AG, den Njis APM. 1989. Effects of genotype and temperature on pollen tube-growth in perennial ryegrass (*Lolium perenne* L.). *Sex Plant Reprod.* 2:225–230.

- Fenner M, Thompson K. 2005. The ecology of seeds. New York: Cambridge University Press.
- Gibson DJ. 2009. Grasses and grassland ecology. New York: Oxford University Press.
- Gilbert LO. 1989. The ecology of urban habitats. London: Chapman and Hall.
- Hampton JG. 1988. Herbage seed production. *Ad Res Technol Seeds*. 11: 1–27.
- Hampton JG, Fairey DT. 1997. Components of seed yield in grasses and legumes. In: Fairey DT, Hampton JG, editors. Forage seed production volume 1: temperate species. Oxon New York: CAB International; p. 219–242.
- Heilinger K, Florineth F. 2003. Qualitätsprüfung von nicht gezüchtetem Saatgut [Quality aspects of uncultivated seed mixtures]. *Rasen-Turf- Gazon*. 3:56–66. German.
- Hill MJ, Hampton JG, Hill KA. 1997. 9. Seed quality of grasses and legumes. In: Fairey DT, Hampton JG, editors. Forage seed production volume 1: temperate species. Oxon New York: CAB International; p. 45–70.
- Ishii HS, Hirabayashi Y, Kudo G. 2008. Combined effects of inflorescence architecture, display size, plant density and empty flowers on bumble bee behaviour: experimental study with artificial inflorescences. *Oecologia*. 156:341–350.
- ISTA (International seed testing association). 2008. International rules for seed testing. Basserdorf, Switzerland.
- Klotz S, Kuhn I, Durka W. 2002. Biolflor: eine Datenbank mit biologischökologischen Merkmalen zur Flora von Deutschland [Biolflor: search and information system on vascular plants in Germany]. Bonn: Bundesamt für Naturschutz. German.
- Lorenzetti F. 1993. Achieving potential herbage seed yields in species of temperate regions. *Proceedings of the XVII International Grassland Congress*. Palmerston North, N.Z.: New Zealand Grassland Association; p. 1621–1628.
- McDonald M. 2002. A philosophy of dormancy testing in native species. *Seed Technol*. 24(1):26–35.
- Pignatti S. 1982. *Flora d'Italia* [Flora of Italy]. Bologna: Edagricole. Italian.
- PlantWorldSeeds. 2017. *Scabiosa columbaria*. [accessed 2017 Nov 24]. <https://www.plant-world-seeds.com>.
- Schmid W, Bolzern H, Guyer C. 2007. Mahwiesen - Ökologie und Bewirtschaftung. Flora, Fauna und Bewirtschaftung am Beispiel von elf Luzerner Mahwiesen [Cut meadows – Ecology and management. Flora, Fauna and management with examples of eleven Lucerne cut meadows]. Luzern: Kanton Luzern. German.
- Schroder R, Prasse R. 2013. Cultivation and hybridization alter the germination behavior of native plants used in revegetation and restoration. *Restor Ecol*. 21:793–800.
- Schutz W. 2000. The importance of seed regeneration strategies for the persistence of species in the changing landscape of central Europe. *Zeitschrift für Ökologie und Naturschutz*. 9(1/2):73–83.

- Scotton M. 2016. Establishing a semi-natural grassland: Effects of harvesting time and sowing density on species composition and structure of a restored *Arrhenatherum elatius* meadow. *Agric Ecosyst Environ.* 220:35–44.
- Scotton M. 2018. Seed production in grassland species: Morphobiological determinants in a species-rich semi-natural grassland. *Grass Forage Sci.* 73:764–776. doi:10.1111/gfs.12359.
- Scotton M, Piccinin L, Dainese M, Sancin F. 2009. Seed production of an *Arrhenatherion elatioris* hay-meadow in the eastern Italian Alps. *Grass Forage Sci.* 64:208–218.
- Scotton M, Sicher L, Kasal A. 2014. Semi-natural grasslands of the Non Valley (Eastern Italian Alps): Agronomic and environmental value of traditional and new Alpine hay-meadow types. *Agric Ecosyst Environ.* 197:243–254.
- Sokal RR, Rohlf FJ. 2012. Biometry, the principles and practice of statistics in biological research. 4th ed. New York: W.H Freeman and Co.
- Steiner L. 2011. Einfluss von Fragmentierung und Isolation auf die Populationsbiologie und Diasporenbank von Kalk-Magerrasen (*Mesobrometum erecti*) in Südwestdeutschland [Effect of fragmentation and isolation on population biology and seed bank of calcareous meadows (*Mesobrometum erecti*) in south Germany]. Dissertation der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg. German.
- Tegegnetwork W, Shanwad UK, Wubayehu G. 2015. Nutrient Management in Sunflower (*Helianthus annuus* L.). Saarbrücken: LAP LAMBERT Academic Publishing.
- Ter Borg SJ. 2005. Dormancy and germination of six *Rhinanthus* species in relation to climate. *Folia Geobot.* 40:243–260.
- Thomas RG. 1987. Reproductive development. In: Baker MJ, Williams WM, editors. White clover. Wallingford: CAB International; p. 63–123.
- Thompson K, Bakker JP, Bekker RM. 1997. The soil seed banks of North West Europe: methodology, density and longevity. Cambridge: Cambridge University Press.
- Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. SMATR 3 – an R package for estimation and inference about allometric lines. *Methods Ecol Evol.* 3:257–259.
- Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biol Rev Camb Philos Soc.* 81:259–291.
- Weiner J, Campbell LG, Pino J, Echarte L. 2009. The allometry of reproduction within plant populations. *J Ecol.* 97:1220–1233.
- Westbury DB. 2004. *Rhinanthus minor* L. *J Ecol.* 92(5):906–927.
- Westoby M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil.* 199:213–227.
- Westoby M, Leishman M, Lord J. 1997. Comparative ecology of seed size and dispersal. In: Silvertown J, Franco M, Harper JL, editors. Plant life histories. Cambridge: Cambridge University Press; p. 143–162.
- Wiens D. 1984. Ovule survivorship, brood size, life history, breeding systems and reproductive success in plants. *Oecologia.* 64:47–53.

Annexes

Table S1. Site, management and botanical traits of the collection grasslands.

Collection grassland	Candaten	Pianari	Rubbio
Province	Belluno	Vicenza	Vicenza
Latitude E	12°07'33"	11°37'55"	11°40'18"
Longitude N	46°12'04"	45°46'38"	45°48'08"
Elevation (m a.s.l.)	420	435	1030
Slope (%)	0	20	25
Aspect	-	S	SW
Fertilization (Kg N-P ₂ O ₅ -K ₂ O year ⁻¹)	0-54-108	20-12-27	15-9-21
No. cuts year ⁻¹	2	2-3	1
No. grasses-forbs (100 m ²)	11-33	10-38	13-37
Geological substratum	calcareous, alluvial	calcareous, rocky	calcareous, rocky
Soil pH	7.5	7.5	6.6
Soil depth (cm)	21	15	15
Soil texture	loamy-sand	sandy- loam	sandy-loam
Mean yearly rainfall (mm)	1366	1266	1440
Mean yearly temperature (°C)	10.6	11.1	7.5

Figure S1 Relationships between ovule number per shoot and corymb number (*Achillea roseo-alba*), receptacle area (*Centaurea nigrescens* and *Tragopogon pratensis*), head height (*Scabiosa columbaria*) and raceme length (*Salvia pratensis* and *Rhinanthus freynii*) in collections from North-Eastern Italy. Collection codes: see legend for Table I. All equations were significant at $p \leq 0.05$. Symbol/symbol after an equation indicate that the slope and elevation significantly differed from 1 and 0, respectively at $p \leq 0.05$ (*) or were not significantly different (ns) from 1 and 0, respectively.

Chapter 4

Large-scale grassland restoration in Italian alpine areas: species transfer and vegetation development during the first years after sowing

Valentina Rossetti & Michele Scotton

Abstract

Conservation and restoration of species-rich grassland are important issues of the current European agricultural policy. In landscapes where enough surfaces of semi-natural grassland still exist, restoration can be implemented with the method of the seed-rich green hay transfer. To analyse the potentials of this technique on the large scale, the species richness and structure of the donor and restored grasslands at in two wide restorations implemented on twenty-seven areas in Trentino (eastern Italian Alps) were studied over three-four years after sowing. The absolute transfer rate of donor site species was 82-73% respectively in the two restoration sites corresponding to 60-61 species per restored area but after initially high values it decreased to about 46-45% in the last year, mainly due to the poor persistence of many forbs. Low forb sowing density, high grass competitiveness during the establishment years and a more difficult forb adaptation to the raw soils were probable reasons for this result. However, beside the species loss, a significant gain of grassland species was observed especially in the not fertilized and autumn-grazed restoration site (+ eight species in the last year). In the considered highly natural contexts of the studied restorations, only few exotic species were recorded. In both restorations the species composition developed towards vegetation comparable with the standard meadows in the region, even if the dynamic interactions between transferred species and site created plant communities more adapted to the specific traits of the restoration sites. As most surveyed traits were still significantly changing three-four years after sowing, the study needs to be continued. However, it confirms the efficiency of the green hay transfer method but raises the problem of the forbs transfer as main topic to be faced in both experimental and professional grassland restorations in mountain raw soils.

Keywords: green hay transfer, restoration, species-rich grassland, target vegetation, transfer rate.

Introduction

The species-rich semi-natural grassland created by extensive grazing and haymaking are among the European habitats with the highest biodiversity (EU, 2011). Their composition and structure allow the colonization and survival of many native plants beside rare animals, like birds, insects and mammals (Vickery et al., 2001; Stromberg, 2007).

In the last decades, beside abandonment and forest recolonization, the increase of fertilisation changed profoundly the botanical composition of semi-natural grasslands as shown by many studies (e.g. Scotton et al., 2014). The consequences of this alteration involve the whole society and include the landscape banalisation and the loss of natural and cultural heritage and organoleptic value of the agricultural products.

Beside the crucial efficient conservation of the few remnants of high-quality species-rich grasslands, also the creation of new high-nature value grasslands through ecological restoration has become an important tool for grassland improvement (Pärtel et al., 2015).

Ecological restoration requires that species and ecotypes used are native, i.e. they are used in the same geographical region where they were collected and that the restored vegetation

resembles a natural or semi-natural ecosystem in the region (SER, 2014). The native ecotype requirement is often an obstacle for grassland restoration as seeds of native ecotypes are frequently unavailable in the conventional seed market. This often forces to the use of commercial seed mixtures of genetically improved varieties (Bullock et al., 2002) with the consequent creation of plant communities without any natural value and the risk of contaminating the native gene pools through hybridization (McKay et al., 2005).

In areas where high-quality semi-natural grasslands are still available, one of the most efficient way to obtain seed of native species and ecotypes is harvesting seeds directly from donor grasslands at the stage of seed maturation of as many species as possible. Several harvestings can be carried out on the same grassland area in order to collect species maturing their seed at different times of the growing season.

Among the different methods available, green hay, dry hay and seed stripping are best adapted to the often sloping and little meadows of mountain areas (Scotton et al., 2012). Green hay transfer is an inexpensive method as it can be carried out with equipment normally available to mountain farmers (mower, hay loader wagon). It was demonstrated to be an efficient restoration method in many European experiments (see e.g. Kiehl et al., 2010) and in the Italian Alps (Scotton, 2019). The seed harvested from semi-natural grasslands was always found to be of good quality as regards germinability and viability and allowed for usually high initial species establishment. However, some critical points were also highlighted in past researches.

The number of species transferred from the donor grasslands and permanently established at the restored areas was normally significantly less than the donor site species. One reason for this was that mowing is usually not enough to collect donor site species maturing their seed very early or late in the season (Scotton, 2016). Another important limit was the poor permanent establishment of a significant part of transferred species. For example, in a review of central Europe experiments, three-thirteen years after sowing Kiehl et al. (2010) found final transfer rates of 68% on average on ex-arable fields with topsoil removal. Poor establishment affected above all forb species while grasses were found to suffer much less from the problem. The explanations provided were that grasses normally establish very efficiently through vegetative propagation (tillering), therefore behaving very competitively against forbs which propagate mainly by seed (Klotz et al., 2002) and that the seed content of the propagation material harvested directly from existing grassland is usually much lower for most forbs than for grasses, therefore often allowing the establishment of no or very few forb plants (Scotton, 2016). On coarse soils, the final transfer rate was found to be even less (e.g. 47% and 67% at mining areas and quarries in Kiehl et al., 2010 and Scotton, 2018 respectively), again mainly due to poor forb persistence, so that also a lower ability to grow at raw soils can be suspected for grassland forbs compared to grasses.

A second critical issue is that at least in the first years after sowing the vegetation obtained was not always the initially planned target vegetation. This problem arises from the variability of the specific site and weather conditions of the restoration areas and years (Stuble et al., 2017). For example, dry vs. wet years affect significantly and at various degree the seed germination and establishment of individual grassland species (Bakker et al., 2003) and also of grasses vs. forbs, so that restoration practitioners often recognize “grass and forb restoration years” (e.g. Zavaleta et al., 2003). In mountain areas, this problem is often exacerbated by the low fertility of coarse and sloping soils which acts selectively on the

establishment of species with different ecological behaviour (Scotton, 2018). Further, the time elapsed after sowing is always an important factor for the vegetation achieved. Similarly to the long time needed for the formation of existing grasslands, the species richness of restored grasslands was found to start to resemble that of the target vegetation only 20–50 years after sowing (Pärtel et al., 2015) mainly due to many grassland specialists being able to establish only in the long-term (Waldén & Lindborg, 2018).

Most published studies on grassland restoration were conducted under experimental conditions, whereas restoration carried out by practitioners were less frequently considered (Waldén & Lindborg, 2018), mainly due to the difficulty to exactly set and measure the influencing variables. However, the analysis of the last type of restorations can supply important information for improving the restoration projects. Firstly, it can help check the effectiveness of the knowledge acquired from experimental researches when applied on large areas. Secondly, it can integrate the knowledge acquired from experimental researches with the information obtainable from large-scale projects under real condition (Daoust et al., 2014) where factors usually less important for experimental researches (e.g. high site heterogeneity, administrative problems, insufficient funding) often affect substantially the restoration results.

Therefore, main aim of this study was to monitor the first years results of two large-scale mountain grassland restorations implemented by the Trentino (Italy) Forest Service with the use of the green hay technique. The specific aims of the study were:

- + analysing the efficiency of the green hay technique with regard to the total number of species transferred from the donor sites;
- + studying the time pattern and causes of the changing species richness observed in the first years after restoration;
- + analysing the time pattern of the vegetation structure as regards the balance of the two main functional groups of grasses and forbs;
- + comparing the vegetation obtained to the target vegetation of donor grasslands and the standard grassland types of the region.

Materials and methods

The restoration sites

In years 2014 and 2015 two large restorations were carried out in the Primiero Valley (Trento Province, North-Eastern Italy) by the Public Forest Administration.

The 2014 restoration has an extent of 3.8 ha and was located along the left side of the Vanoi river (46.15946N, 11.72231E), in the municipality of Canal San Bovo (abbreviated CSB) (Annex 1). Prior to the restoration, the vegetation was a spruce (*Picea excelsa*) wood spontaneously established on grassland areas abandoned after the big 1966 flooding. The restoration site is surrounded by spruce forests on the north-eastern site and by the river on the south-west side. The elevation is 670 m a.s.l. and the mean slope about 10%. The geological substratum is alluvium of siliceous rocks and the soil is stony and shallow (average depth of 11 cm). The rainfall is 1342 mm per year with sub-equinoctial distribution and monthly amounts always higher than 100 mm in the growing period. The mean year

temperature is 9.6 °C. Compared to the multiyear mean, the rainfall was high and the temperature low in the months July-September of the sowing year (Annex 2).

The 2015 restoration is located in locality Poline (abbreviated POL: 46.20420N, 11.83245E), Natural Park Paneveggio Pale di San Martino (Annex 1). Prior to the restoration, the vegetation was a spruce forest originated from grassland abandonment in the sixties-seventies of the last century. After the restoration, the site borders with spruce forests to the east and west sides and with grasslands to the north and south sides. The extent is 3 ha. The average altitude is 1250 m a.s.l. and the slope between 24 and 51%. The soil has developed on calcareous moraines and is about 50 cm (25-65 cm) deep. The mean year rainfall is about 1543 mm with sub-equinoctial distribution and monthly precipitation in the growing period always higher than 100 mm. The mean year temperature is 6.4°C. In the sowing year, rainfall and temperature of the months June-July were respectively considerably lower and higher than the multiyear mean (Annex 2).

Restorations and field surveys

The CSB restoration was carried out in June-July 2014, time of optimal seed maturation at the end of the first growth period of the donor grasslands. The restored site was subdivided into eleven areas which were sown with green hay mown from unfertilised *Arrhenatherion elatioris* grasslands located no more than four km away in an altitudinal range of 580-1160 m a.s.l. (Annex 3). The seed-rich hay was cut at the donor sites and spread on the same day on the receptor sites with a surface ratio donor/receptor site of 1:1. The restored areas were fertilized with 30 m³/ha of slurry in June 2014 and 8 t/ha of solid manure in November 2014. Since 2015 the management of the site was shared among three farmers who cut the restored grasslands two or three times per year and fertilised them with slurry or solid manure (100-253 kg of N per ha and year).

The POL restoration was carried out in July-August 2015 on sixteen areas. The donor sites were unfertilised hay-meadows with *Mesobromion* or *Arrhenatherion elatioris* vegetation located no more than 2,6 km away from the restoration site in an altitudinal range of 1200-1300 m a.s.l. (Annex 3). The green hay was cut at the donor sites and the same day spread at the receptor site with a surface ratio of 2:1. No fertilisers were added before the restoration. Since August 2016 the site was managed by one farmer who cut it once per year and fertilised it with solid manure on 1.3 ha (50 kg di N per ha and year).

In 2015 and 2016, the vegetation of each CSB and POL donor grassland was surveyed for vascular plants composition. The same was done for each restored area at the CSB and POL restorations in years 2015-2018 and 2016-2018 respectively. The surveys were carried out twice per year at the end of the first and second growth periods (June and August for CSB and July and September for POL). The surfaces surveyed were the whole donor or restored area (only species presence-absence) and one-two 10x10 m sampling plots per restored area (presence-absence and visual estimation of the percent species abundance). Along with species composition, the percent cover of vegetation, bare soil and mosses and lichens was surveyed. Species were identified and named according to Pignatti (1982). Elevation, aspect and slope of each donor and restored area were obtained from geographical maps available on the cartographic portal of the Trento Province.

In 2015 soil sampling was carried out. Three soil depth measures were taken at each donor and restored area using a graded iron stick. In the same positions, three soil sub-samples were

collected from the 0-10 cm soil layer and mixed to obtain one sample for each donor and restored area. The soil samples were then analysed for texture according to the Italian official methods (G.U., 1999).

Data analysis

The aims of the data analyses were checking the restoration success in terms of amount of species transferred from the donor to the receptor (=restored) areas and traits of the created vegetation; and studying the causes of the results obtained.

In a preliminary analysis, donor and receptor sites of each restoration were compared for differences in soil and site traits. After checking the normal data distribution and variance homogeneity, parametric one-way analyses of variance (ANOVA) with four levels (CSB and POL donor and restored sites) and Tukey tests were performed for each trait. In ANOVA, an orthogonal contrast CSB vs. POL was added to check for among restorations differences.

According to Cochran & Cox (1950), as the two restoration differed with respect to the number of surveying years available, the following analyses were performed separately for CSB and POL.

For each restored area a list of species found at least once in all surveying years and four (CSB) or three (POL) lists of species found in each year after sowing were compiled. Each list was then divided into four categories: 1. species found also at the donor area (D) divided into grasses and forbs, 2. species not found at the donor area (receptor site species) and typical for grassland vegetation (RG) and 3. species not found at the donor area and not typical for grassland vegetation (ruderal or wood species: RO). A similar list was created for the species found at each donor grassland. From the lists, the number of D grasses and forbs (all areas) and RG and RO species (only restored areas) were calculated. Separately for the two restorations and species category, the data sets of species richness obtained were checked for homoscedasticity and normality, if necessary log or square-root transformed and, similarly as done by Waldén & Lindborg (2016), compared with parametric tests (one-way ANOVA and Tukey tests). In this and all following tests, each restored area and donor grassland was considered as a replicate and the factor tested was a combination of site (donor or restored site) and year after sowing with six (CSB) or five (POL) levels (donor grassland; restored area all years: restored area 1st to 4th year in CSB and 1st to 3th in POL). The year after sowing was considered as a fixed factor, as its effect on the studied restoration traits was interesting in itself.

The total and percentage numbers of species transferred (D species found at least once in each restored area) were then referred to the species richness of the corresponding donor grasslands through linear regression.

In the attempt to explain why the many D species showed different frequencies and a different time pattern of presence at the restored areas, two analyses were performed. For each D species the total frequency calculated in the restored areas for the whole surveying period was related to the mean abundance of the same species in the donor grasslands where it had been surveyed. Further, for each D species a ratio between its last year frequency and its maximum frequency (usually occurring in the first or second year after sowing) was calculated. Values of this last trait lower than 1 indicated species loss during the restoration development. For the values of this trait, one-way ANOVAs were performed by using the

Landolt (1977) indicators of ecological behaviour and species functional group (grasses and forbs) as classification factor.

The restored vegetation was further characterized as regards vegetation cover, species abundance, similarity within donor and restored areas and between donor or restored areas and standard meadow types in the region and ecological characteristics.

The time pattern of percent cover of vegetation, mosses + lichens and bare soil was analysed for each restoration through ANOVA using year as classification factor. The same was done for the following traits: percent abundance of D grasses, D forbs, RG and RO species surveyed at the donor and restored areas. Data were checked for assumptions of parametric ANOVA and, if necessary log or square root transformed. When no transformation produced data suitable for parametric tests, a non parametric ANOVA under the Monte Carlo permutation approach was chosen, where the statistical test was the sum of squares between groups (Qb). A post hoc mean comparison was performed with the Tukey's test (parametric ANOVA) or pairwise contrasts (non-parametric ANOVA: Pillar De Patta, 2006).

To analyse the similarity patterns of the restored vegetation, separately for each restoration and year after sowing two types of similarity were calculated: 1. between each restored area and the corresponding donor grassland; 2. between each restored area and the other restored areas. The Jaccard's coefficient (Legendre and Legendre, 1998) which uses presence-absence data was referred to as similarity function. The data set obtained for each restoration site was analysed with a two-way ANOVA with year and type of similarity as class factors. The Tukey post-hoc test was used for pairwise comparison of the individual means. By using again the Jaccard's coefficient, the vegetation of each donor and restored area was compared to the standard species composition of the meadow type in the region (Scotton et al., 2012).

A last analysis aimed at finding whether the time patterns of the species establishment could reveal the particular ecological traits of the restored grasslands in comparison to the donor grasslands. Separately for each restoration and year, the mean values of the eight Landolt's ecological indicators (Landolt, 1977) retrieved from the database of the Mulva-5 software (Wildi & Orloci, 1996) were calculated for each restored area and donor grassland. The obtained values were subjected to ANOVA with year as classification factor.

The statistical software used were: SAS (1985) with procedures GLM for parametric ANOVA, Tukey tests and regression analysis; Multiv (Pillar De Patta, 2006) for non-parametric ANOVA and related post hoc pairwise contrasts.

Results

Soil and site traits of the donor and receptor sites

The two restorations differed significantly for several traits (Table 1). The altitude was lower for CSB and the slope higher for POL. In CSB the slope was much lower in the restored areas than in the donor grasslands. The soils were shallower on the CSB siliceous alluvia than on the POL calcareous moraines. The soil gravel and sand contents were much higher and the silt and clay contents much lower at CSB than at POL. On average, at the CSB donor and receptor sites soils were respectively sandy and gravelly: at the POL donors and receptor sites

they were much finer, sandy loams and loams respectively. At both CSB and POL, the pH was significantly higher in the restored area soils than in the donor grasslands. In general, site and soil differences between donor and receptor areas were very pronounced at the CSB restoration and negligible at the POL restoration.

Restoration Type of site	Canal San Bovo		Poline	
	Donor	Receptor	Donor	Receptor
Altitude (m s.l.m.)	860 b	670 c	1247 a	1250 a
Slope (%)	27 b	11 c	30 ab	35 a
Soil depth (cm)	39 a	11 b	44 a	48 a
% of gravel in the soil	37 b	73 a	13 c	19 c
% of sand in the fine earth	70 b	84 c	59 a	49 a
% of silt in the fine earth	19 c	9 d	24 b	27 a
% of clay in the fine earth	11 b	7 c	16 a	23 a

Table 1. Mean values of the site traits of the donor and receptor sites of two grassland restorations in the Italian Eastern Alps. Anova performed to compare the means in each line was always significant at $p < 0.0001$. The letters show the result of the Tukey's test: different letters mean significant differences at $p < 0.05$. The contrast CSB vs. POL was significant at $p = 0.0147$ for pH and at $p < 0.0001$ for all other traits.

Transfer and loss of species from the donor grasslands

In front of the average number of 73 and 84 species found at the CSB and POL donor grasslands, the total number of species transferred was respectively 59.8 and 61.1 (Fig. 1). The absolute transfer rate (the above values divided by the total number of D species) was therefore 82 and 73%. The percentage of transferred species was higher for grasses (84% in both CSB and POL) than for forbs (81% and 70% in CSB and POL respectively).

The number of D species found on the restored areas was maximum in the first (CSB: 46.7 D species) or second (POL: 53.4 D species) year after sowing and minimum in the last surveying year (34 and 39 species with final transfer rates equal to 46 and 47% in CSB and POL respectively). The loss was due especially to forb species which decreased to 41-43% of the number found in the donor grasslands, while grasses decreased to 61-63%.

The mean number of RG species increased with time in both restorations becoming in the last surveying year about five in CSB and eight in POL. Conversely, the number of RO species was maximum in the first year after sowing (about 12 and 4 in CBS and POL respectively) and minimum (1-2) in the last year.

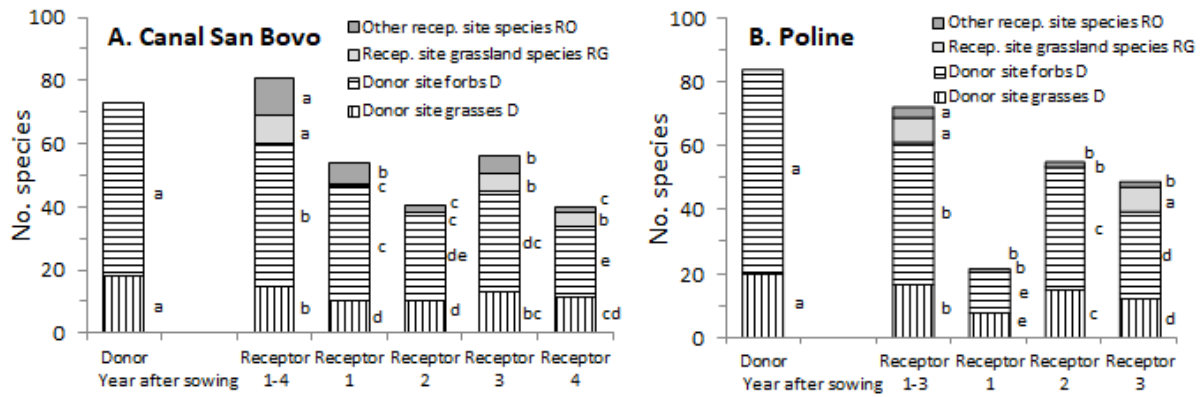


Figure 1. Number of donor and receptor site species in two grassland restoration in the Italian Eastern Alps. Means of eleven (680-5530 m²) and sixteen (700-4400 m²) restored areas in A and B respectively. The letters show the result of the Tukey's test or pairwise non parametric contrasts: different letters mean significant differences at $p < 0.05$.

In both restorations the total number of D species transferred was linearly related to the species richness of the donor grasslands (Fig. 2). However, as the slope of the linear relationship was lower than 1, the percent number of transferred species was inversely related to the species richness of the donor grasslands (Fig. 2). This relationship was much closer at CSB than at POL (Fig. 2) and much clearer in the first years after sowing than in the last year (results not shown).

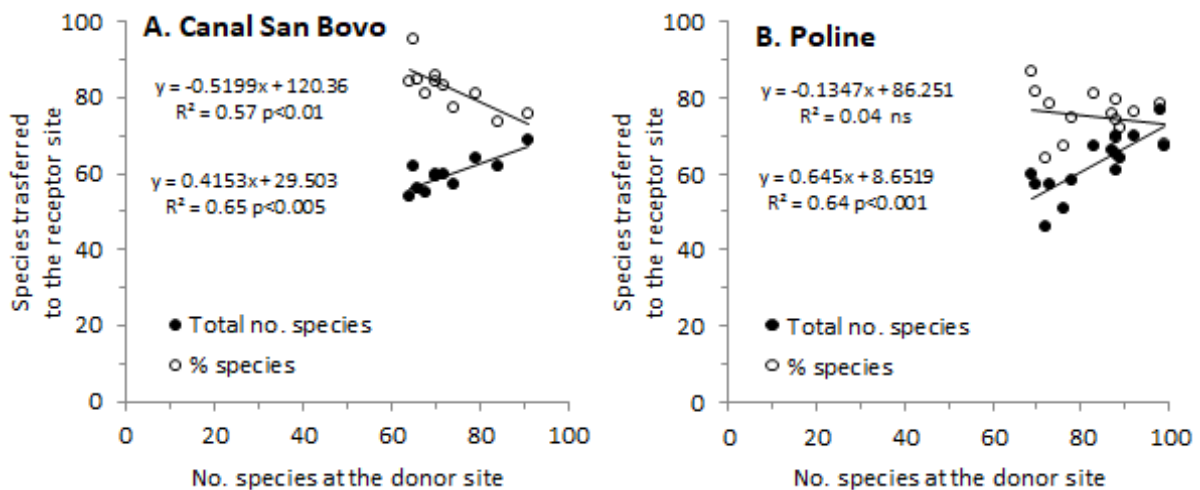


Figure 2. Relationship between number or percentage of donor site species transferred and species richness of the donor grasslands in two grassland restorations in the Italian Eastern Alps. Values from eleven (680-5530 m²) and sixteen (700-4400 m²) restored areas in A and B respectively.

The total frequency of D species in the restored areas was related to the mean abundance of the same species at the donor grasslands and this relationship was also much closer in CSB ($y = 0.48 + 0.16x$, $r^2 = 0.48$, $p < 0.0001$) than in POL ($y = 0.16 + 0.33x$, $r^2 = 0.04$, not significant) (Annex 4).

In CSB the ratio between the last year frequency and the year maximum frequency of D species still significantly related to the mean abundance of the same species at the donor grasslands ($y = 0.128 \ln(x) + 0.53$, $r^2 = 0.40$, $p < 0.0001$). In addition, forbs got lost much more than grasses and species with low abundance at the donor site and of more humid soils almost disappeared while species of dry and medium soils reduced much less (Table 2). In POL only the soil reaction had a significant effect: species of neutral pH soils increased while species of acid or sub acidic soils decreased (Table 2).

RESTORATION				
Canal San Bovo			Poline	
Soil humidity	1	0.63 a	Soil pH 1	-
Soil humidity	2	0.87 a	Soil pH 2	0.72 b
Soil humidity	3	0.79 a	Soil pH 3	0.73 b
Soil humidity	4	0.1 b	Soil pH 4	1.06 a
Soil humidity	5	-	Soil pH 5	-
Grasses		0.57 a	-	-
Forbs		0.37 b	-	-

Table 2. Ratio between the last year frequency and the year maximum frequency of donor site species of different categories found in the restored areas. Results from two grassland restorations in the Italian Eastern Alps. In the grasses vs. forbs comparison, all species were considered, in the soil humidity and pH comparison only very frequent species (species with total multiyear frequency higher than 0.4 and 0.3 in CSB and POL respectively) were taken into account. For letters beside means see legend of Table 1.

Traits of the restored vegetation

In the donor grasslands the soil was 100% covered by vascular plants (data not shown) and forbs (respectively 64 and 56% in CSB and POL) were dominant over grasses (Fig. 3.)

In CSB, the vegetation cover was 68% already from the Autumn of the sowing year and almost 100% in years 2-4 after sowing (Annex 5). Initially, grasses were dominant (56% at the first year after sowing, Fig. 3: especially *Holcus lanatus*: data not shown), in the following phase, forbs increased but in the last year grasses increased again especially due to a strong increment of *Dactylis glomerata*.

In POL, the vegetation cover was low in the Autumn of the sowing year (21%: Annex 5) but increased to 77% in the following Spring and to more than 85% in years 2 and 3. In POL, too, grasses dominated in the first phase (80% in the second year after sowing, Fig. 3, with high *Holcus lanatus* abundance) but later forbs increased greatly (especially *Leontodon hispidus*, *Plantago lanceolata*, *Trifolium repens*, *T. pratense* and *Anthyllis vulneraria*: data not shown). R species played some small role only in the last surveying year (6-7%) and were above all RO species (5.5%) in CSB and RG species (4.8%) in POL (Fig. 3). Mosses and lichens did never play in either restoration, an important role (cover about 0 in all donor and restored grasslands).

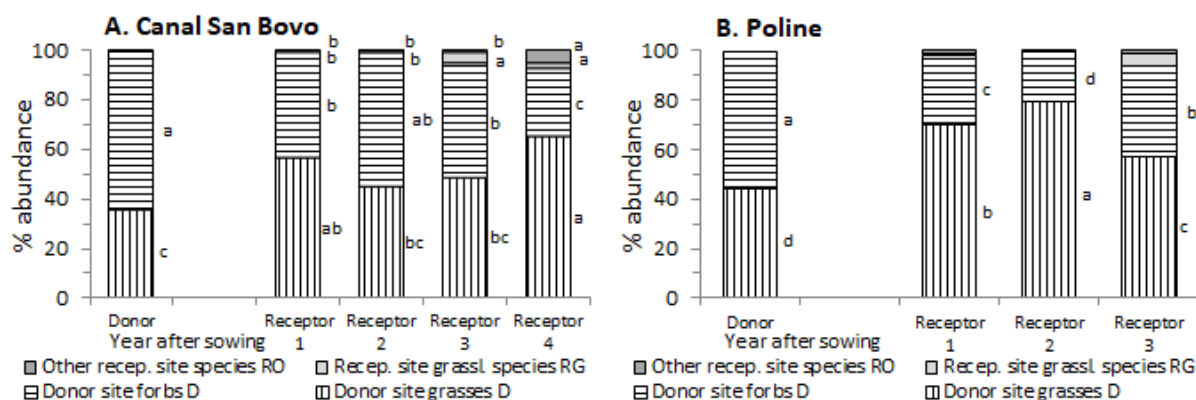


Figure 3. Time pattern of the abundance of different plant species categories in two grassland restorations in the Italian Eastern Alps. Means of eleven and sixteen restored areas in A and B respectively. The letters beside bars show the results of the Tukey test or pairwise non parametric contrasts performed among the values of the same species category. Means with a common letter are not significantly different at $p \leq 0.05$.

After the year of maximum D species number surveyed in the restored areas (year 1 and 2 after sowing in CSB and POL respectively), in both restorations the mean similarity between restored area and the corresponding donor grassland decreased significantly while the mean similarity among restored areas increased (Fig. 4).

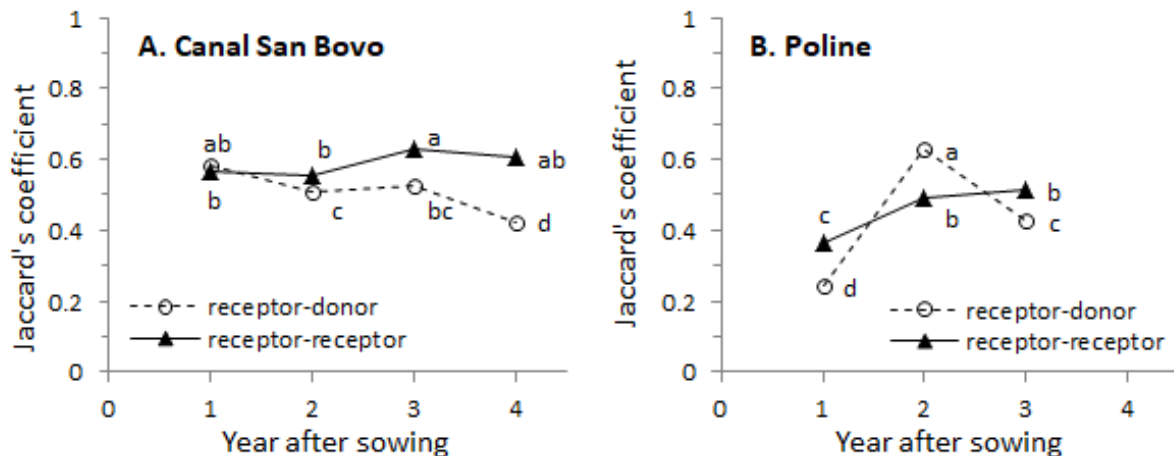


Figure 4. Time pattern of the similarity (Jaccard's coefficient) among restored areas (receptor-receptor) and restored and corresponding donor grasslands (receptor-donor) in two grassland restorations in the Italian Eastern Alps. For letters besides means see legend of Table 1.

The meadow type of the donor grasslands was almost exclusively Ar1 (medium fertilised *Arrhenatherum elatius* meadow) and Br (unfertilised *Bromus erectus* meadow) in CSB and POL respectively (Annex 6). The Jaccard's coefficients between the donor grasslands and the standard meadow types were on average 0.56 e 0.49 respectively for CSB and POL (Table 3). In the CSB restored areas, the maximum values of the Jaccard's coefficient between the vegetation and the standard meadow types increased with time up to values of 0.54-0.59 (Table 3), which were similar to the donor grasslands. However, the vegetation resembled in most cases the donor site vegetation (Ar1) in the first two years but evolved often to meadows of less fertile soils (Av o Ar0: Annex 6) in the last years.

In POL, too, the similarity of the restored areas to the standard meadow types was in the last surveying year only by little less (0.45 vs. 0.49: Table 3), than for the donor grasslands. However, differently from CSB, the more similar vegetation remained the same as in the corresponding donor grasslands in only five out of sixteen cases in the second year and in only one case in the third year when most restored areas became more similar to meadow of soil richer in nutrients (Ar1 e TT0: Annex 6).

	Donor grass- lands	Restored areas			
		Year after sowing			
		1	2	3	4
CANAL SAN BOVO					
Mean Jaccard's coeff.*	0.56	0.49	0.51	0.59	0.54
No. correspondences R-D **	-	10	10	8	6
POLINE					
Mean Jaccard's coeff. *	0.49	0.20	0.39	0.45	-
No. correspondences R-D **	-	1	5	1	-

Table 3. Similarity between donor or restored areas and the Trentino meadow types (Scotton et al., 2012) found in two grassland restorations in the Italian Eastern Alps.

* Mean Jaccard's coeff. is the mean of the maximum values of the Jaccard's coefficient between the grassland and the Trentino meadow types. ** No. correspondences R-D is the number of correspondences between meadow type of the restored area and the corresponding donor grassland.

The mean values of the Landolt's ecological indicators were always significantly different among donor and receptor areas surveyed in the different years (Table 4). Often, such differences were inter-annual variations which did not show a clear trend to values different from the donor grasslands. In other cases, however, such variation led to a significant shift from the donor grasslands. In CSB, this was the case of the soil nitrogen, soil humidity and temperature indicators. Compared to donor grasslands, the first two indicators tended to decrease while the third one tended to increase. Instead, in POL the soil nutrient indicator tended significantly to increase and the pH indicator was initially low and increased in the last year.

Resto- ration	Donor or Receptor (D, R)	Year after sowing	Humi- dity	Light	Tempe- rature	Continen- tality	Soil pH	Soil ni- trogen	Spoil humus	Soil texture
Canal San Bovo	D	-	2.72 a	3.62 b	3.20 b	3.10 a	3.00 b	3.19 bc	3.23 a	4.03 ab
	R	1	2.69 abc	3.61 b	3.33 a	3.09 a	3.05 a	3.34 a	3.17 bc	4.00 b
	R	2	2.71 ab	3.65 ab	3.30 a	3.04 b	3.03 ab	3.28 ab	3.21 ab	4.06 a
	R	3	2.66 bc	3.68 a	3.29 a	3.10 a	3.06 a	3.23 bc	3.16 c	4.00 ab
	R	4	2.64 c	3.66 ab	3.30 a	3.10 a	3.07 a	3.13 c	3.18 bc	4.05 ab
Poline	D	-	2.68 b	3.67 a	3.08 cd	3.12 ab	3.19 a	2.80 c	3.25 a	4.06 ab
	R	1	2.88 a	3.52 b	3.07 d	3.09 b	2.95 c	3.08 a	3.20 ab	4.12 a
	R	2	2.66 b	3.66 a	3.14 bc	3.14 a	3.12 b	2.86 bc	3.18 b	4.02 b
	R	3	2.63 b	3.64 a	3.20 a	3.15 a	3.16 ab	2.94 b	3.20 ab	4.06 ab

Table 4. Time pattern of the Landolt's ecological indicators calculated for the donor and receptor areas in two grassland restorations in the Italian Eastern Alps. For letters beside means see legend of Table 1.

Discussion

The analysis of the site traits highlighted clear differences among the two restorations: lower altitude and coarser soils at CSB vs. higher altitude and finer soils at POL; soil differences between donor and receptor grasslands high at CSB vs. low at POL. In front of these considerable site differences, the study found many aspects of both similarity and divergence between the two restorations.

The found values of relative transfer rate were similar in the two restorations (83-73%). They lied in about the same range of the experiments of grassland restoration in central Europe reviewed by Kiehl et al. (2010) who found ranges of 66-85 and 49-80% respectively in topsoil removal sites and mining areas. A similar value was found by Scotton (2018) in a mining area restoration. These relatively high values seem to be characteristic of restorations using propagation materials containing seeds of all donor site species, as for example material from the first cut of poor-soil grasslands where the seed production is concentrated in the first growing period (see the references above) or from the first and second cut of more mesic grassland with seed production extended also to the second growth period (e.g. Scotton, 2016: 81%). Restoration with materials from only one of the growth periods producing seed resulted usually in lower absolute transfer rates (e.g. Scotton, 2016: 63-65%).

In both restorations, the total number of transferred species was positively related to the species richness of the donor grassland, as usually found (e.g. Kiel et al., 2010). However, in both CSB and POL the absolute transfer rate was instead negatively related to the species richness of the donor grassland, i.e. the species transfer from more species-rich grasslands was less efficient. The main reason for this might be that more species at the donor grassland means an average lower abundance and therefore also less seed per species. This explanation is confirmed at least for CSB by the positive relation found between the species abundance at the donor site and the species frequency at the restored areas. Some past experiments where the sowing density was also measured (Münzbergová, 2012; Scotton, 2016 and 2018) found that this last trait was related to the species establishment at the restored area, indicating 10 seeds per m² being the threshold under which the species establishment is often unsuccessful. In both restorations the maximum and minimum number of D species at the restored areas were found respectively in the first years after sowing and in the last surveying year, so that the final transfer rate (about 46%) was considerably lower than the absolute transfer rate. As in the restorations studied here, such low final transfer rates were found in past experiments carried out at raw soils, e.g. at mined areas (47%: Kiehl et al., 2010) or at quarry waste dumps (63%: Scotton, 2018), whereas on better soils final transfer rates were higher (67% in experiments with topsoil removal, Kiehl et al., 2010; 78% in an experiment on an ex-arable field, Scotton 2016). It is probable that on raw soils many grasslands species are unable to establish permanently even if the transferred seed can successfully germinate. The results from the CSB restoration would confirm this hypothesis as the frequency decrease was much sharper for species typical of more humid than for less humid soils. The same results show that the decrease was stronger for species less abundant (and therefore with less transferred seed) at the donor site and for forbs than for grasses. The last findings underline: that the sowing density is probably important not only for the species transfer but also for the species persistence (Dickson et al., 2009), as small populations are more sensitive to the negative effects of the demographic stochasticity (Harmon & Braude, 2010); and that the higher establishment ability of grasses through tillering (beside their higher mean abundance, i.e. seed production, at the donor sites) should also negatively affect the forb persistence (Kindscher & Fraser, 2000). The homogeneous soil conditions created in both restorations with the use of excavators might also have negatively affected the species richness recorded in the last surveying year, as a large variety of microhabitats within a grassland usually increases the coexistence of more species (Gazol et al., 2012). The POL results suggested only the soil pH as factor affecting the species persistence.

In front of the D species loss, both restorations gained from the second-third year onwards some RG species (grassland species arriving from close restored areas or meadows surrounding the restorations). This occurrence could partially counterbalance the species loss and was more important in POL than in CSB (eight vs. four species on average). The species gain was possible for two main reasons. After restoration the many restored areas were managed (mown) by only three (CSB) and one (POL) farmers. In this way, as found by Strykstra et al. (1996), seeds of species present only in some restored areas could arrive through the mowing operations to other areas where it had not been sown. In POL, a second reason was that after the first mowing the restored areas were grazed in September. Indeed, grazing enhances the species movement within the grazed area through the deposition of faeces containing seeds of the ingested species and the topsoil disturbance due to trampling which favors the establishment of new plants (Smith et al., 2002). Contrary to RG species, RO species (mainly ruderal or invasive alien plants) were only very few (5-6 and 1-2 in the first and last year respectively), which is a low number if compared to other studies in agricultural or quarry sites (e.g. 5 species in the fifth year in Scotton 2016 and 2018). This result was probably due to the land use of the restored sites prior to the restorations, i.e. forest, whose few herbaceous species were not able to establish in open grasslands.

Further traits shared by the two restorations were that the vegetation created was assimilable to standard meadow types of the region and that the vegetation similarity between each restored site and the corresponding donor grassland decreased with time, while the similarity within restored areas increased. This meant that areas sown with propagation materials harvested at different donor grasslands tended to evolve towards a same vegetation typical for the specific restoration site. This occurrence was due to both the selective species loss in each restored area and to the entry of unsown (but evidently site adapted) RG species (see the discussion above). These results may seem to contradict the findings of Stuble et al. (2017) who found that plant communities resulting from sowing the same seed mixture differed significantly across sites and establishment years. However, the Stuble's results were based on considering only the species abundance of a small number (eight) of sown species and his conclusions did not take into account that in perennial-dominated grassland ecosystems the cover and even presence/absence of some species can vary substantially across years due to changing weather conditions, even if the overall species composition does not differ significantly across years (Cleland et al., 2013).

In front of the above similarities, the two restorations showed some important differences as regards the dynamics of the grassland structure and vegetation development.

At the end of the sowing year, the vegetation cover was already very high at CSB (68%) but still low at POL (21%). Two reasons can be suggested for this result. In POL, sowing was carried out one month later (July-August) than in CSB (June-July) and due to the higher altitude, the growing season ended 20 days before. Therefore, in POL plants established had about fifty days less available for growing. Secondly, the 2015 summer, when the POL restoration was sown, was considerably drier than the 2014 summer, when the CSB site was restored. As often observed in grasslands on coarse substrata where the soil dry out very rapidly compared to fine soils (Chapin & Bliss, 1989; Rummel & Holscher, 1955), the 2015 weather caused the death of most seedlings emerged in POL during summer. However, in the Spring of the first year after sowing the vegetation cover was similar in the two restorations mainly due to the grass seed germination and seedling establishment in POL during Autumn

and Spring. This grass behavior in POL confirms the “asynchronous seed germination” of these species (Gasque & Garcia-Fayos, 2003) due to the presence of many dormant and non-dormant seeds in the same seed population. This behavior is typical for grasses (Fenner, 1985) and is a guarantee against the risk of death of the whole progeny which could occur in the case that all seeds germinate at the same time and unfavourable weather conditions after germination compromise the seedlings survival (Guttermann, 1992). The high grass abundance in POL found in Spring 2016 after a relatively dry year does not resemble the results by Stuble et al. (2017) who found grass establishment being favored under wetter conditions and forbs under drier weather. Probably, in POL the dry 2015 summer was equally unfavorable for grass and forb seedlings but the asynchronous seed germination could still allow a strong grass establishment.

The time development of the abundance ratio grasses/forbs was only partially similar in the two restorations. At both sites there was a first phase of high grass abundance followed by a forb increase. However, in a second phase grasses increased again only in CSB. An initial high grass abundance was often observed in restorations where the propagation material was harvested during the first growth period (Thormann et al., 2003; Scotton, 2016), as in temperate grasslands most grass seeds are produced in Spring (see e.g. Martinková et al., 2002). Further, the initial grass predominance was also due to the grass ability of fast establishment through tillering (Pywell et al., 2003). The forb increase observed for the second-to-third year after sowing in both restorations led to grass/forb ratios more similar to the donor grasslands. However, differently from POL, grasses tended to increase again in CSB. This late divergent development of the two restorations was probably due to the different post-restoration management: whereas in POL restored areas were not fertilised, the CSB restored areas received significant amount of organic fertiliser which notoriously increases the grass abundance.

The time development of the vegetation was also different in the two restorations. The trajectory of the CSB vegetation was only partially expected. The trend to a meadow type with a drier character than the donor grasslands (lower Landolt humidity indicator) was in line with the much coarser soil texture at the restored site. A similar result was obtained on a very coarse soil of a quarry waste dump (Scotton, 2018). Instead, the observed frequent formation of a meadow type typical for nutrient-poorer soils was inconsistent with the fertiliser application by farmers in the years after restoration and also with the grass increase (especially the N-loving *Dactylis glomerata*) observed in the last year. As reported by Tilman (1987), one explanation could be that in mixed grasslands fertilisation increases the abundance of more competitive species in a first stage and eliminates the less competitive species only in a second stage.

At POL, too, the vegetation showed an unexpected trend. The restored areas were not fertilised after restoration but the surveys in the last year revealed a vegetation more similar to meadow types of soil richer in nutrients than the donor grasslands. This result is difficult to explain at this stage of the study and needs further analysis. However, maybe that it is due to the difficult establishment of many D (sub-) acidophilus forbs on the soils with higher pH in the restored site.

Conclusion

The current study demonstrated that under conditions of large-scale professional practice semi-natural meadows can be restored with the technique of the green hay transfer with the same efficiency as under experimental conditions. Furthermore, it confirmed that, even if with a significant reduction of the species number, using extensive grasslands as donor sites makes it possible to obtain grassland compositions able to adapt the specific conditions of the restored sites.

The study demonstrated also that already from the third year after sowing the green hay transfer can create grasslands which are comparable for species composition with the standard meadow vegetation of the geographical region, which is one main aim of ecological restoration. However, it also showed that the creation of the new meadows is the result of a dynamic interaction between the transferred species and the specific site traits leading to a vegetation which is not necessarily the same as the donor grassland.

All vegetation traits surveyed in the study were found to change significantly during the first three-four years of restoration development, as it was always found in similar studies. For this reason the current study can be considered only the first part of a longer monitoring which should last at least until ten years after sowing, as it is suggested for a proper development monitoring of grassland restoration (Urbanska & Chambers, 2002).

In particular, beside the soil traits which will need a much longer monitoring, the following topics should be further analysed in detail in the next years:

- + the presence of D species: it was found to change strongly until the last surveying year, so it is important to check when and how it will become stable: this is particularly true for forbs which showed a very strong dynamic;
- + the cover time pattern of the grass and forb functional groups: this trait, too, was strongly variable, probably due to the interaction of several factors, as the different ability of initial species establishment, the site (especially soil) traits and the management;
- + the time development of the vegetation: to this regard, the first years monitoring showed some results which were surprising if compared with the management implemented after restoration: future analyses should reveal when a stable vegetation will install and the traits of the achieved vegetation.

Acknowledgments

This study was funded by the Padova University with doctorate funding. The authors thank Sara Zenatti and Beatrice Zeni for contributing to the field surveys; the Forestry Service of the Trento Autonomous Province (dr. Luigi Gottardo) for supplying the information about the restoration works; the owners of the donor and restored grasslands for permitting surveying and supplying the information about the grassland management.

Annexes 1-6.

Supplementary data can be found after the References section.

References

- Bakker J.D., Wilson S.D., Christian J.M., Li X., Ambrose L.G. and Waddington J., 2003. Contingency of grassland restoration on year, site, and competition from introduced grasses. *Ecological Applications* 13, 137-153.
- Bullock J.M., Moy I.L., Pywell R.F., Coulson S.J., Nolan A.M. & Caswell H., 2002. Plant dispersal and colonization processes at local and landscape scales. In Bullock J.M., Kenward R.E. and Hails R.S. (Eds.), *Dispersal ecology*. Oxford: Blackwell. pp. 297–302.
- Chapin D.M. & Bliss L.C., 1989. Seedling growth, physiology, and survivorship in a subalpine, volcanic environment. *Ecology* 70, 1325-1324.
- Cleland E.E., Collins S.L., Dickson T.L., Farrer E.C., Gross K.L., Gherardi L.A., Hallet L.M., Hobbs R.J., Hsu J.S., Turnbull L. & Suding K.N., 2013. Sensitivity of grassland plant community composition to spatial vs. temporal variation in precipitation. *Ecology* 94(8), 1687–1696.
- Cochran, W.G., Cox, G.M., 1950. *Experimental Designs*. New York, John Wiley and Sons, Inc. Chapman and Hall, Limited, London.
- Daoust R., Doss T., Gorman M., Harwel M.I & Ulrich C., 2014. A 10-year ecosystem restoration community of practice tracks large-scale restoration trends. *S.A.P.I.E.N.S* [Online], 7.2 | 2014, Online since 22 April 2014, connection on 20 April 2019. URL : <http://journals.openedition.org/sapiens/1569>.
- Dickson T.L. & Busby W.H., 2009. Forb species establishment increases with decreased grass seeding density and with increased forb seeding density in a Northeast Kansas, U.S.A., experimental prairie restoration. *Restoration Ecology* 17, 597– 605.
- EU, 2011. Communication from the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions Our life insurance, our natural capital: an EU biodiversity strategy to 2020. COM 2011/0244.
- Fenner M., 1985. *Seed Ecology*. Chapman & Hall, London.
- Gasque M. & Garcia-Fayos P., 2003. Seed dormancy and longevity in *Stipa tenacissima* L. (Poaceae). *Plant Ecology* 168, 279 –290.
- Gazol A., Tamme R., Takkis K., Kasari L., Saar L., Helm A. et al., 2012. Landscape- and small-scale determinants of grassland species diversity: direct and indirect influences. *Ecography* 35, 944–951. doi:10.1111/j.1600-0587.2012.07627.x.
- G.U. (Gazzetta Ufficiale dello Stato Italiano), 1997. Approvazione dei Metodi ufficiali di analisi fisica del suolo. Decreto Ministeriale (1 agosto Suppl.) G.U., 204, 2 settembre 1997.
- Guttermann Y., 1992. Maternal effects on seed germinability: phenotypic maternal effects during seed maturation. *Israel Journal of Botany* 29, 105 –117.
- Harmon L.J. & Braude S., 2010. Conservation of small populations: effective population sizes, inbreeding, and the 50/500 rule. In: Braude S. and Low B.S. (Eds.), *An Introduction to Methods and Models in Ecology, Evolution, and Conservation Biology*. Princeton University Press.

- Kiehl K., Kirmer A., Donath T.W., Rasran L. & Hölzel, N., 2010. Species introduction in restoration projects—evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology* 11, 285–299.
- Kindscher K. & Fraser A., 2000. Planting forbs first provides greater species diversity in tallgrass prairie restorations (Kansas). *Ecological Restoration* 18, 115–116.
- Klotz S., Kühn I. & Durka W., 2002. *Biolflor: eine Datenbank mit biologisch-Ökologischen Merkmalen zur Flora von Deutschland* [Biolflor: search and information system on vascular plants in Germany]. Bonn: Bundesamt für Naturschutz. German.
- Landolt E., 1977. *Ökologische Zeigerwerte zur Schweizer Flora*, Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübel, 64. Heft, Zürich.
- Legendre P. & Legendre L., 2012. *Numerical Ecology*, Third English edition. Elsevier Science BV, Amsterdam.
- Martínková J., Šmilauer P. & Mihulka S., 2002. Phenological pattern of grassland species: relation to the ecological and morphological traits. *Flora* 197, 290–302.
- McKay J.K., Christian C.E., Harrison S. & Rice K.J., 2005. “How local is local?”—A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13, 432–440.
- Münzbergová Z., 2012. Seed density significantly affects species richness and composition in experimental plant communities. *PLoS One* 7 (10), e46704. doi: <http://dx.doi.org/10.1371/journal.pone.0046704>.
- Pärtel M., Bruun H.H. & Sammuli M., 2015. Biodiversity in temperate European grasslands: origin and conservation. Integrating efficient grassland farming and biodiversity. *Grassland Science in Europe* 10, 1-13.
- Pignatti S., 1982. *Flora d'Italia*. Edagricole, Bologna.
- Pillar De Patta, V., 2006. *MULTIV. Multivariate Exploratory Analysis, Randomization Testing and Bootstrap Resampling. User's Guide v. 2.4*. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
- Pywell R.F., Bullock J.M., Roy D.B., Warman L., Walker K.J. & Rothery P., 2003. Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology* 40, 65– 77.
- Rummel R.S. & Holscher C.E., 1955. *Seeding Summer Ranges in Eastern Oregon*. U.S. Dep. Agr. Farmer's Bull. 2091.
- SAS Institute Inc 2002-2012. *SAS/STAT® 9.4* Cary, NC: SAS Institute Inc.
- Scotton M., 2016. Establishing a semi-natural grassland: Effects of harvesting time and sowing density on species composition and structure of a restored *Arrhenatherum elatius* meadow. *Agriculture, Ecosystems and Environment* 220, 35-44. Doi: 10.1016/j.agee.2015.12.029.

- Scotton M., 2018. Calcareous grassland restoration at a coarse quarry waste dump in the Italian Alps. *Ecological Engineering* 117, 174–181. <https://doi.org/10.1016/j.ecoleng.2018.04.012>.
- Scotton M., 2019. Mountain grassland restoration: Effects of sowing rate, climate and soil on plant density and cover. *Science of The Total Environment*, 651, 3090-3098.
- Scotton M., Kirmer A. & Krautzer Bernhard (eds.), 2012. Practical handbook for seed harvest and ecological restoration of species-rich grasslands. CLEUP, Cooperativa Libreria Editrice Università di Padova, ISBN 9788861298002.
- Scotton M., Pecile A. & Franchi R., 2012. I tipi di prato permanente in Trentino. Tipologia agroecologica della praticoltura con finalità zootecniche, paesaggistiche e ambientali. (Types of semi-natural meadows in Trentino (ITA). Agro-ecological typology with zootechnical, environmental and landscape aims) Fondazione Edmund Mach, Centro Trasferimento Tecnologico. 200 pp. e Appendici. ISBN 978-88-7843-038-9.
- Scotton M., Sicher L. & Kasal A., 2014. Semi-natural grasslands of the Non Valley (Eastern Italian Alps): Agronomic and environmental value of traditional and new Alpine hay-meadow types. *Agriculture, ecosystems & environment* 197, 243-254.
- SER International Primer on Ecological Restoration [Internet]. 2004. Available: <http://www.ser.org>.
- Smith R.S., Shiel R.S., Millward D., Corkhill P. & Sanderson D.A., 2002. Soil seed banks and the effects of meadow management on vegetation change in 10-year meadow field trial. *Journal of Applied Ecology* 39, 279–293.
- Stromberg M.R., D'Antonio C.M., Young T.P., Wirka J. & Kephart P.R., 2007. California grassland restoration. In Stromberg M., Corbin J. and D'Antonio C. (Eds.), *California grasslands: ecology and management*. University of California Press, Berkeley. Pp. 254-280.
- Strykstra R.J., Bekker R.M. & Verwcij G.L., 1996. Establishment of *Rhinanthus angustifolius* in a successional hayfield after seed dispersal by mowing machinery. *Acta Botanica Neerlandica* 45, 557-562.
- Stuble K.L., Fick S.E. & Young T.P., 2017. Toward prediction in the restoration of biodiversity. Every restoration is unique: testing year effects and site effects as drivers of initial restoration trajectories. *Journal of Applied Ecology* 54, 1051–1057.
- Thormann A., Kiehl K. & Pfadenhauer J., 2003. Einfluss unterschiedlicher Renaturierungsmaßnahmen auf die langfristige Vegetationsentwicklung neu angelegter Kalkmagerrasen. *Angewandte Landschaftsökologie* 55, 73–106.
- Tilman D., 1987. Secondary Succession and the Pattern of Plant Dominance Along Experimental Nitrogen Gradients. *Ecological Monographs* 57(3), 189-214.
- Urbanska K. & Chambers J., 2002. High-elevation ecosystems. In Perrow M. R. and Davy A.J. (Eds.), *Handbook of Ecological Restoration*, Vol. 2. Cambridge University Press. Pp. 376-400.
- Vickery J.A., Tallowin J.R., Feber R.E., Asteraki E.J., Atkinson P.W., Fuller R.J. & Brown V.K., 2001. Distribution and abundance of breeding and wintering birds and their food

resources with special reference to recent changes in fertilizer, mowing and grazing practices on lowland neutral grasslands in Britain. *Journal of Applied Ecology* 38, 647–664.

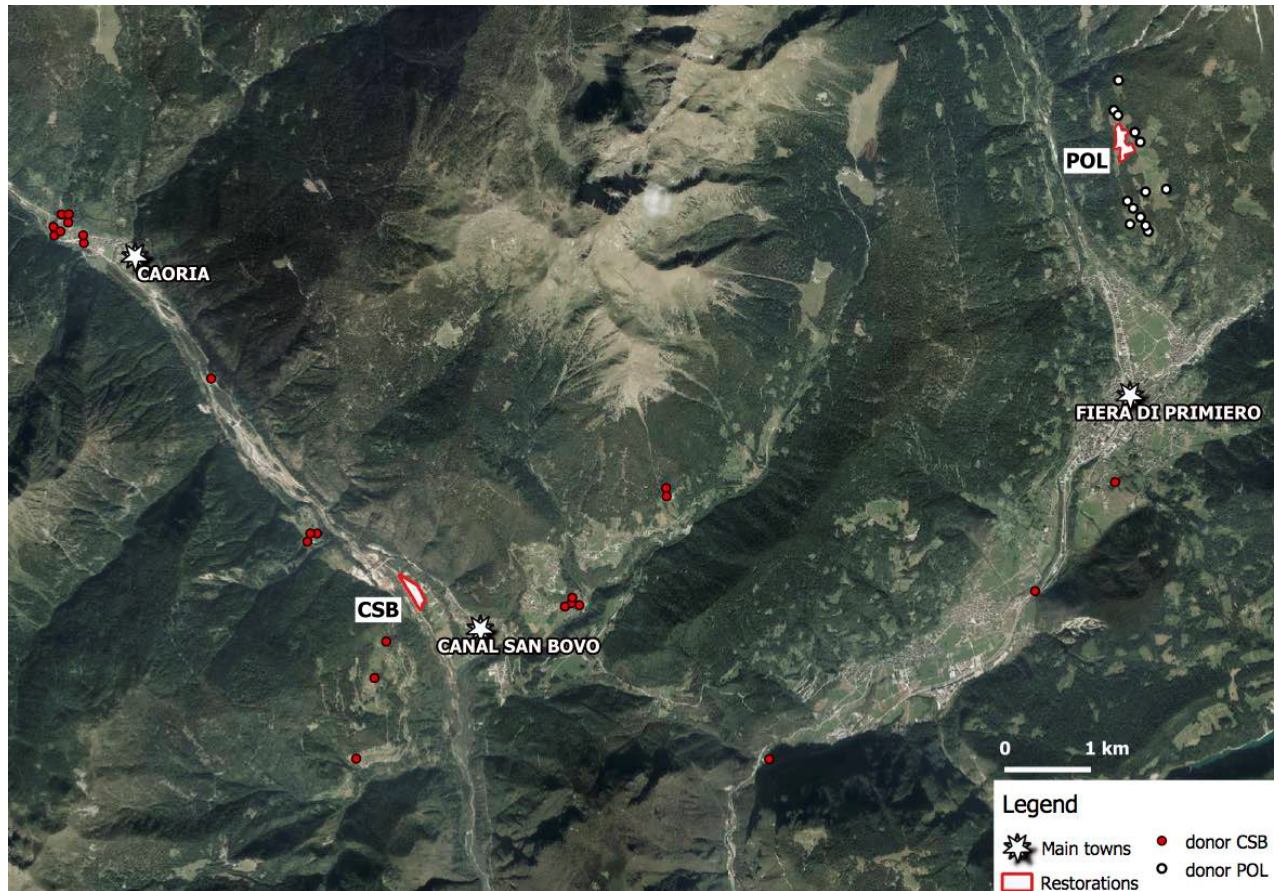
Waldén E. & Lindborg R., 2016. Long Term Positive Effect of Grassland Restoration on Plant Diversity - Success or Not? *PLoS ONE* 11(5): e0155836. doi:10.1371/journal.pone.0155836.

Wildi O. & Orloci L., 1996. *Numerical Exploration of Community Patterns, A Guide to the Use of MULVA-5*. SPB Academic Publishing, Amsterdam.

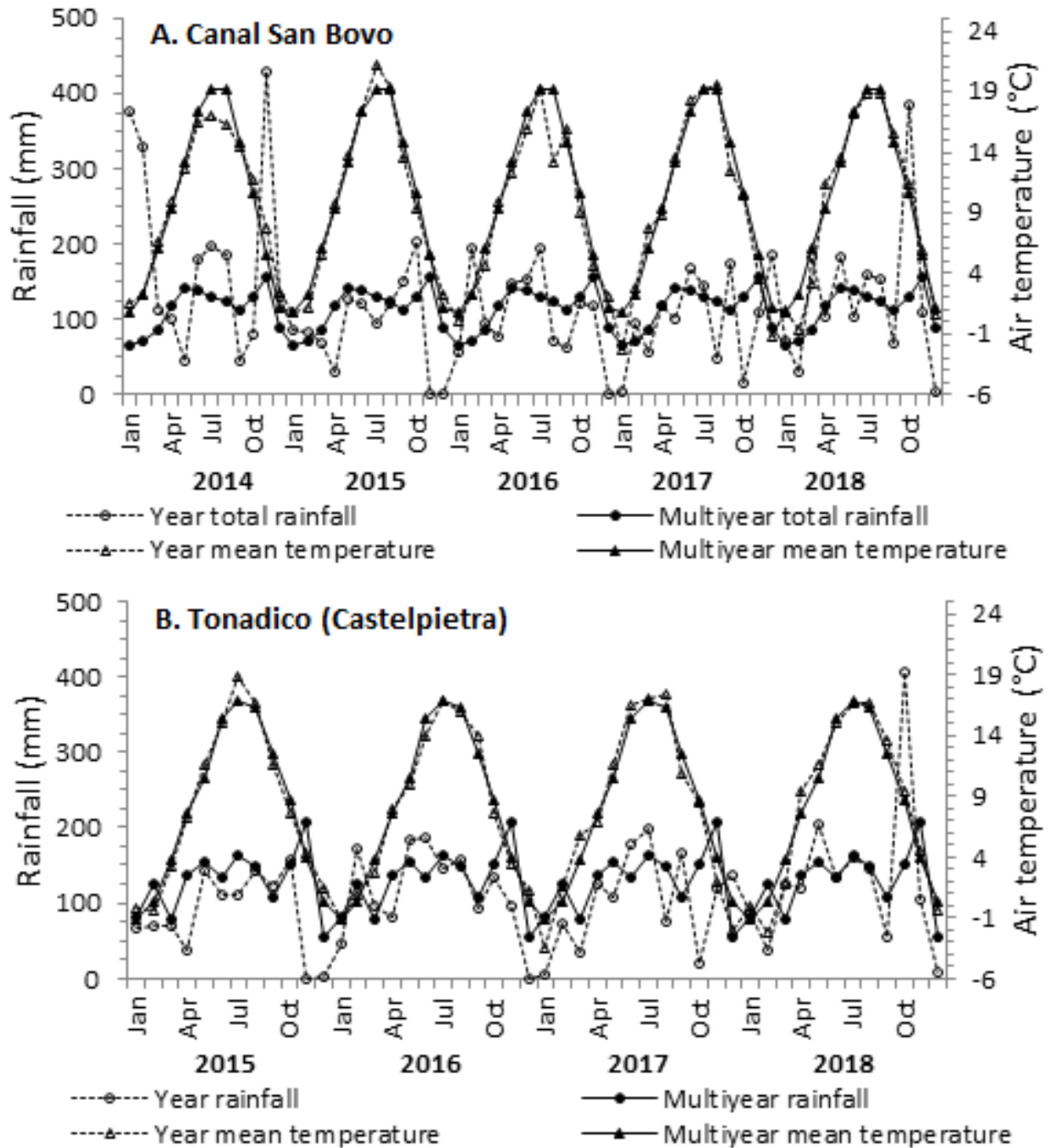
Zavaleta E.S., Shaw M.R., Chiariello N.R., Thomas B.D., Cleland E.E., Field C.B. & Mooney H.A., 2003. Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs* 73, 585–604.

Annexes

Annex 1 Geographical distribution of the donor and receptor areas of two restorations in the Italian Eastern Alps.



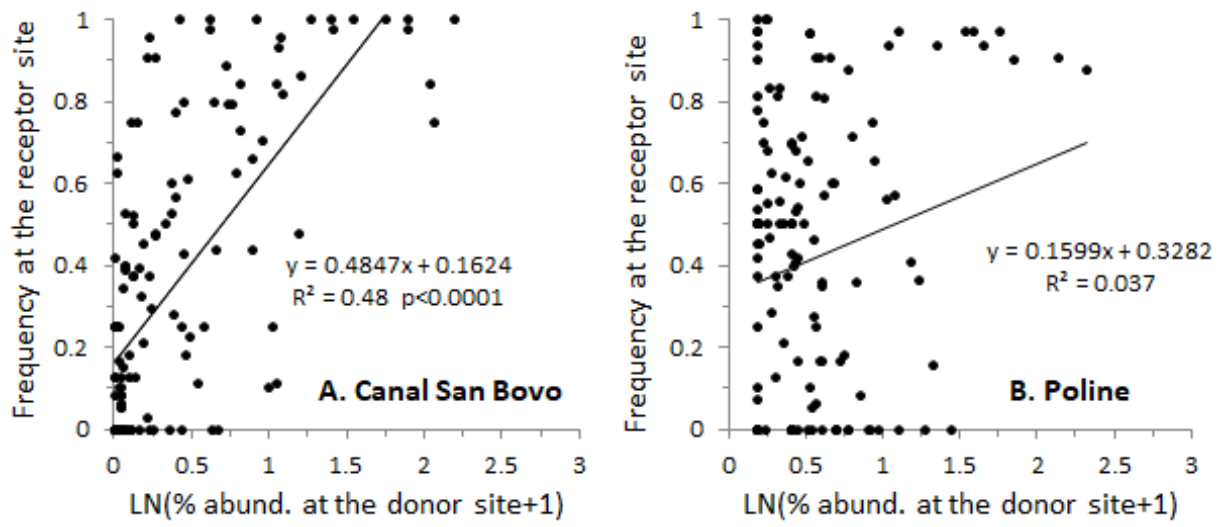
Annex 2 Climatic and weather conditions at meteorological stations close to the restoration sites. The station of Canal San Bovo is close to the Canal San Bovo restoration. The Tonadico (Castelpietra) station is close to the Poline restoration. Meteorological data retrieved from <http://storico.meteotrentino.it>.



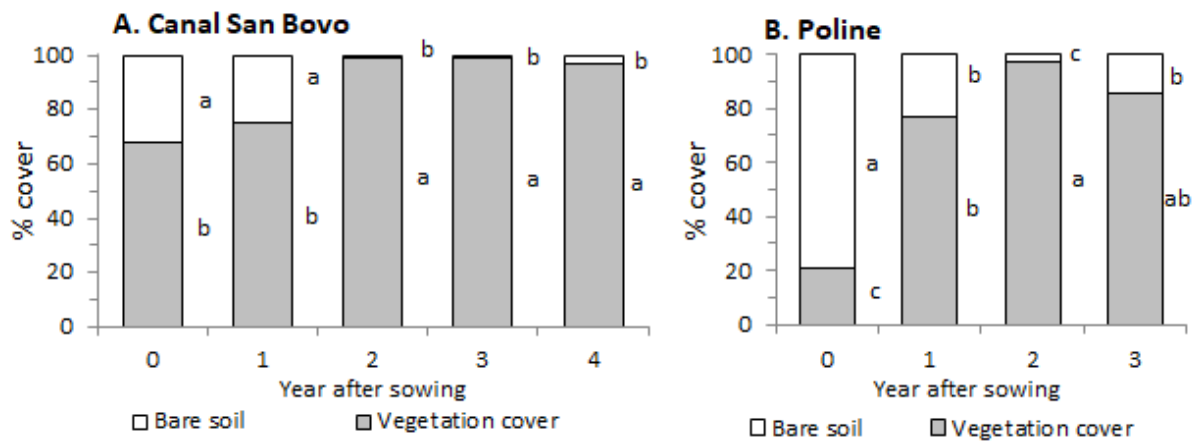
Annex 3 Main site traits of the donor grasslands of two restorations in the Italian Eastern Alps.

Restoration	Donor site code	Area (m ²)	Latitude	Longitude	Altitude (m a.s.l.)	Aspect	Slope (%)	Soil depth (cm)	Soil % gravel	Soil % fine earth	Soil % sand	Soil % silt	Soil % clay	No. cuts per year
Canal San Bovo	EM1	9122	46.15792	11.74603	900	E SE	39	55	33.4	66.6	74.8	16.6	8.6	2
Canal San Bovo	EM2	1010	46.15734	11.7451	830	S	47	80	35.3	64.7	81.0	12.5	6.5	2
Canal San Bovo	EM3	381	46.15738	11.74604	890	S	47	49	53.4	46.6	58.8	26.6	14.6	2
Canal San Bovo	EM4	773	46.15744	11.74738	880	S SE	33	38	29.3	70.7	57.0	29.5	13.5	2
Canal San Bovo	EM5	915	11.76103	46.16906	1160	S	51	71	14.8	85.2	59.0	27.5	13.5	2
Canal San Bovo	EM6	1003	11.76119	46.16861	1150	S	51	72	14.0	86.0	74.7	15.7	9.7	2
Canal San Bovo	ES1	503	46.1543	11.71786	830	E	41	44	46.6	53.4	74.4	16.8	8.8	2
Canal San Bovo	ES2	1153	46.1505	11.7159	900	E NE	22	24	52.2	47.8	74.4	18.8	6.8	2
Canal San Bovo	GR1	10493	46.1657	11.70673	810	S SE	18	18	60.8	39.2	66.8	22.6	10.6	3
Canal San Bovo	GR2	781	46.16596	11.70792	810	N NE	16	16	31.8	68.2	74.7	15.7	9.7	3
Canal San Bovo	GR3	7319	46.16512	11.7064	810	S SW	16	21	21.9	78.1	60.8	26.6	12.6	3
Canal San Bovo	PG1	824	46.20052	11.67078	910	S	20	33	36.0	64.0	68.8	20.6	10.6	2
Canal San Bovo	PG2	776	46.20013	11.6714	910	S	20	46	39.7	60.3	70.8	18.6	10.6	2
Canal San Bovo	PG3	332	46.19975	11.67152	910	S SE	20	34	36.8	63.2	70.8	18.6	10.6	2
Canal San Bovo	PG4	419	46.19874	11.6705	870	S SE	18	39	42.2	57.8	68.8	20.6	10.6	2
Canal San Bovo	PG5	1375	46.19833	11.66956	840	S	35	28	54.5	45.5	72.8	18.6	8.6	2
Canal San Bovo	PG6	3013	46.19924	11.66948	880	S SW	22	22	41.6	58.4	68.4	18.8	12.8	2
Canal San Bovo	PL1	1698	11.71236	46.14201	890	S SE	41	57	45.9	54.1	72.7	15.7	11.7	1
Canal San Bovo	SA1	1725	46.1405	11.77537	580	S	2	28	23.4	76.6	61.0	27.5	11.5	2
Canal San Bovo	SA2	877	46.15722	11.81663	650	S	7	18	36.9	63.1	69.0	19.5	11.5	2
Canal San Bovo	SA3	3721	46.16843	11.82937	760	W NW	30	38	2.6	97.4	60.7	17.6	21.6	2
Canal San Bovo	SI1	305	46.19824	11.67394	840	S SE	14	61	46.3	53.7	70.7	17.7	11.7	2
Canal San Bovo	SI2	1742	46.19743	11.67401	850	S	14	9	57.9	42.1	84.4	6.8	8.8	2
Canal San Bovo	SI3	2463	46.18271	11.69265	770	S	30	26	40.2	59.8	80.8	10.6	8.6	2
Poline	1	4403	46.19558	11.83303	1200	S SW	45	49	9.0	91.0	72.7	13.7	13.7	1
Poline	2	4464	46.19527	11.83531	1200	S	35	51	6.5	93.5	64.7	21.7	13.7	1
Poline	3	2713	46.19578	11.83469	1200	S	33	50	8.7	91.3	43.0	31.5	25.5	1
Poline	4	2516	46.20628	11.83155	1250	W	43	73	14.2	85.8	42.8	32.6	24.6	1
Poline	5	4136	46.19609	11.83431	1200	S SW	35	24	31.6	68.4	66.7	21.7	11.7	1
Poline	6	8431	46.19678	11.83351	1200	S SW	39	20	14.9	85.1	47.0	31.5	21.5	1
Poline	7	7953	46.20755	11.83126	1250	W SW	26	47	0.0	100.0	53.0	29.5	17.5	1
Poline	8	6419	46.2108	11.83206	1300	S SW	14	44	39.0	61.0	58.8	26.6	14.6	1
Poline	9	1033	46.19894	11.83557	1300	S	30	29	24.6	75.4	70.7	17.6	11.6	1
Poline	9bis	2960	46.19894	11.83557	1300	S	30	64	10.3	89.7	64.8	22.6	12.6	1
Poline	10	8926	46.22205	11.82435	1300	W SW	24	20	26.0	74.0	71.0	17.5	11.5	1
Poline	11	2423	46.20454	11.83324	1300	W	28	41	0.0	100.0	47.0	31.5	21.5	1
Poline	12	11179	46.20636	11.83265	1300	W SW	28	73	0.0	100.0	47.0	31.5	21.5	1
Poline	13	9522	46.20671	11.83084	1200	S SW	22	57	13.4	86.6	66.8	20.6	12.6	1
Poline	14	9846	46.19764	11.83335	1200	S	26	23	1.8	98.2	72.7	15.7	11.7	1

Annex 4 Relationship between total multiyear frequency of donor site species in the restored areas and % abundance of the same species in the donor grasslands. Results from two grassland restorations in the Italian Eastern Alps.



Annex 5 Time pattern of the soil cover in two two grassland restorations in the Italian Eastern Alps. The letters show the result of the Tukey's test or pairwise non parametric contrasts: different letters mean significant differences at $p < 0.05$.



Annex 6 Hay meadow type of the donor grasslands and restored areas. The mean Jaccard's coefficients of similarity between donor or restored areas (sampling areas of 100 m²) and the Trentino meadow types are shown.

	Restored area	Donor grassland	Restored area				
			Year 2015	Year 2016	Year 2017	Year 2018	
CANAL SAN BOVO							
Grassland type ¹	3	Ar1	Ar1	Ar1	Ar0	Ar1	
	4	Ar1	Ar1	Ar0	Ar1	Ar1	
	5	Ar1	Ar1	Ar1	Ar1	Ar1	
	6	Ar1	Ar1	Ar1	Ar1	Ar0	
	7	Ar1	Ar1	Ar1	Ar0	Av	
	8	Ar1	Ar1	Ar1	Ar1	Ar1	
	9	Ar1	Ar1	Ar1	Ar1	Ar1	
	10	Ar1	Ar1	Ar1	Ar1	Av	
	11	Ar0	Ar1	Ar1	Ar1	Ar1	
	12	Ar1	Ar1	Ar1	Ar1	Av	
	13	Ar1	Ar1	Ar1	Ar1	Ar1	
	Mean Jaccard's coeff. ²		0.56	0.49	0.51	0.59	0.54
	No. correspondences R-D ³		-	10	10	8	6
POLINE							
Grassland type ¹	1	Ar0	-	Ar2Alo	TT0	TT0	
	2	Br	-	Ar2Alo	Av	Ar0	
	3	Br	-	FA	Ar1	Ar1	
	4	TT0	-	TT0	TT0	Br	
	5	Br	-	Tr1	TT0	Ar1	
	6.2	Br	-	Tr1	Br	TT0	
	6.3	Br	-	Tr1	Ar0	TT0	
	7.1	Br	-	Lo	TT0	-	
	7.3	Br	-	Ar0	TT0	Ar1	
	8	Br	-	Tr1	Na	Ar1	
	9	Br	-	Ar1	Br	Ar1	
	10	Br	-	Ag	Ar0	Ar1	
	11.1	Br	-	Ar2Alo	Ar0	TT0	
	12	Ar1	-	Tr1	Ar0	Ar1	
	13.1	Br	-	TT0	Br	Ar1	
14	Br	-	Ar1	Br	Ar1		
Mean Jaccard's coeff. ²		0.49	-	0.20	0.39	0.45	
No. correspondences R-D ³		-	-	1	5	1	

Legend. 1. Hay meadow types according to the Trentino meadow typology (Scotton et al., 2012).

Code	Definition	Code	Definition
Br	<i>Bromus erectus</i> meadow	Lo	<i>Lolium multiflorum</i> meadow
Av	<i>Helictotrichon pubescens</i> meadow	Ag	<i>Agropyron repens</i> meadow
Ar0	<i>Arrhenatherum elatius</i> meadow, poor soil form	Na	<i>Nardus stricta</i> meadow
Ar1	<i>Arrhenatherum elatius</i> meadow, typical form	FA	<i>Festura rubra</i> and <i>Agrostis capillaris</i> meadow
Ar2Gra	<i>Arrhenatherum elatius</i> meadow, rich soil form with grasses	TT0	<i>Trisetum flavescens</i> meadow, transition, poor soil form
Ar2Alo	<i>Arrhenatherum elatius</i> meadow, rich soil form with <i>Alopecurus pratensis</i>	TT1	<i>Trisetum flavescens</i> meadow, transition, typical form
Ar2Agp	<i>Arrhenatherum elatius</i> meadow, rich soil form with <i>Agropyron repens</i>	Tr0	<i>Trisetum flavescens</i> meadow, poor soil form
Al0	<i>Alopecurus pratensis</i> meadow, poor soil form	Tr1	<i>Trisetum flavescens</i> meadow, typical form
Al1	<i>Alopecurus pratensis</i> meadow, typical form	-	-

2. Mean Jaccard coefficient, mean of the maximum values of the Jaccard coefficient between the grassland and the Trentino meadow types. 3. No. correspondences, number of meadow type correspondences between receptor and donor areas.

Chapter 5

General conclusions

The main aims of this study were to increase the knowledge of the seed production of the main species of lowland semi-natural grasslands and the potentiality of using it for grassland restoration. The seed production at the fertile shoot level was studied for a hay-meadow fertilised at different rates and for some important forbs collected in different years and sites. The success of using seed from semi-natural grasslands for grassland restoration was analysed on high-extent restoration areas.

Forb transfer resulted the most important problem of the ecological restoration. Compared to grasses, forbs are negatively affected by the fertilisation, increasing less (or decreasing) the viable seed production at higher fertilisation level. This is a strong limitation especially in lowland and plain meadows, where the grassland management is usually characterised by high fertilisation rate. Further, forbs showed a high inter-species and intra-species variability. Therefore, also predicting the amount of their seed production is difficult.

The green hay technique permitted to create in few years grasslands with a composition comparable with the standard meadow vegetation of the geographical region, which is one main aim of ecological restoration.

The creation of the new meadows is the result of a dynamic interaction between the transferred species and the specific site traits leading to a vegetation which is not necessarily the same as the donor grassland.

An important issue was the transfer limitation of species less represented in the donor site. In fact, the percentage of transferred species resulted inversely related to the species richness of the donor sites. A first obvious reason for this result is that species rare in the donor site also produce less seeds, so their establishment at the restored site is less safe. However, probably a second reason can be that species that occupy a narrow niche and have particular ecological requirements for germination, seedling establishment and reproduction, like forbs, have less possibilities to be transferred. This problem can become a particular obstacle for successful restoration when the transfer of rare or threatened species is necessary.

Future effort has to be done to improve our knowledge of: the seed production of semi-natural grassland and its relations to management practices (fertilisation and cutting frequency) especially with reference to forb seed production; the factors that influence the quality of the botanical composition of the restored sites; the methods to increase the number of forbs transferred species and achieve.