

Evolution of productive and biodiversity features in lucerne fields of different ages

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Abstract. *Medicago sativa* is a legume forage crop characterized by high production of forage, with a notable nutritive value, but in mountain areas duration of the crop could be remarkably affected by severe environmental conditions. To assess the vegetation evolution of lucerne crops in relation to crop age, data from fields of lucerne of different ages were collected. The aim is the evaluation of lucerne productive performances, evolution of forage quality and assessment of recovery by autochthonous species that naturally recolonize the studied areas in relation to age of the cropped species. With increasing years, lucerne population was significantly decreased and replaced by different functional types of plants, such as perennial graminoids and short-lived forbs. Biodiversity increased significantly along time, and evolution of similarity indices demonstrated an evolution of vegetation toward that represented by reference grassland of the area. Productive characteristics of forage, in terms of aboveground biomass and quality, were negatively affected by age. Results permitted to assess the evolution of different features of lucerne for a mountain environment and to hypothesize the appropriate management for this resource, that could contemplate also the evolution towards the reconstitution of the reference habitat for the studied area.

Key words: aboveground biomass, botanical composition, *Medicago sativa*, sown meadows, succession.

INTRODUCTION

Lucerne (*Medicago sativa*) is one of the most important forage crops in temperate environments, for its productivity and nutritive value (Hakl et al., 2021) and it has been used in different environments for thousands of years (Sheaffer et al., 2020). Its role inside agricultural systems is not only to provide good quality forage (Pacchioli & Fattori, 2014), but also to be included in crop rotations as an improving crop that covers the ground for several years while providing fodder production and other ecosystem services such as increasing chemical soil fertility (Głab & Gondek, 2013) or reduction of weed invasion for following crops (Meiss et al., 2010).

Its high capacity for protein synthesis, resulting from nitrogen-fixing capacity due to symbiosis with rhizobia (Jáuregui et al., 2019), makes lucerne one of the most interesting forage species for its nutritional value when feeding dairy cows especially in

intensive dairy farms (Giustini et al., 2007; Tabacco et al., 2018). In Italy, it is currently cultivated on about 700,000 ha (ISTAT, 2021), one third of which is in the Emilia-Romagna region alone, as this is the production area of Parmigiano-Reggiano, one of the best known Italian agri-food products in the world (Lovarelli et al., 2019). The origin area of this PDO (Protected Designation of Origin) is restricted to only a few very specific areas of the region, often in very extensive plains or hills (Mancini et al., 2019). Nevertheless, there is a production district corresponding to some mountainous areas in which lucerne plays an important and irreplaceable role in the provision of quality fodder coupled with grass-legumes mixtures (Argenti et al., 2021). Under these conditions, however, given its considerable sensitivity to cold (Bertrand et al., 2017), lucerne undergoes severe thinning and can reduce its duration in sown meadows sown with this legume (Belanger et al., 2006). Forage resources are actually encroached along time by various types of weeds species (mainly grasses but also species belonging to other families) and this vegetation development can reduce the productive capacity of a grassland (Ponzetta et al., 2010), the quality of the forage and the profitable longevity of the crop (Chataigner et al., 2010).

Based on the above considerations, the aim of the following research was to investigate, on lucerne fields characterized by different ages, both the level and the speediness of recolonization by spontaneous vegetation. Furthermore, the study is aimed to identify production and quality trends according to the age of the crop, to define appropriate forms of management and duration of the meadows sown with lucerne for the studied area.

MATERIALS AND METHODS

Study area comprises fields cropped with *Medicago sativa* of different ages inside the district of ‘Terre di Montagna’, a Consortium that assembles mountain farms that produce Parmesan cheese over 600 m a.s.l., in Emilia-Romagna Region (north Italy). In the area, climate is temperate, with an annual average temperature of 10.1 °C and a rainfall of about 930 mm (Fig. 1). Climatic information is referred to the meteorological station of Montese, the main town approximately occurring in the centre of the district (Regione Emilia-Romagna, 2021). Soils range from acidic to calcareous and developed on different kind of rocks (sandstone, limestone and marl).

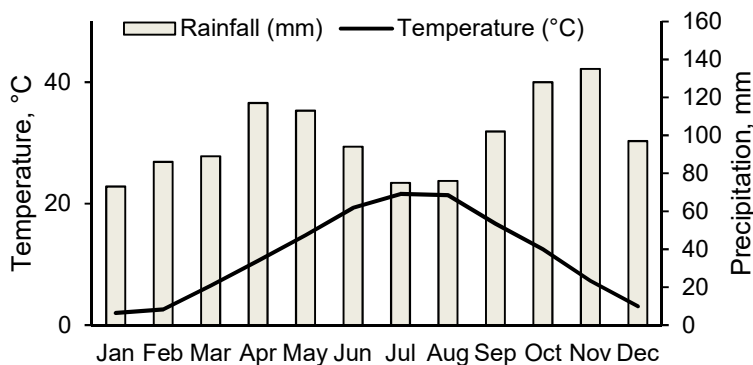


Figure 1. Average monthly data for temperature and precipitation for the studied area.

Main forage crops of the area are temporary meadows of lucerne and grass-legumes mixture, both binary than complex, utilized for hay production by means of 2 or 3 cuts per year (Argenti et al., 2021). Duration of the crops varies a lot according to forage species involved and farmer options, but, even if meadows are generally cropped for 4–5 years since sowing, it is common to find also lucerne fields older than the ordinary duration. Concerning fertilization management, only organic manure at sowing is performed and no further additional fertilization is generally added during crop cultivation. In this research, 4 different fields of 2, 4, 8 and 12 years has been selected, included at an altitude of about 700–800 m a.s.l., on flat or poorly sloped areas. Fields were within a radius of 5 km from the centre of the area (N 44° 16', E 10° 58') and with similar environmental and climatic characteristics. Soils of the four study sites (Table 1) are quite homogeneous, with a clay-loam texture and an average pH of 6.7 in water (Soilgrids, 2022).

For each survey point, data collection was performed on three different subplots where many productive and botanical parameters were recorded. All the samplings reported refer to the early vegetative stage (end of May), before the first mowing as performed in previous similar research (Török et al., 2011).

Aboveground biomass was clipped from a square of 0.5×0.5 m, as reported by Mikhailova et al. (2000). Fresh biomass was stored in sealable bags and then was dried in a forced air oven at 80 °C to constant weight for dry matter determination as usually performed in forage science (Wang et al., 2019). On the same area, Leaf Area Index (LAI, m²·m⁻²), a variable related to the complex structure of the canopy and to its biomass allocation (Francone et al., 2014), was collected by means of a ceptometer (LI-COR, USA). Each dried forage sample was grounded through a mill (Brabender OHG, Germany) to pass 1 mm and analysed for the following parameters: crude protein (CP) and Acid Detergent Fibre (ADF) as main drivers of quality (Lemaire & Belanger, 2020) according to standard laboratory procedures (AOAC, 2012).

Vegetation was monitored on the same sample areas. Cover of all vascular plants was recorded by means of visual estimation (Boob et al., 2019a). In the following data elaboration, species were grouped to the following functional types: *Medicago sativa* (sown crop), perennial forbs (excluding lucerne), perennial graminoids, short lived forbs and short lived graminoids according to the classification adopted by Török et al. (2011).

Species diversity was assessed by means of parameters such the number of species observed in each sample, Shannon diversity index (H') calculated by Eq. 1:

$$H' = - \sum(p_i \times \ln p_i) \quad (1)$$

and by Pielou evenness index (J', Eq. 2):

$$J' = H' / \ln S \quad (2)$$

where 'p_i' is the percentage proportion (expressed as decimal fraction) of the *i*-species in the canopy and 'S' is the total number of species in each sample, or richness (Pruchniewicz, 2017).

Finally, to evaluate the level of naturalization reached by lucerne fields as a function of age, the list of species from each sample was compared to the list of characteristic species of a given reference habitat (Raduła et al., 2020), *i.e.* those species native and

Table 1. Main soil properties for the investigated sites

Parameter	Value
Sand (%)	25.4
Clay (%)	33.5
Silt (%)	41.1
Type	Clay Loam
pH (water)	6.7
Bulk density (g cm ⁻³)	1.31

typical of a particular community occurring in a specific area (Helm et al., 2015). In our case the list of characteristic species was derived from Habitat 6510 (Lowland hay meadows), considered as the reference habitat of the area, as reported in a Guide to habitat of community interest for the Emilia-Romagna Region (Bassi et al., 2015). Comparison between lucerne fields and the reference habitat was performed by means of two of the most used similarity indices (Eqs 3 and 4, Magurran, 2004):

$$\text{Sørensen Index (S}_s\text{): } 2a/(2a + b + c) \quad (3)$$

$$\text{Jaccard Index (J}_j\text{): } a/(a + b + c) \quad (4)$$

where ‘a’ is the number of species shared by the two lists (lucerne fields and reference habitat), and ‘b’ and ‘c’ are the number of species unique to the two lists. Thus, calculation of these parameters needs only presence/absence of the species and not their percentage of occurrence. Both indices range from 0 (no similarity) to 1 (identity).

To compare data from different field age, ANOVA was performed adopting Tukey test or LSD as post hoc tests. Results were used to assess possible relationships among monitored parameters and evolution of a specific parameters along time. Analyses were performed with the SPSS statistical software (release 27, IBM, 2020).

RESULTS AND DISCUSSION

Table 2 reports evolution of percentage presence of identified functional groups of species in the lucerne fields of different age. Ground cover of cropped species differs significantly along time, with a highly decreased cover in oldest fields (5.7% and 6.3% for 8 and 12 years old areas respectively). The functional group that benefitted mostly from this evolution was that of perennial graminoids (such as *Lolium perenne*, *Dactylis glomerata* or *Arrhenatherum elatius*), that increased from 6.0 to more than 50% in oldest samples. Other functional groups differed in a less relevant way or did not show any significant difference along time.

Table 2. Percentage cover of species belonging to different functional groups in relation to age of fields. Values are means \pm standard error

	Age (years) of lucerne fields			
	2	4	8	12
<i>Medicago sativa</i>	77.3 \pm 4.3 ^a	34.3 \pm 2.7 ^b	5.7 \pm 2.3 ^c	6.3 \pm 2.1 ^c
Perennial forbs	4.7 \pm 0.6 ^{ns}	7.7 \pm 1.6 ^{ns}	9.0 \pm 4.7 ^{ns}	19.7 \pm 1.6 ^{ns}
Perennial graminoids	6.0 \pm 2.0 ^b	36.6 \pm 1.3 ^{ab}	58.6 \pm 8.5 ^a	53.0 \pm 12.1 ^a
Short lived forbs	1.7 \pm 0.3 ^b	6.7 \pm .7 ^a	8.0 \pm 1.9 ^a	7.7 \pm 1.3 ^a
Short lived graminoids	10.3 \pm 3.2 ^{ns}	14.7 \pm 1.2 ^{ns}	18.7 \pm 3.3 ^{ns}	13.3 \pm 5.9 ^{ns}

Values with the same letters are not significantly different according to Tukey test ($P < 0.05$); ns = not significant.

Development of vegetation along years interested also forage production, with an aboveground biomass that decreased significantly from recent to old fields (Fig. 2). Simultaneously to this evolution, a reduction in the average quality of the forage has been recorded, with a significant decrease in crude protein content and an increase in the fibrous components, evidenced by increasing ADF values. Decrease of crude protein content (from 22.3% to 12.5%) was more rapid than reduction of above ground biomass.

Concerning LAI, a general reduction of this parameter was recorded along time with a less remarkable relation to years since sowing.

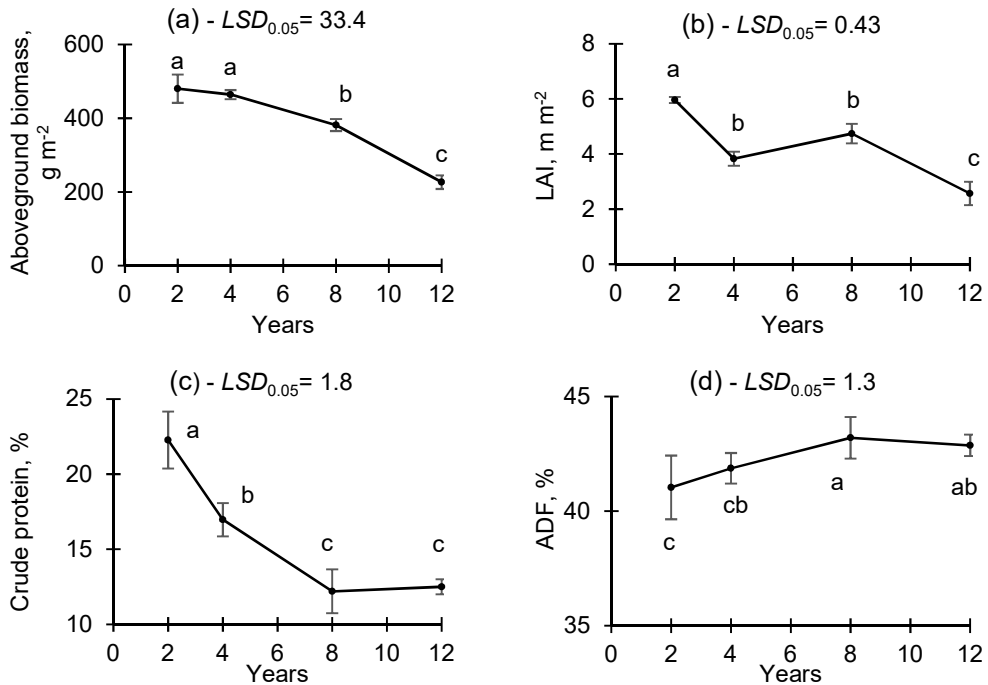


Figure 2. Evolution of aboveground biomass (AGB) (a), Leaf Area Index (LAI) (b), crude protein content (c) and ADF (d) in lucerne according to different age fields. Bars represent standard errors. *LSD* is the least significant difference ($P = 0.05$) for each analysed variable.

Evolution along time of botanical composition and forage quality is significantly related to age of fields (Fig. 3). Presence of weeds increases constantly in relation to age with a very accurate logarithmic curve ($R^2 = 0.91$). Infestation by weeds is faster at the beginning of naturalization process. At the same time crude protein content decreases along time with a similar pattern but with a lower accuracy ($R^2 = 0.77$).

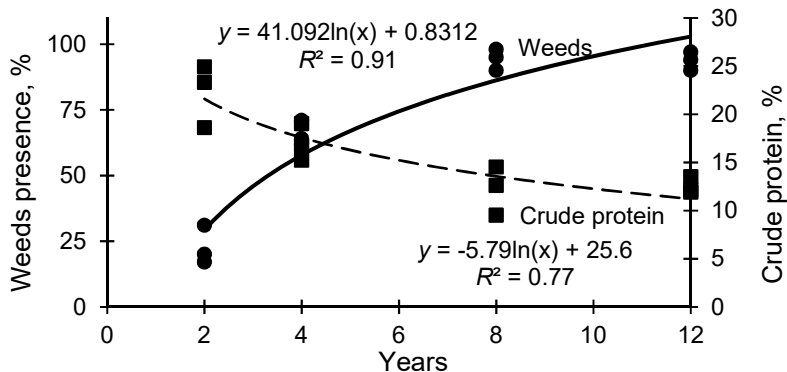


Figure 3. Regressions of presence of weeds and crude protein content with age of lucerne fields.

Evolution recorded did not differ from that observed in other studies related to mowed grasslands. Utilization itself can improve recovery of native species inside sown forage crops, as mowing is acknowledged to introduce seeds from other fields into grasslands or favour some kinds of species according to time and frequency of cutting (Gaujour et al., 2012). Reduction of lucerne cover in relation to age observed in this survey is similar to that reported by Török et al. (2011) that analysed lucerne fields from 1 to 10 years of age. Native species recolonization is limited in the first years by high density of the seeded plant as reported by Li et al. (2008). High presence of standing biomass can also explain suppression of species belonging to native flora that develop more remarkably in older stage of evolution, according to Güsewell & Edwards (1999). Perennial species are the most represented category in older stage of evolution (Štolcová, 2002) and especially perennial grasses are the most frequent in secondary succession (Török et al., 2008) and our results are consistent to previous studies concerning this aspect (Feng et al., 2007).

Reduction of productive and qualitative features in lucerne as consequence of age is reported by many authors (Teixeira et al., 2007) and this behaviour is due to different causes, such as self-thinning for competition, specific environmental conditions (such as acid pH or waterlogging), presence of pests or diseases (Burnett et al., 2020). Especially in mountain areas, also climatic conditions can affect development and diffusion of grassland resources (Dibari et al., 2015) and this is particularly true for lucerne which is acknowledged to be very sensitive to cold (Ta et al., 2020). Our results are in line with what is commonly performed in many hilly areas of Italy where a duration of 3–4 years is envisaged, since after this period a strong decline is observed with impact on productive features (Parrini & Bonari, 2002), while some authors report a reduction in yield of lucerne that starts earlier, with a third year aboveground biomass higher in grass/legumes mixtures than in monoculture of lucerne (Dhakai & Anowarul Islam, 2018). Decrease of aboveground biomass with increasing fields age recorded in our trial follows almost the same pattern reported by Török et al. (2011). In our case, yield reduction is due to the replacement of lucerne by grasses that are not adequately supported by fertilization due to the very extensive management, as it is known that grasses have high requirements of N for maintaining a high productive level (Helgadóttir et al., 2018).

Evolution of forage quality in sown meadows is closely related to their botanical composition and high presence of weeds, that present lower nutritive value than sown species, produces a decrease of protein content (Reiné et al., 2020). Thus, changes in cover of lucerne or, in general, legumes along time affects nutritive value of the forage biomass (Movedi et al., 2019). Assessing pure stands of *Medicago sativa* with different proportion of grasses, Pacchioli & Ligabue (2013) found a reduction of 34% of crude protein and an increase of 15% of ADF in lucerne fields highly encroached by grasses compared to pure stands. These results are consistent with our findings and demonstrate the higher variation along time of crude protein content respect to fibre components, in particular cellulose and lignin included in ADF parameters. Anyway, presence of grasses inside a pure stand of lucerne can also play a positive role, for instance to reduce productive decline of this legume, due to higher persistency of grasses (Berzins et al., 2011) and to protect lucerne from low temperature (Aponte et al., 2019). Due to the different nutritional value of lucerne hay available over the years (Boob et al., 2019b), the different chemical composition, especially the lower content of crude protein and the

higher content of fibre fraction, should be properly evaluated, at least with rapid tools, in order to balance the diet intended for dairy cows (Parrini et al., 2022).

Table 3. Diversity indices in relation to age of fields. Values are means \pm standard error

	Age (years) of lucerne fields			
	2	4	8	12
Number of perennial species	4.7 \pm 0.3 ^b	5.3 \pm 0.3 ^b	6.3 \pm 0.9 ^b	10.0 \pm 1.1 ^a
Number of short lived species	3.0 \pm 0.5 ^{ns}	5.0 \pm 1.1 ^{ns}	4.7 \pm 0.4 ^{ns}	4.1 \pm 1.5 ^{ns}
Total richness	7.7 \pm 0.6 ^b	10.3 \pm 0.8 ^{ab}	11.0 \pm 0.6 ^{ab}	14.1 \pm 2.1 ^a
H'	0.86 \pm 0.07 ^b	1.60 \pm 0.07 ^a	1.79 \pm 0.08 ^a	1.89 \pm 0.22 ^a
Pielou index	0.42 \pm 0.04 ^b	0.69 \pm 0.01 ^a	0.75 \pm 0.02 ^a	0.72 \pm 0.07 ^a

Values with the same letters are not significantly different according to Tukey test ($P < 0.05$); ns = not significant.

Studied diversity indices are reported in Table 3 in order to analyse the evolution of biodiversity as affected by naturalization development. Number of species in botanical samples increased significantly along time, ranging from an average of 7.7 species in two year old fields to 14.1 in oldest meadows. Notably, only the first value is significantly different from the last one. Species that perform natural recolonisation of lucerne fields are mainly perennial, with an average value of 10 species per sample at last data collection significantly different from previous ages. On the contrary, species number per sample of short lived species (*i.e.* annual or biennial) are not significantly different for all fields age. Concerning biodiversity, Shannon index is significantly lower in the youngest fields (2 years old) with respect to oldest and the same trend is observed for Pielou index. Thus, a constant naturalization of old lucerne fields can be recorded, with a very high rapidity of this process in the first years of development, confirming what was observed in terms of ground cover.

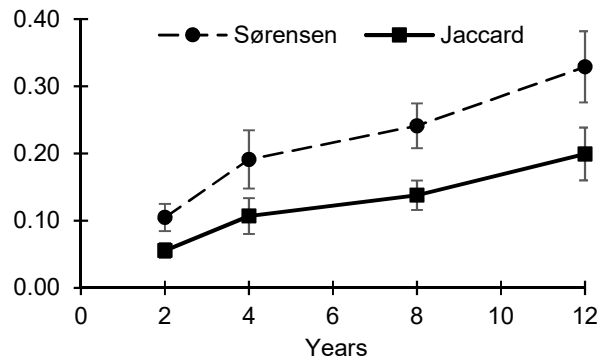


Figure 4. Evolution of similarity indices (Sørensen and Jaccard) of lucerne fields with reference grassland habitat (6510) according to different age. Bars represent standard errors.

Some characteristic species of the chosen reference grasslands were found in all different fields. Assessment of evolution of similarity among lucerne fields and reference plant community was performed by two different indices (Fig. 4). Both represented in the same way and with the same pattern the evolution towards the reference habitat composition, with a very reduced similarity in most recent fields (0.10 and 0.06 for

Sørensen and Jaccard respectively) with respect to oldest one (0.33 and 0.20). Also these parameters point out, by means of analysis of the slope of the regression line, a higher velocity of recovery in the first years of naturalisation.

Results are generally consistent with previous studies. Colonisation of native species are mainly represented, in terms of number, by perennial species as pointed out by Török et al. (2011). These authors state a possible recovery in roughly 10 years, even if other studies pointed out very different time to reach an almost stable vegetation composition (Fonseca & Ganade, 2001). Kelemen et al. (2017) observed that recent field are dominated by the sown crop (*Medicago sativa*) for its competitiveness in using available resources, and the other species are able to use other kind of resources occupying different ecological niches. Different factors are involved in the naturalization process, such as ordinary management of the grasslands or specific restoration techniques (Kiehl et al., 2010). Also, absence of soil tillage for many years, as occur in lucerne crop, can favor diversity and richness along time, mainly represented by development of perennial species that profited of absence of this kind of disturbance (Meiss et al., 2010). In absence of specific active intervention, anyway, secondary succession can lead in the long term to seminatural vegetation (Csecserits & Rédei, 2001), even if a factor that enhances naturalization process is the presence of spontaneous grasslands in the surroundings of the managed fields (Öster et al., 2009).

Comparison of species composition in different age fields and in the reference grassland by means of similarity indices allowed to evaluate that dissimilarity tends to reduce with increasing age. Török et al. (2010) found the same results in restoration interventions on previous lucerne fields and this tendency was mainly due to changes in life forms (shift from short lived to perennials as reported before). It is anyway possible to have lower similarity performances in lucerne fields from 5 to 10 years old compared to reference grasslands (Török et al., 2011). Increasing biodiversity in old lucerne field along time, measured by means of Shannon index, was assessed by Kelemen et al. (2012), which considered this as a promising evolution towards a stable vegetation typical of seminatural grasslands for that investigated area. Yuan et al. (2015), on lucerne-rich stands in an arid environment, stated that time is the most important environmental factor affecting evolution of vegetation in these conditions.

CONCLUSIONS

The study make available data concerning development of lucerne fields as consequence of age since sowing. Production and nutritive value were strongly affected by age, and this was mainly due to two different and contrasting processes: the lucerne innate self-thinning that produces a sharp decline of presence along time, and a natural recolonization of space left by sown crop that is occupied by native species, mainly represented by perennial species. This evolution is responsible of the naturalization process described that can recover, in the long time, the natural plant communities of the area. This development, pointed out also in several previous studies, can produce two distinct outcomes. In some conditions, it is possible to produce a lucerne renovation, at least with a one-year cereal species before a new lucerne stand, to enhance the productive services provided by this crop. In this case, a maximum duration of 4–5 years should be envisaged, in order to face productive and qualitative decline of lucerne fields. Eventual intensification with fertilization to maintain a high level of productive and qualitative

features in meadows enriched of grasses could be an option to prolong the duration of these sown resources. In other situations, where the agricultural activity is abandoned or highly reduced and where provision of other ecosystem services by grasslands is a priority, a recovery of local plant communities is possible through the naturalisation of the old fields left to colonization by autochthonous species of the surroundings. The destiny of this process is able to recover a stable plants community, even if the time of a definitive naturalisation is not certain, as it depends on many variables, such as environmental characteristics, mowing frequency and boundary management, and at least 10 years seem necessary. The development towards these reference habitats can anyway be improved and favoured by extensive management (reduced number of cutting, release of mulch biomass from mowing, etc.) or direct improvement utilising seeds from donor sites rich in target species, as suggested by some authors.

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Supplementary material

Table A1. ANOVA results for investigated parameters presented in Table 1 and 2

Variable	Source of variation	DF	MS	F test value	<i>p</i> -value
<i>Medicago sativa</i>	Age	3	3408.08	129.41	< 0.001
Perennial forbs	Age	3	128.09	1.23	0.362
Perennial graminoids	Age	3	1676.52	9.96	0.004
Short lived forbs	Age	3	26.00	5.78	0.021
Short lived graminoids	Age	3	35.86	0.83	0.515
Number of perennial species	Age	3	16.97	9.69	0.005
Number of short lived species	Age	3	2.33	0.76	0.549
Total richness	Age	3	20.31	4.59	0.038
H'	Age	3	0.66	13.19	0.002
Pielou index	Age	3	0.67	10.13	0.004