Received: 15 February 2022 | Accepted: 25 November 2022
DOI: 10.1111/1365-2664.14344

Journal of Applied Ecology
BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Experimental extensification of mountain grasslands restores plant species richness but not species composition in the mid-term

Ségolène Humann-Guilleminot[1,2] | **Steffen Boch**[3] | **Gerard Martínez-De León**[1] | **Malie Lessard-Therrien**[1] | **Aline Hayoz-Andrey**[1] | **Alberto Serres-Hänni**[1] | **Jean-Yves Humbert**[1]

[1]Division of Conservation Biology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

[2]Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum d'Histoire Naturelle, CNRS, SU, EPHE, UA, Paris, France

[3]WSL Swiss Federal Research Institute, Birmensdorf, Switzerland

Correspondence
Ségolène Humann-Guilleminot
Email: segolene.humann@gmail.com

Funding information
Canton of Grisons, Switzerland; Canton of Valais, Switzerland; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A_172953/1; Swiss Federal Offices for Agriculture and the Environment

Handling Editor: Alistair Auffret

Abstract

1. The traditional grasslands that characterize the cultural landscapes of the Palaeartic mountain massifs represent biodiversity hotspots. Yet, they are currently threatened by the intensification of farming practices, notably excesses in fertilization and irrigation.
2. We experimentally investigated the passive restoration of montane and subalpine hay meadows after 6 years of management intensification, with different levels of fertilization and irrigation, followed by 5 years of release of intensive management, that is, extensification. More specifically, relying on a full randomized block-design replicated at 11 Swiss study sites constituted of extensively managed meadows, we exposed during 6 years (2010–2015) four 20 m diameter plots to three levels of intensification (low, medium and high inputs), while a fourth plot served as a control (no inputs). In the second phase of the experiment (2016–2020), all study meadows underwent farming extensification.
3. We monitored total species richness and plant diversity (Simpson diversity), indicator plant species as well as the composition and variability of the plant communities based on Bray–Curtis dissimilarity distances.
4. We found that total species richness decreased in the most intensified plots after 6 years of intensification, but all plots retrieved their baseline species richness after 5 years of re-extensification. In addition, we found no difference between the years in plant diversity (Simpson diversity) among the treatments. Yet, intensification led to different plants communities' compositions in all three levels of intensification in 2015 compared to the extensive plots, and this structural difference remained after 5 years of re-extensification.
5. *Synthesis and applications.* Land-use intensification induces a rapid impoverishment of the flora of mountain meadows. Our results demonstrate the potential of mountain hay meadows to passively restore plant species richness after

---

re-extensification; however, plants communities did not fully recover. We recommend maintaining fertilization inputs as low as possible and operating active restoration on grasslands formerly intensified.

KEYWORDS
agricultural intensification, ecological restoration, functional groups, montane grassland, passive restoration, plant communities, species richness

1 | INTRODUCTION

The decline of semi-natural, cultivated grasslands is one of the primary drivers of biodiversity loss globally (Green et al., 2005; Stoate et al., 2001; Tilman et al., 2001), calling for more sustainable agricultural management capable of retaining plant diversity as an insurance for long-term ecosystem stability (Grime, 1998; Hooper et al., 2012; Soliveres et al., 2016). Anthropogenic disturbances of grassland vegetation originate mostly from the management of grasslands with the aim of producing fodder, with their impact varying depending on the degree of intensification (Humbert et al., 2016; Newbold et al., 2015).

In Western Europe, it has been estimated that more than two-thirds of the previously traditionally managed secondary grasslands have been intensified (Dengler et al., 2020). In montane and subalpine regions of Europe, extensively managed hay meadows, dedicated to fodder production for livestock, as well as pastures have typically dominated the agricultural landscape for centuries (Zabel, 2019). In addition to being emblematic of the cultural landscapes of the Palaearctic, these semi-natural grasslands are recognized as habitats of exceptional biodiversity value (Biurrun et al., 2021; Török & Dengler, 2018; Veen et al., 2009) and the maintenance of high species diversity is essential to maintain stable ecosystem functioning and services (Isbell et al., 2011; Soliveres et al., 2016; Tilman et al., 2006). Yet, the multi-functionality of mountain grassland farming, which was for long economically viable and ecologically sustainable, is nowadays threatened by a rapid expansion of agricultural intensification towards higher elevations (Pecher et al., 2017; Schermer et al., 2016).

Grassland intensification among mountain hay meadows, notably in the Swiss Alps, is achieved through increases in inputs of fertilizers (nowadays in the form of liquid manure, i.e. slurry) and in dry regions combined with irrigation (nowadays using mainly sprinklers) that results in increasing mowing frequency (Britschgi et al., 2006) and altered grazing regimes with higher livestock densities. These marked land-use changes—from extensively to intensively managed grasslands—operate as an environmental filter against biological diversity, whereby generalist species take over other specialists, ultimately leading to a sheer simplification or degradation of ecological communities at all levels of the food chain (Andrey et al., 2016; Boch et al., 2021; Gámez-Virués et al., 2015; Gossner et al., 2016; Klein et al., 2020; Schirpke et al., 2020). Therefore, once species diversity has declined as a result of prolonged high-intensity management, the grassland ecological community would require a long period of time to recover or ends up with irreversible changes (Isbell et al., 2013), impeding any return to a species-richer stable state as long as the artificially added mineral nutrients remain non-limiting.

To remedy grassland degradation, various restoration techniques are currently proposed or under development (e.g. Hedberg & Kotowski, 2010; Jongepierová et al., 2007; Kiehl et al., 2010; Královec et al., 2009; Ludewig et al., 2021; Wagner et al., 2021). Their aim is to reinstate stable assemblages of plants comparable to their non-intensified counterparts (van Andel & Aronson, 2012), further offering room for other associated living forms (Schaffers et al., 2008; Scherber et al., 2010). The capacity of a degraded semi-natural grassland to be reconverted into an integral system is generally greater if the surrounding matrix is made up of a complex and rich plant community (Johanidesová et al., 2015; Lencová & Prach, 2011; Török et al., 2018) and if the exposure to a perturbation has been brief (Bekker et al., 1997). However, any effort towards the retro-conversion target may be compromised by the dynamic of the interspecific interactions taking place in the community, notably those revolving around species dominance and outcompetition that may eventually lead to a total shift in community composition (Fynn et al., 2005; Zhou et al., 2020). Given this complexity, evaluating the appropriateness of different grassland restoration techniques necessitate truly controlled, replicated experiments. Unfortunately, most insights so far came from purely empirical studies carried out mostly at only one site. This is what decided us to launch a long-term research programme on grassland restoration in mountain agro-ecosystems.

The potential for self-recovery of montane grassland communities is likely to be greater than in monotonous landscape matrices where fields are subjected to long-term high-intensity agriculture. This is due to the capacity of a grassland that was species rich in the past but has suffered from a change in its management to recover without any action beyond halting the disturbance. Although most studies have investigated the shifts in plant communities in species-poor, over-simplified landscapes (e.g. grasslands in the lowland), we still lack investigations that experimentally tested the response of montane meadow plant communities to the release of management intensity in traditional, complex agricultural landscapes.

In this context, we experimentally investigated the passive restoration of montane hay meadows (800–1800 m a.s.l.), embedded in a naturally richly structured agricultural Alpine landscape, 4 years after intensive agricultural management had been halted. In particular, we investigated the response of total species richness and plant diversity (Simpson diversity). In addition, we looked at indicator plant species, the relative species abundance and community structure because they reflect long-term ecosystem stability (Foley et al., 2005; Ives et al., 2003; Ives & Carpenter, 2007). We designed a full randomized block-design experiment split in two phases. During the first phase (2010–2015), three randomly chosen plots, within 11 extensively managed hay meadows scattered across the south-western Swiss Alps, were exposed to different degrees of intensification, while a fourth plot serving as a control was not subjected to intensification at all. In the second phase (2016–2020), intensification was totally halted, allowing the plant community to reconstitute. Vegetation relevés were conducted in 2010, 2015 and 2020 to measure, via longitudinal data, the response of plants to releasing management intensity, that is, the restoration potential of montane grasslands.

2 | MATERIALS AND METHODS

2.1 | Study sites and experimental design

In 2010, 11 extensively managed meadows (>4000 m^2 each) were selected as study sites in Valais, SW Swiss Alps (800–1800 m elevation; Table S1). Each meadow had been extensively managed (no or very low levels of fertilizer, only in the form of organic solid manure) for at least 10 years prior to the onset of the experiment. Within each meadow, four circular plots of 20 m diameter were delimited, their edges being 5 m distant at their closest proximity. Three different experimental treatments matching a gradient of fertilization plus irrigation in combination, as well as a control (no such inputs, i.e. extensive management; hereafter C-plot) were randomly allocated to the four plots of a meadow (Figure S1). The treatment plots received low, medium or high inputs, corresponding to a combination of aerial irrigation (sprinklers) and fertilization (slurry) that mimicked a gradient of intensification, with, respectively, 1/3 (low intensity; I+F 1/3), 2/3 (mid intensity; I+F 2/3) and 3/3 (high intensity; I+F 3/3) of the quantity of inputs that would be necessary to achieve the maximum local hay yield in a given meadow according to its agronomic properties. The exact amount of slurry applied per plot are presented in Table S2, and in Andrey et al. (2014, 2016) and Lessard-Therrien et al. (2017). Mowing regimes had to be adapted to phytomass production to simulate local farming practice: C- and I+F 1/3 plots were mown once a year, the other plots twice a year (see Andrey et al., 2014, 2016; Lessard-Therrien et al., 2017 for details). From 2016 onwards, we stopped fertilization and watering operations, that is, totally released intensification, thus eliciting a phase of ‘re-extensification’.

All farmers granted us access to their meadows for the duration of the study. No other authorization was required to conduct the fieldwork.

2.2 | Vegetation relevés

In each circular treatment plot, a permanent subplot of 2×4 m was placed at a distance of 4 m from the plot centre, with the rectangle being oriented longitudinally parallel to the slope axis (see figure 1 in Andrey et al., 2016 and Figure S1). The upper proximal corner of the subplot was permanently marked belowground with a nail enabling that the sampling area could subsequently be retrieved. Baseline vegetation relevés have been conducted prior to treatments, in 2010 and in the last year with experimental inputs, in 2015. In June 2020, we conducted new vegetation relevés, following the exact same procedure as in 2010 and 2015, at nine of the 11 study sites (two meadows having in-between unfortunately been converted into other land-uses). Plant species richness was assessed from field vegetation relevés while the cover of each species within the plot was estimated visually before the first mowing in June–July. Plant species covering only <0.1% were arbitrary attributed a percentage of 0.03.

2.3 | Statistical analyses

All analyses were performed using R v. 3.5.3 (R Core Team, 2020).

2.3.1 | Treatment effects on plant species richness, diversity and cover

First, we tested the effect of the three experimental treatments against control with a four-level factorial analysis (low, mid- and high intensity, i.e. I+F 1/3, I+F 2/3, I+F 3/3). Second, we tested the difference between sampling dates, that is, 2010–2015, 2010–2020 and 2015–2020 with a three-factorial analysis and by changing the contrast. For both analyses, the response variables were as follows: (1) plant species richness and (2) plant diversity as measured by Simpson's diversity (D_1) where $D_1 = 1 - \Sigma p_i^2$, with p_i being the cover of each species *i* (Morris et al., 2014). Additional analyses on (3) species richness of three different functional groups (legumes, forbs and grasses) and (4) cover of the three functional groups are presented in the supplemental results of the supporting information. We opted for a combination of species richness and Simpson diversity indices in order to account for biases typically induced by either rare or abundant plant species (Morris et al., 2014).

We ran linear mixed-effects models (LMMs), with restricted maximum likelihood for parameter estimation, using the *lmer* function from the LME4 R package (Bates et al., 2015). The validity of each LMM was verified by comparing the Akaike information criterion (AIC) of the full model against the AIC of the null model (intercept only), using the maximum likelihood estimation function (Forstmeier & Schielzeth, 2011). Modelling assumptions (normality and homoscedasticity of residuals, homogeneity of variance and normality of random effect) were further validated by visual inspection using the *check_model* function of the PERFORMANCE R package (Lüdecke et al., 2021). Variables were transformed when necessary (indicated in the respective summary Tables 1 and 2 and Tables S4–S7).

For facilitating the interpretation of the results and because the design and the inclusion of random factors rendered insufficient degrees of freedom to run a full model fitting an interaction term *treatment* × *year*, that would include both the repeated measures in each plot and the study sites as random intercepts, we first ran separate models for each year with study site included as a random intercept. We then ran separate models for each treatment but including all 3 years with study site included as a random intercept. To avoid inflating the family-wise type I rate from multiple models testing the same hypothesis, we controlled the false discovery rate, that is, the proportion of incorrect rejections among all rejections of the null hypothesis (Benjamini & Hochberg, 1995), and corrected all p-values with the *fdr* method of the *p.adjust* function (STATS package; R Core Team, 2020). All linear mixed model outputs, variable transformation and ΔAIC are reported in Tables 1 and 2 and Tables S4–S7.

2.3.2 | Multivariate analyses of plant community composition and variability

All multivariate analyses were performed using the VEGAN R package (Oksanen et al., 2020). Here again, for an easier interpretation of the results and due to a lack of degrees of freedom to run a full model including an interaction term *treatment* × *year*, we ran separate models for each year of sampling.

We tested the effect of treatment on plant community composition with a permutational analysis of variance (*permanova*; 999 permutations) using Bray–Curtis dissimilarity with the function *adonis*. It tests the null hypothesis that the centroids and dispersion of the species (as defined by the measure of space) within each group (i.e. treatment) are equivalent. A rejection of the null hypothesis means that either the centroid and/or the spread of the species is different between groups. To compare specific groups, we ran pairwise analyses between each experimental treatment and the control and corrected p-values applying the false discovery rate (*p.adjust*) as described above.

To test for homogeneity of dispersion among groups (i.e. beta diversity), we created a distance matrix with a square-root transformed Bray–Curtis dissimilarity index using *vegdist* and performed an analysis of multivariate homogeneity of group dispersions using the *betadisper function*. This function calculates the average distance of group constituents (plant species within each treatment) to the group spatial median in a multivariate space. To test if one or more levels of our experimental treatment is/are more variable than the other(s), we performed a permutation test (ANOVA; 999 permutations) of the distances to group spatial medians under the null hypothesis of no difference in dispersion between the four levels of the experimental treatment. Statistical significance was obtained from post-hoc

TABLE 1 Linear mixed model outputs testing for an effect of the three levels of intensification on total plant species richness and Simpson diversity fitted using a restricted maximum likelihood function. Transformation of the response variable is indicated in the estimate column and corresponding year. All the *p*-values presented are corrected for the false discovery rate. Significant effects ($p < 0.05$) are in bold. The difference between the AIC of each full model against the AIC of the null model (intercept only) is indicated in the ΔAIC column, a negative ΔAIC meaning that the full model better explains the data

	Plant species richness					Simpson diversity				
	Estimate	SE	df	*p*	ΔAIC	Estimate	SE	df	*p*	ΔAIC
2010					0.300					−0.300
Intercept (control)	47.455	2.593	21.257	**<0.001**		0.819	0.030	19.260	**<0.001**	
I + F 1/3	4.727	2.481	30.000	0.199		0.032	0.027	30.000	0.305	
I + F 2/3	3.364	2.481	30.000	0.556		−0.021	0.027	30.000	0.443	
I + F 3/3	4.522 E-15	2.481	30.000	1.000		−0.031	0.027	30.000	0.388	
2015					−6.000	*Logit*				1.600
Intercept (control)	48.091	2.678	28.960	**<0.001**		1.251	0.237	38.806	**<0.001**	
I + F 1/3	−0.455	3.039	30.000	0.882		0.475	0.318	30.000	0.305	
I + F 2/3	−2.455	3.039	30.000	0.638		0.310	0.318	30.000	0.443	
I + F 3/3	−9.727	3.039	30.000	**0.010**		−0.101	0.318	30.000	0.753	
2020					4.700					2.100
Intercept (control)	47.889	2.777	18.533	**<0.001**		0.847	0.031	30.838	**<0.001**	
I + F 1/3	−0.667	2.798	24.000	0.882		0.044	0.042	24.000	0.305	
I + F 2/3	−0.889	2.798	24.000	0.753		0.071	0.042	24.000	0.304	
I + F 3/3	−2.889	2.798	24.000	0.468		0.067	0.042	24.000	0.357	

TABLE 2 Linear mixed model outputs testing for an effect of each year on total plant species richness and Simpson diversity. Transformation of the response variable is indicated in the estimate column and corresponding treatment. All the *p*-values presented are corrected for the false discovery rate. Significant effects ($p < 0.05$) are in bold and marginally non-significant effects ($0.05 < p < 0.1$) in italics. The difference between the AIC of each full model against the AIC of the null model (intercept only) is indicated in the ΔAIC column, a negative ΔAIC meaning that the full model better explains the data

	Plant species richness					Simpson diversity				
	Estimate	**SE**	**df**	***p***	**ΔAIC**	**Estimate**	**SE**	**df**	***p***	**ΔAIC**
Control	*Log*				3.400					1.200
Intercept (2010)	3.846	0.067	18.236	**<0.001**		0.819	0.049	17.688	**<0.001**	
2015 vs. 2010	0.009	0.065	17.720	0.888		−0.070	0.048	16.804	0.358	
2020 vs. 2010	−0.041	0.069	18.221	0.577		−0.001	0.051	17.343	0.981	
Intercept (2015)	3.855	0.067	18.236	**<0.001**		0.749	0.049	17.688	**<0.001**	
2020 vs. 2015	−0.050	0.069	18.221	0.958		0.069	0.051	17.343	0.199	
I+F 1/3	*Log*				0.700					0.000
Intercept (2010)	3.944	0.059	22.838	**<0.001**		0.850	0.025	26.181	**<0.001**	
2015 vs. 2010	−0.107	0.067	18.519	0.167		−0.026	0.032	18.719	0.577	
2020 vs. 2010	−0.103	0.071	19.221	0.326		0.042	0.034	19.651	0.309	
Intercept (2015)	3.836	0.059	22.838	**<0.001**		0.825	0.025	26.181	**<0.001**	
2020 vs. 2015	0.004	0.071	19.221	0.958		0.068	0.034	19.651	*0.081*	
I+F 2/3	*Log*				0.000	*Gamma**				−4.000
Intercept (2010)	3.915	0.048	24.330	**<0.001**		1.258	0.047		**<0.001**	
2015 vs. 2010	−0.103	0.053	20.397	0.156		−0.029	0.054		0.587	
2020 vs. 2010	−0.090	0.056	21.132	0.326		−0.155	0.054		**0.018**	
Intercept (2015)	3.812	0.048	24.330	**<0.001**		1.229	0.046		**<0.001**	
2020 vs. 2015	0.014	0.056	21.132	0.958		−0.126	0.054		**0.039**	
I+F 3/3	*Log*				−4.300	*Gamma**				−5.500
Intercept (2010)	3.843	0.057	27.299	**<0.001**		1.311	0.090		**<0.001**	
2015 vs. 2010	−0.221	0.076	15.907	**0.043**		0.095	0.071		0.358	
2020 vs. 2010	−0.046	0.081	17.145	0.577		−0.139	0.068		*0.083*	
Intercept (2015)	3.622	0.057	27.299	**<0.001**		1.405	0.092		**<0.001**	
2020 vs. 2015	0.175	0.081	17.145	0.179		−0.234	0.071		**0.004**	

*GLMM applied.

tests of pairwise differences using the Tukey Honest Significant Differences test with *TukeyHSD*.

To account for our full block design, that is, we tested the effect of treatment within each study site, permutation tests were performed within experimental sites (i.e. meadows) but never across them. Restricted permutation could be achieved by setting a strata directly on the model generated, either using the function *adonis* from the vegan package or with the function *how* from the package permute (Simpson, 2019).

To visualize the plants community composition and homogeneity (i.e. beta diversity), we extracted the scores of each site of the groups as well as the distances to their centroids (medians) along the first two main axes of a principal coordinates analysis and displayed the four treatments with different colours (Figure 2). All summary statistics of both analyses are reported in Table 3 and Table S8.

2.3.3 | Indicator species analysis

We ran an indicator species analysis (*multipatt* function of the indicspecies package; Cáceres & Legendre, 2009) to identify which species (1) reflect the biotic or abiotic state of the local environment; (2) provide evidence for the impact of changes resulting from the experimental treatments and (3) best predict overall plant species diversity within the treatment plots (Cáceres & Legendre, 2009). We further relied on a correlation index to assess the positive or negative ecological preference of a plant species for a given treatment; the statistical significance of these relationships was tested via permutation tests of the underlying plant abundance matrix (999 permutations). Corrections for multiple testing (i.e. multiple species tested) were applied using the false discovery rate (*p.adjust*) described above. Summary statistics of the indicator species analysis are reported in Table S9.

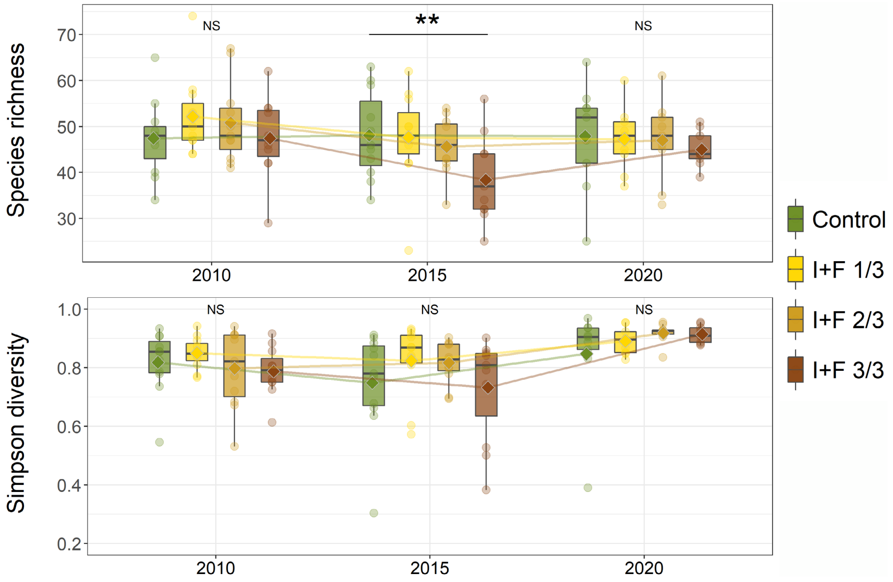

FIGURE 1 Effects of different levels of combined irrigation and fertilization (I + F, with 1/3, 2/3 and 3/3 corresponding to low-, medium- and high-intensity management, respectively) on plants species richness and Simpson diversity index in 2010, 2015 and 2020 as measured from vegetation relevés carried out on a reference area of 8 m^2 per plot. Non-significant differences between treatments and control are indicated by the annotation 'NS' and significant effects are indicated by asterisks (**$p \leq 0.01$).

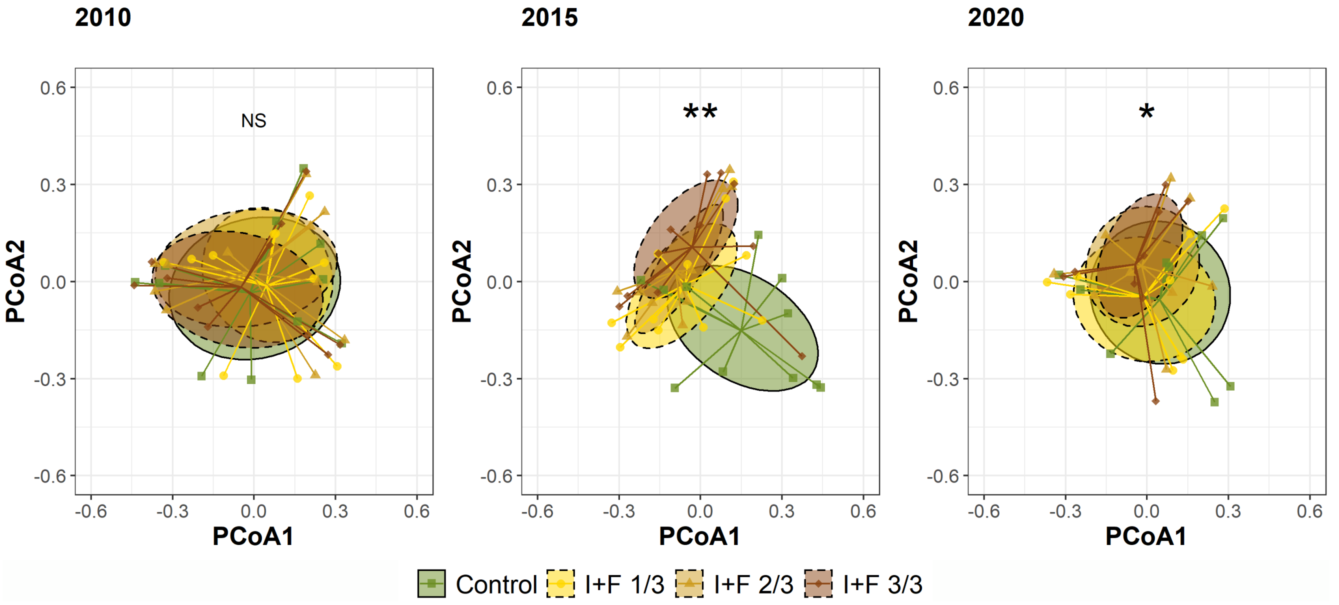

FIGURE 2 Principal coordinates analysis (PCoA) ordination plot of the first two axes based on Bray–Curtis dissimilarities of plants community variabilities among the three levels of intensification (see legend of Figure 1 for more details) in 2010, 2015 and 2020. Non-significant differences between treatments and control are indicated by the annotation 'NS' and significant effects are indicated by asterisks (*$p \leq 0.05$, **$p \leq 0.01$).

3 | RESULTS

We recorded a total of 310 species of plants from 39 families over the three sampling dates and treatments. All recorded species and respective cover are presented in the Dryad Digital Repository (Humann-Guilleminot et al., 2022). Basic descriptive statistics are presented in Table S3.

3.1 | Treatment effects on plant species richness and diversity

At the onset of the experiment, in 2010, there were no statistically significant differences in plant species richness and diversity (Simpson D_1 index) between any of the experimental treatments and the controls (Table 1, Figure 1), indicating that the random allocation

TABLE 3 Summary statistics of permutational multivariate analysis of variance using the Bray–Curtis distance indices testing the effects of experimental management treatment on plant community composition. Presented are degrees of freedom, variance explained (R^2), F-values and p-values. Significant effects ($p < 0.05$) are in bold and marginally non-significant effects ($0.05 < p < 0.1$) in italics. p-values from the pairwise analyses are presented with the false discovery rate correction

	df	*F*-value	R^2	*p*
2010				
Treatment	3	0.574	0.041	*0.089*
Control vs. I+F 1/3	1	0.626	0.030	0.154
Control vs. I+F 2/3	1	0.352	0.017	0.390
Control vs. I+F 3/3	1	0.419	0.021	0.317
2015				
Treatment	3	1.671	0.111	**0.001**
Control vs. I+F 1/3	1	1.997	0.091	**0.002**
Control vs. I+F 2/3	1	2.883	0.126	**0.002**
Control vs. I+F 3/3	1	2.797	0.123	**0.002**
2020				
Treatment	3	0.726	0.064	**0.011**
Control vs. I+F 1/3	1	0.452	0.032	0.467
Control vs. I+F 2/3	1	1.014	0.066	**0.015**
Control vs. I+F 3/3	1	1.156	0.079	**0.015**

of treatments and control to plots induced no bias. In 2015, we found a significant decrease (by 20.2%) in plant species richness of the most intensified (I+F 3/3) plots, compared to the control plots, but no difference as regards the Simpson diversity (Table 1, Figure 1). In 2020, after 5 years of re-extensification, there was no statistically significant difference anymore between any of the three levels of intensification and the controls, neither for plant species richness nor for diversity (Table 1, Figure 1).

Species richness significantly decreased by 5.2% in the high-intensive treatment between 2010 and 2015 (Table 2, Figure 1). However, we did not find any other significant results regarding the species richness between any years (Table 2, Figure 1). We found a significant and marginally non-significance difference in Simpson diversity between 2015 and 2020 in the mid- and high-intensive and low-intensive treatments, respectively (Table 2, Figure 1). There was no statistical difference in Simpson diversity in any of the treatments between 2010 and 2015 nor 2010 and 2020.

Results on the effects of treatments on plant species richness and cover of the three functional groups are presented in the supplementary Results and Discussion, and in Tables S4–S7 and Figures S2 and S3.

3.2 | Treatment effects on plant community composition and variability

While the permutational multivariate analysis of variance using Bray–Curtis distance index did not detect any significant effect of our experimental treatment on plant species composition in 2010, there was a significant effect of treatment in 2015 and 2020. More specifically, our experimental treatments explained 11% and 6% of the compositional variance in 2015 and 2020, respectively (Table 3, Figure 2). When conducting pairwise comparisons between the three levels of intensification and the control plots, we found that, in 2015, the plant species composition of all three intensification levels differed from that of the control plots, with our treatment explaining 9%–13% of the compositional variance (Table 3, Figure 2). Moreover, in 2020, the mid-intensive plots and the high-intensive plots significantly differed from the control plots in terms of plant species composition, with our experimental treatment explaining 7% and 8% of the compositional variance, respectively. Yet, plant species composition was not significantly different between the low-intensive and the control plots (Table 3, Figure 2).

The analysis of multivariate homogeneity of group dispersions using Bray–Curtis distance index did not reveal any significant effect of our experimental treatment on the variability of plant communities, neither in 2010, 2015 nor in 2020 (Table S8, Figure 2).

Results of the indicator species analysis are presented in the supplementary Results and Discussion, and in Table S9.

4 | DISCUSSION

The effects of land-use intensification and re-extensification upon grassland biodiversity are still poorly documented, which represents an impediment to the development of sound grassland restoration strategies. Our long-term, spatially replicated field experiment, which involved 6 years of farming intensification (with slurry addition and aerial irrigation), followed by 5 years of re-extensification, established that plant species richness of Alpine hay meadows dramatically declined under the highest level of agricultural intensification, but was passively restored 5 years after intensification had been halted. In contrast, plant diversity (Simpson D_1 index) showed no significant pattern all along years and across treatments and controls. After 6 years of intensification followed by 5 years of re-extensification, however, the composition of the plant communities still remained affected by intensification. Plant communities had not fully returned to their initial composition, suggesting some mid-term retention of the effects of formerly intensified farming practices. We shall first discuss these findings in the light of the extant literature, before drawing preliminary recommendations for biodiversity-friendly management of mountain hay meadows.

Intensification induces a rapid impoverishment of the flora of montane and subalpine meadows in the short term (Andrey et al., 2014) and mid-term (Boch et al., 2021; Lessard-Therrien et al., 2017), as demonstrated by our earlier work within the same experimental set up and corroborated in this study. In addition, effects of intensification are more pronounced in species-rich grasslands (e.g. extensive grasslands) compared to species-poor grasslands because species-rich grasslands are home to a larger number of rare species, more vulnerable to increasing land-use intensity (Kleijn et al., 2009; Suding et al., 2005). Although plant species richness could be passively reversed after 5 years of re-extensification, plant communities' compositions were not completely restored. The analyses of the composition of the plant communities (i.e. beta diversity) and of the relative species-specific abundances show that intensification did not affect the floral composition but influenced their relative abundance. In effect, fairly similar across all plots in 2010, the relative species-specific abundances started to diverge after 5 years of intensification, with a legacy effect still observable in the mid- and the high-intensive plots in 2020, that is, after 5 years of re-extensification. Nonetheless and in contrast, these differences have vanished already in 2020 in low-intensive plots, compared to control plots, exemplifying the potential resilience of the hay meadow plant community to mid-term intensification of management and mid-term re-extensification, provided that the amount of fertilizer applied remained low. In addition, the indicator species analysis revealed that plant species with a propensity to grow in nitrogen-rich soils (e.g. *Taraxacum officinale aggr.*, *Trifolium repens* or *Ranunculus acris*) were more abundant in 2015 and 2020 in the plots that had received mid to high amounts of fertilizers and irrigation before 2016 (see supplementary Discussion for details). This suggests that the environmental conditions in the two most intensified plots were not fully restored after 5 years: the accumulation of nutrients in the soil was still impacting plant communities after 5 years of re-extensification, engendering a mid-term legacy effect of intensive agricultural management. The rapid convergence in species richness and slow convergence in species composition has also been found in other grassland experiments where communities were allowed to passively reassemble after manipulation of the initial plant community composition (Fukami et al., 2005; Roscher et al., 2009).

In the light of this contrasted pattern, two main questions emerge. First, did the fairly short periods of intensification (6 years) and re-extensification (5 years) of our experiment explain the high mid-term resilience of our plant species richness and diversity to farming intensification? Second, did the embedment of our meadows among heterogenous and naturally richly structured farmed landscapes, natively particularly rich in biodiversity, contribute to enhance the recovery of the hay meadow flora, thanks to what has been termed an insurance effect (Yachi & Loreau, 1999)? The short-term history of grasslands intensification and the landscape matrix itself may play a decisive role when it comes to regeneration. Intensified mountain meadows are most of the time embedded in a heterogeneous environment that still consists of small fields, subjected to various levels of management intensity, interspersed with natural structures such as hedges, stone walls, etc. A greater landscape heterogeneity involves an increased number of potential sources of plant species via the dispersion of propagules over sometimes long distances (Geertsema et al., 2002). It may therefore increase the regional species pool, enhance colonization probability of the target grassland and likely contributed to the partial recovery of our experimental plots (Bakker & Berendse, 1999; Bekker et al., 1997; Benton et al., 2003; Lencová & Prach, 2011; Riedener et al., 2015; Török et al., 2018; Wagner & Edwards, 2001). However, the establishment of species adapted to disturbed environments is a slow process and species sometimes need several decades to establish even when the seed pool is available in the immediate vicinity (Berendse et al., 2021; Isbell et al., 2013). Although grasslands can eliminate accumulated nitrogen following fertilization episodes, full recovery may take several years before formerly fertilized soils return to concentrations similar to that of unfertilized grasslands (Clark et al., 2009; Stevens, 2016). Furthermore, the recovery of species richness does not necessarily predict the restoration of species composition, that may take centuries to recover once the grasslands are destroyed (Isbell et al., 2019; Nerlekar & Veldman, 2020). To fasten the process of grassland restoration, multiple reliable approaches for active restoration are available. Methods include, for instance, the addition of hay or seeds collected from a species-rich donor grassland (Bischoff et al., 2018; Slodowicz et al., 2019; Valkó et al., 2022), topsoil transfer from a species-rich donor grassland or topsoil removal from the degraded grassland (Buisson et al., 2022; Piqueray et al., 2020; Rasran et al., 2007) or the activation of the soil seed bank (Klaus et al., 2018; Ludewig et al., 2021). So far, seed addition via hay transfer appears to be an effective measure for grassland restoration, provided that the donor sites are sufficiently species rich and close enough to the receiver grassland to ensure compatibility (Albert et al., 2019; Durbecq et al., 2022; Valkó et al., 2022).

The present robust experimental study, because spatially replicated and fully controlled, demonstrates the potential of montane and subalpine hay meadows to passively restore the flora species richness in the mid-term. However, it also demonstrates that more time is needed for intensified meadows to retrieve their original plants composition. We therefore recommend keeping grasslands management as extensive as possible while maintaining a surrounding undisturbed area. When considering the recovery of former intensified montane and subalpine grasslands, we recommend operating active restoration of grasslands by means of a relaxation of farming intensification, together with a transfer of hay from adjacent species-rich donor meadows, which are still readily available in the Alps.

AUTHOR CONTRIBUTIONS

Jean-Yves Humbert conceived the study. Jean-Yves Humbert, Aline Hayoz-Andrey, Malie Lessard-Therrien and Ségolène Humann-Guilleminot designed the study. Ségolène Humann-Guilleminot, Malie Lessard-Therrien, Aline Hayoz-Andrey, Alberto Serres-Hänni, Steffen Boch, Gerard Martinez-De León and Jean-Yves Humbert conducted the experiment and collected the data. Ségolène Humann-Guilleminot performed the statistical analyses and wrote the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGEMENTS

We would like to thank all the students and volunteers who helped us in the field as well as the farmers who agreed to collaborate with us. We especially thank Stephane Mettaz for his great help in the field over the 10 years of the experiment, Dr. Sylvain Losdat and Dr. Roel van Klink for their valuable advice on statistical analysis and Prof. Eric Allan for his comments on the manuscript. This study was funded principally by an Excellence grant from the Swiss National Science Foundation (credit number 31003A_172953/1) allocated to Prof. Raphaël Arlettaz (head of the division of conservation biology at the university of Bern, Switzerland), and co-funded by the Swiss Federal Offices for Agriculture and the Environment, and by the States of Valais and Grisons. Note that while the study was funded by a grant allocated to Prof. Raphaël Arlettaz, he has decided to withdraw from the senior co-authorship of the present paper for personal reasons.

CONFLICT OF INTEREST

The authors bear sole responsibility for the content and declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.ksn02v787 (Humann-Guilleminot et al., 2022).

ORCID

Ségolène Humann-Guilleminot https://orcid.org/0000-0003-4888-0847
Steffen Boch https://orcid.org/0000-0003-2814-5343
Gerard Martínez-De León https://orcid.org/0000-0001-5890-7391
Malie Lessard-Therrien https://orcid.org/0000-0002-1152-1755
Jean-Yves Humbert https://orcid.org/0000-0002-5756-6748

REFERENCES

Albert, Á.-J., Mudrák, O., Jongepierová, I., Fajmon, K., Frei, I., Ševčíková, M., Klimešová, J., & Doležal, J. (2019). Grassland restoration on exarable land by transfer of brush-harvested propagules and green hay. *Agriculture, Ecosystems & Environment*, *272*, 74–82.

Andrey, A., Humbert, J.-Y., & Arlettaz, R. (2016). Functional response of leaf- and planthoppers to modern fertilisation and irrigation of hay meadows. *Basic and Applied Ecology*, *17*, 627–637.

Andrey, A., Humbert, J.-Y., Pernollet, C., & Arlettaz, R. (2014). Experimental evidence for the immediate impact of fertilization and irrigation upon the plant and invertebrate communities of mountain grasslands. *Ecology and Evolution*, *4*, 2610–2623.

Bakker, J. P., & Berendse, F. (1999). Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution*, *14*, 63–68.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Bekker, R. M., Verweij, G. L., Smith, R. E. N., Reine, R., Bakker, J. P., & Schneider, S. (1997). Soil seed banks in European grasslands: Does land use affect regeneration perspectives? *Journal of Applied Ecology*, *34*, 1293–1310.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B: Methodological*, *57*, 289–300.

Benton, T. G., Vickery, J. A., & Wilson, J. D. (2003). Farmland biodiversity: Is habitat heterogeneity the key? *Trends in Ecology & Evolution*, *18*, 182–188.

Berendse, F., Geerts, R. H. E. M., Elberse, W. T., Bezemer, T. M., Goedhart, P. W., Xue, W., Noordijk, E., ter Braak, C. J. F., & Korevaar, H. (2021). A matter of time: Recovery of plant species diversity in wild plant communities at declining nitrogen deposition. *Diversity and Distributions*, *27*, 1180–1193.

Bischoff, A., Hoboy, S., Winter, N., & Warthemann, G. (2018). Hay and seed transfer to re-establish rare grassland species and communities: How important are date and soil preparation? *Biological Conservation*, *221*, 182–189.

Biurrun, I., Pielech, R., Dembicz, I., Gillet, F., Kozub, Ł., Marcenò, C., Reitalu, T., Van Meerbeek, K., Guarino, R., Chytrý, M., Pakeman, R. J., Preislerová, Z., Axmanová, I., Burrascano, S., Bartha, S., Boch, S., Bruun, H. H., Conradi, T., De Frenne, P., et al. (2021). Benchmarking plant diversity of Palaearctic grasslands and other open habitats. *Journal of Vegetation Science*, *32*, e13050.

Boch, S., Kurtogullari, Y., Allan, E., Lessard-Therrien, M., Rieder, N. S., Fischer, M., Martínez De León, G., Arlettaz, R., & Humbert, J.-Y. (2021). Effects of fertilization and irrigation on vascular plant species richness, functional composition and yield in mountain grasslands. *Journal of Environmental Management*, *279*, 111629.

Britschgi, A., Spaar, R., & Arlettaz, R. (2006). Impact of grassland farming intensification on the breeding ecology of an indicator insectivorous passerine, the whinchat *Saxicola rubetra*: Lessons for overall alpine meadowland management. *Biological Conservation*, *130*, 193–205.

Buisson, E., Archibald, S., Fidelis, A., & Suding, K. N. (2022). Ancient grasslands guide ambitious goals in grassland restoration. *Science*, *377*, 594–598.

Cáceres, M. D., & Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. *Ecology*, *90*, 3566–3574.

Clark, C. M., Hobbie, S. E., Venterea, R., & Tilman, D. (2009). Long-lasting effects on nitrogen cycling 12 years after treatments cease despite minimal long-term nitrogen retention. *Global Change Biology*, *15*, 1755–1766.

Dengler, J., Biurrun, I., Boch, S., Dembicz, I., & Török, P. (2020). Grasslands of the palaearctic biogeographic realm: Introduction and synthesis. In M. I. Goldstein & D. A. DellaSala (Eds.), *Encyclopedia of the World's biomes* (pp. 617–637). Elsevier.

Durbecq, A., Rocher, L., Jaunatre, R., Dupré la Tour, A., Buisson, E., & Bischoff, A. (2022). Mountain grassland restoration using hay and brush material transfer combined with temporary wheat cover. *Ecological Engineering*, *174*, 106447.

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. A., Kucharik, C. J., Monfreda, C., Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K. (2005). Global consequences of land use. *Science*, *309*, 570–574.

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: Overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology*, *65*, 47–55.

Fukami, T., Martijn Bezemer, T., Mortimer, S. R., & van der Putten, W. H. (2005). Species divergence and trait convergence in experimental plant community assembly. *Ecology Letters*, *8*, 1283–1290.

Fynn, R. W. S., Morris, C. D., & Kirkman, K. P. (2005). Plant strategies and trait trade-offs influence trends in competitive ability along gradients of soil fertility and disturbance. *Journal of Ecology*, *93*, 384–394.

Gámez-Virués, S., Perović, D. J., Gossner, M. M., Börschig, C., Blüthgen, N., de Jong, H., Simons, N. K., Klein, A.-M., Krauss, J., Maier, G., Scherber, C., Steckel, J., Rothenwöhrer, C., Steffan-Dewenter, I., Weiner, C. N., Weisser, W., Werner, M., Tscharntke, T., & Westphal, C. (2015). Landscape simplification filters species traits and drives biotic homogenization. *Nature Communications*, *6*, 8568.

Geertsema, W., Opdam, P., & Kropff, M. J. (2002). Plant strategies and agricultural landscapes: Survival in spatially and temporally fragmented habitat. *Landscape Ecology*, *17*, 263–279.

Gossner, M. M., Lewinsohn, T. M., Kahl, T., Grassein, F., Boch, S., Prati, D., Birkhofer, K., Renner, S. C., Sikorski, J., Wubet, T., Arndt, H., Baumgartner, V., Blaser, S., Blüthgen, N., Börschig, C., Buscot, F., Diekötter, T., Jorge, L. R., Jung, K., … Allan, E. (2016). Land-use intensification causes multitrophic homogenization of grassland communities. *Nature*, *540*, 266–269.

Green, R. E., Cornell, S. J., Scharlemann, J. P. W., & Balmford, A. (2005). Farming and the fate of wild nature. *Science*, *307*, 550–555.

Grime, J. P. (1998). Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *Journal of Ecology*, *86*, 902–910.

Hedberg, P., & Kotowski, W. (2010). New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. *Journal for Nature Conservation*, *18*, 304–308.

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E. K., Hungate, B. A., Matulich, K. L., Gonzalez, A., Duffy, J. E., Gamfeldt, L., & O'Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, *486*, 105–108. https://doi.org/10.1038/nature11118

Humann-Guilleminot, S., Boch, S., Martínez-De León, G., Lessard-Therrien, M., Hayoz-Andrey, A., Serres-Hänni, A., & Humbert, J.-Y. (2022). Data from: Experimental extensification of mountain grasslands restores plant species richness but not species composition in the mid-term. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.ksn02v787

Humbert, J.-Y., Dwyer, J. M., Andrey, A., & Arlettaz, R. (2016). Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: A systematic review. *Global Change Biology*, *22*, 110–120.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., van Ruijven, J., Weigelt, A., Wilsey, B. J., Zavaleta, E. S., & Loreau, M. (2011). High plant diversity is needed to maintain ecosystem services. *Nature*, *477*, 199–202.

Isbell, F., Tilman, D., Polasky, S., Binder, S., & Hawthorne, P. (2013). Low biodiversity state persists two decades after cessation of nutrient enrichment. *Ecology Letters*, *16*, 454–460.

Isbell, F., Tilman, D., Reich, P. B., & Clark, A. T. (2019). Deficits of biodiversity and productivity linger a century after agricultural abandonment. *Nature Ecology & Evolution*, *3*, 1533–1538.

Ives, A. R., & Carpenter, S. R. (2007). Stability and diversity of ecosystems. *Science*, *317*, 58–62.

Ives, A. R., Dennis, B., Cottingham, K. L., & Carpenter, S. R. (2003). Estimating community stability and ecological interactions from time-series data. *Ecological Monographs*, *73*, 301–330.

Johanidesová, E., Fajmon, K., Jongepierová, I., & Prach, K. (2015). Spontaneous colonization of restored dry grasslands by target species: Restoration proceeds beyond sowing regional seed mixtures. *Grass and Forage Science*, *70*, 631–638.

Jongepierová, I., Mitchley, J., & Tzanopoulos, J. (2007). A field experiment to recreate species rich hay meadows using regional seed mixtures. *Biological Conservation*, *139*, 297–305.

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.

Klaus, V. H., Hoever, C. J., Fischer, M., Hamer, U., Kleinebecker, T., Mertens, D., Schäfer, D., Prati, D., & Hölzel, N. (2018). Contribution of the soil seed bank to the restoration of temperate grasslands by mechanical sward disturbance. *Restoration Ecology*, *26*, S114–S122.

Kleijn, D., Kohler, F., Báldi, A., Batáry, P., Concepción, E. D., Clough, Y., Díaz, M., Gabriel, D., Holzschuh, A., Knop, E., Kovács, A., Marshall, E. J. P., Tscharntke, T., & Verhulst, J. (2009). On the relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 903–909.

Klein, N., Theux, C., Arlettaz, R., Jacot, A., & Pradervand, J.-N. (2020). Modeling the effects of grassland management intensity on biodiversity. *Ecology and Evolution*, *10*, 13518–13529.

Královec, J., Pocová, L., Jonášová, M., Macek, P., & Prach, K. (2009). Spontaneous recovery of an intensively used grassland after cessation of fertilizing. *Applied Vegetation Science*, *12*, 391–397.

Lencová, K., & Prach, K. (2011). Restoration of hay meadows on ex-arable land: Commercial seed mixtures vs. spontaneous succession. *Grass and Forage Science*, *66*, 265–271.

Lessard-Therrien, M., Humbert, J.-Y., & Arlettaz, R. (2017). Experiment-based recommendations for biodiversity-friendly management of mountain hay meadows. *Applied Vegetation Science*, *20*, 352–362.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). Performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, *6*(60), 3139.

Ludewig, K., Hansen, W., Klinger, Y. P., Eckstein, R. L., & Otte, A. (2021). Seed bank offers potential for active restoration of mountain meadows. *Restoration Ecology*, *29*, e13311.

Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., Meiners, T., Müller, C., Obermaier, E., Prati, D., Socher, S. A., Sonnemann, I., Wäschke, N., Wubet, T., Wurst, S., & Rillig, M. C. (2014). Choosing and using diversity indices: Insights for ecological applications from the German biodiversity Exploratories. *Ecology and Evolution*, *4*, 3514–3524.

Nerlekar, A. N., & Veldman, J. W. (2020). High plant diversity and slow assembly of old-growth grasslands. *Proceedings of the National Academy of Sciences*, *117*, 18550–18556.

Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., Börger, L., Bennett, D. J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M. J., Feldman, A., Garon, M., Harrison, M. L. K., Alhusseini, T., … Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature*, *520*, 45–50.

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry Stevens, H. H., Szoecs, E., & Wagner, H. (2020). *The vegan package: Community ecology package*.

Pecher, C., Bacher, M., Tasser, E., & Tappeiner, U. (2017). Agricultural landscapes between intensification and abandonment: The expectations of the public in a central-alpine cross-border region. *Landscape Research*, *43*, 428–442.

Piqueray, J., Gilliaux, V., Wubs, E. R. J., & Mahy, G. (2020). Topsoil translocation in extensively managed arable field margins promotes plant species richness and threatened arable plant species. *Journal of Environmental Management*, *260*, 110126.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rasran, L., Vogt, K., & Jensen, K. (2007). Effects of topsoil removal, seed transfer with plant material and moderate grazing on restoration of riparian fen grasslands. *Applied Vegetation Science*, *10*, 451–460.

Riedener, E., Melliger, R. L., Rusterholz, H.-P., & Baur, B. (2015). Changes in landscape composition of differently irrigated hay meadows in an arid mountain region. *Applied Vegetation Science*, *18*, 242–251.

Roscher, C., Temperton, V. M., Buchmann, N., & Schulze, E.-D. (2009). Community assembly and biomass production in regularly and never weeded experimental grasslands. *Acta Oecologica*, *35*, 206–217.

Schaffers, A. P., Raemakers, I. P., Sýkora, K. V., & ter Braak, C. J. F. (2008). Arthropod assemblages are best predicted by plant species composition. *Ecology*, *89*, 782–794.

Scherber, C., Eisenhauer, N., Weisser, W. W., Schmid, B., Voigt, W., Fischer, M., Schulze, E.-D., Roscher, C., Weigelt, A., Allan, E., Beßler, H., Bonkowski, M., Buchmann, N., Buscot, F., Clement, L. W., Ebeling, A., Engels, C., Halle, S., Kertscher, I., … Tscharntke, T. (2010). Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature*, *468*, 553–556.

Schermer, M., Darnhofer, I., Daugstad, K., Gabillet, M., Lavorel, S., & Steinbacher, M. (2016). Institutional impacts on the resilience of mountain grasslands: An analysis based on three European case studies. *Land Use Policy*, *52*, 382–391.

Schirpke, U., Tscholl, S., & Tasser, E. (2020). Spatio-temporal changes in ecosystem service values: Effects of land-use changes from past to future (1860–2100). *Journal of Environmental Management*, *272*, 111068.

Simpson, G. L. (2019). *permute: Functions for generating restricted permutations of data*.

Slodowicz, D., Humbert, J.-Y., & Arlettaz, R. (2019). The relative effectiveness of seed addition methods for restoring or re-creating species rich grasslands: A systematic review protocol. *Environmental Evidence*, *8*, 28.

Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M. M., Renner, S. C., Alt, F., Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S., Böhm, S., Börschig, C., Buscot, F., Diekötter, T., Heinze, J., … Allan, E. (2016). Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature*, *536*, 456–459.

Stevens, C. J. (2016). How long do ecosystems take to recover from atmospheric nitrogen deposition? *Biological Conservation*, *200*, 160–167.

Stoate, C., Boatman, N. D., Borralho, R. J., Carvalho, C. R., Snoo, G. R. d., & Eden, P. (2001). Ecological impacts of arable intensification in Europe. *Journal of Environmental Management*, *63*, 337–365.

Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., Gross, K. L., Milchunas, D. G., & Pennings, S. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 4387–4392.

Tilman, D., Fargione, J., Wolff, B., Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., & Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science*, *292*, 281–284.

Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, *441*, 629–632.

Török, P., & Dengler, J. (2018). Palaearctic grasslands in transition: Overarching patterns and future prospects. In *Grasslands of the world* (1st ed., pp. 15–26). CRC Press.

Török, P., Helm, A., Kiehl, K., Buisson, E., & Valkó, O. (2018). Beyond the species pool: Modification of species dispersal, establishment, and assembly by habitat restoration. *Restoration Ecology*, *26*, S65–S72.

Valkó, O., Rádai, Z., & Deák, B. (2022). Hay transfer is a nature-based and sustainable solution for restoring grassland biodiversity. *Journal of Environmental Management*, *311*, 114816.

van Andel, J., & Aronson, J. (2012). Getting started. In J. van Andel & J. Aronson (Eds.), *Restoration ecology* (pp. 3–8). Blackwell Publishing Ltd.

Veen, P., Jefferson, R., de Smidt, J., & van des Straaten, J. (2009). *Grasslands in Europe of high nature value*. KNNV Publishing.

Wagner, H. H., & Edwards, P. J. (2001). Quantifying habitat specificity to assess the contribution of a patch to species richness at a landscape scale. *Landscape Ecology*, *16*, 121–131.

Wagner, M., Hulmes, S., Hulmes, L., Redhead, J. W., Nowakowski, M., & Pywell, R. F. (2021). Green hay transfer for grassland restoration: Species capture and establishment. *Restoration Ecology*, *29*, e13259.

Yachi, S., & Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 1463–1468.

Zabel, A. (2019). Biodiversity-based payments on Swiss alpine pastures. *Land Use Policy*, *81*, 153–159.

Zhou, M., Yang, Q., Zhang, H., Yao, X., Zeng, W., & Wang, W. (2020). Plant community temporal stability in response to nitrogen addition among different degraded grasslands. *Science of the Total Environment*, *729*, 138886.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Humann-Guilleminot, S., Boch, S., Martínez-De León, G., Lessard-Therrien, M., Hayoz-Andrey, A., Serres-Hänni, A., & Humbert, J.-Y. (2023). Experimental extensification of mountain grasslands restores plant species richness but not species composition in the mid-term. *Journal of Applied Ecology*, *60*, 530–540. https://doi.org/10.1111/1365-2664.14344

Experimental extensification of mountain grasslands restores plant species richness but not species composition in the mid-term.

Ségolène Humann-Guilleminot[1,2*], Steffen Boch[3], Gerard Martínez-De León[1], Malie Lessard-Therrien[1], Aline Hayoz-Andrey[1], Alberto Serres-Hänni[1], Jean-Yves Humbert[1]

[1] Division of Conservation Biology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

[2] Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum d'Histoire Naturelle, CNRS, SU, EPHE, UA, 45 rue Buffon, CP50, 75005 Paris, France

[3] WSL Swiss Federal Research Institute, Zürcherstrasse 111, 8903, Birmensdorf, Switzerland

*Correspondence to: segolene.humann@gmail.com

Supporting Information

Supplementary results

Functional group species richness and cover

In 2010, the species richness of legumes was significantly higher in the mid-intensified plots (I+F 2/3) than in the control plots, while there was no difference for the other two functional groups (Table S4, Figure S2). There was no difference for the cover of the three functional groups (Table S6, Figure S3).

In 2015, after five years of experimental intensification, the species richness of legumes was still slightly higher in the mid-intensified plots compared to the control plots (although marginally non-significant; Table S4, Figure S2). Forb species richness was significantly lower in the highly intensified plots (I+F 3/3) compared to the control plots (Table S4, Figure S2). Grass species richness was lower in the highly intensified plots compared to the control plots, although marginally non-significant (Table S4, Figure S2). Cover of legumes was significantly higher in the mid-intensified plots compared to the extensive, control plots, whilst cover of the two others functional groups were not significantly modified by any treatments (Table S6, Figure S3).

In 2020, after five years of re-extensification, we could not assess any significant difference in the species richness of all three functional groups compared to the controls (Table S4, Figure S2). Cover of legumes was still significantly higher in the mid-intensified plots compared to the control plots, whilst no difference was found for forbs and grasses (Table S6, Figure S3).

We found a significant decrease (29.8%) then a significant increase (35.2%) of legumes species richness in the high-intensive treatment between 2010 and 2015 and between 2015 and 2020, respectively (Table S5, Figure S2). We did not find any other significant results regarding other functional groups species richness in any year.

We found a significant increase of grasses cover in the control (58.0%), mid-intensive (52.5%) and high-intensive treatments (44.5%) between 2010 and 2015 (Table S7, Figure S3). Subsequently, we found a significant decrease in the control (49.7%), mid-intensive (43.6%) and high-intensive (56.8%) treatments between 2015 and 2020 (Table S7, Figure S3). Additionally, we found a marginally non-significant increase and then decrease of grasses cover in the low-intensive treatment between 2010 and 2015 and between 2015 and 2020 respectively (Table S7, Figure S3). There was no other difference for legumes nor forbs cover between any year.

Indicator plant species

In 2010, *Trifolium montanum* and *Festuca rubra aggr.* were identified as indicator plant species in the mid-intensified plots and high-intensified plots, respectively (Table S9). In 2015, *Bromus erectus, Hippocrepis comosa*, *Polygala vulgaris* and *Laserpitium latifolium* were evidenced as indicator species for control plots, *Salvia pratensis* for the low-intensified plots and *Taraxacum officinale aggr.* and *Trisetum flavescens* for the high-intensified plots (Table S9). In 2020, *Viola hirta* was identified as an indicator species for the control plots, *Salvia pratensis* for the low-intensive plots, *Festuca rubra aggr.*, *Trifolium repens* and *Rhinanthus alectorolophus* for the mid-intensive plots, and *Tragropogon pratensis, Ranunculus acris* and *Heracleum sphondylium* for the high-intensive plots (Table S9).

Supplementary discussion

Species richness of forbs was affected at the higher levels of farming intensification, but after five years of re-extensification all study plots again harboured a similarly rich flora, comparable to control plots. In addition, legumes species richness decreased during the farming intensification in the high-intensive treatment, although the difference between the treatments was not visible in 2015. Among the three functional groups, only the cover of legumes seems to benefit from the mid-intensification. As the most species diverse and abundant functional groups in our sample, forbs drove the observed trends, which is in line with former investigations in both lowland and upland agro-ecosystems depicting a negative effect of fertilization on plants (Kleijn et al., 2009, Müller et al., 2016, Gaujour et al., 2012, Socher et al., 2013). However, the cover of legumes benefited from a moderate intensification which is in line with their capacity to fix nitrogen present in the soil and other limiting factors (Onipchenko et al., 2012, Rudmann-Maurer et al., 2008).

Prior to the experimental manipulation in 2010, only two species of plants were associated with two levels of our treatments. *Trifolium montanum*, a species growing in soils poor in nutrients (Ellenberg et al., 2001, Landolt et al., 2010), was associated with mid-intensified management and *Festuca rubra aggr.*, a species growing in all types of soil, with the high-intensified management. Since no other indicator species was put forward by the analysis, we can conclude that plant diversity was initially balanced across all treatment levels. However, after five years of intensification, the situation was much more contrasted. Five species with a preference for soils low to moderately rich in nutrients were associated with our extensive (control) and low-intensified treatments (e.g. *Hippocrepis comosa*, *Salvia pratensis*), while two species, *Taraxacum officinale aggr.* and *Trisetum flavescens*, were associated with the high-intensified treatment. These species-habitat relationships conform to the ecological preferences listed by Ellenberg et al., 2001, Landolt et al., 2010 namely that highly fertilized environments favour some plants. In 2020, after five years of re-extensification, indicator species were even more diverse. *Viola hirta* and *Salvia pratensis*, two species growing in poor-nutrient soils, were associated with control and low-intensive plots, while six species were associated with the medium- (e.g. *Trifolium repens*) and high-intensive plots (e.g. *Ranunculus acris*). Thus, plant species with a propensity to grow in nitrogen-rich soils still operated as indicators for the plots that had received mid to high amounts of fertilizers and irrigation before 2016.

Fertilization of grasslands, combined or not with irrigation, increases the availability of naturally limiting resources for plants. However, according to the niche dimension hypothesis, resource limitation and heterogeneous resource availability are essential for plants species to coexist (Harpole and Tilman, 2007). As a result, a loss of niche dimensions and a shift towards light competition induces the competitive exclusion of slow-growing and low-nitrophilous species, which are unable to grow in the shade (Hautier et al., 2009, Harpole et al., 2016, Zhang et al., 2020). In addition, soil type and soil microbial community composition influence the establishment of target species (i.e. late-colonising grassland species) and in turn determine the success of grassland restoration, although the extent of these effects varies across locations (Fry et al., 2017, Smith et al., 2003). Thus, an effective establishment of species would be facilitated when abiotic conditions in formerly degraded habitats are restored (Török et al., 2018). Only longer-term experimental investigations, for instance a prolongation of the present study, as well as resilience studies carried out in more homogenous, i.e. hostile matrices for biodiversity, would help address these questions (Török et al., 2021).

Supplementary references

Andrey, A., Humbert, J.-Y., & Arlettaz, R. (2016). Functional response of leaf- and planthoppers to modern fertilisation and irrigation of hay meadows. *Basic and Applied Ecology*, **17**, 627–637.

Ellenberg, H., Weber H.E., Düll R., Wirth V. & Werner W. (2001) Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanika*, pp. 1-262.

Fry, E. L., Pilgrim, E. S., Tallowin, J. R. B., Smith, R. S., Mortimer, S. R., Beaumont, D. A., Simkin, J., Harris, S. J., Shiel, R. S., Quirk, H., Harrison, K. A., Lawson, C. S., Hobbs, P. J. & Bardgett, R. D. (2017) Plant, soil and microbial controls on grassland diversity restoration: a long-term, multi-site mesocosm experiment. *Journal of Applied Ecology,* **54,** 1320-1330.

Gaujour, E., Amiaud, B., Mignolet, C. & Plantureux, S. (2012) Factors and processes affecting plant biodiversity in permanent grasslands. A review. *Agronomy for Sustainable Development,* **32,** 133-160.

Harpole, W. S., Sullivan, L. L., Lind, E. M., Firn, J., Adler, P. B., Borer, E. T., Chase, J., Fay, P. A., Hautier, Y., Hillebrand, H., MacDougall, A. S., Seabloom, E. W., Williams, R., Bakker, J. D., Cadotte, M. W., Chaneton, E. J., Chu, C., Cleland, E. E., D'Antonio, C., Davies, K. F., Gruner, D. S., Hagenah, N., Kirkman, K., Knops, J. M. H., La Pierre, K. J., McCulley, R. L., Moore, J. L., Morgan, J. W., Prober, S. M., Risch, A. C., Schuetz, M., Stevens, C. J. & Wragg, P. D. (2016) Addition of multiple limiting resources reduces grassland diversity. *Nature,* **537,** 93-96.

Harpole, W. S. & Tilman, D. (2007) Grassland species loss resulting from reduced niche dimension. *Nature,* **446,** 791-793.

Hautier, Y., Niklaus, P. A. & Hector, A. (2009) Competition for light causes plant biodiversity loss after eutrophication. *Science,* **324,** 636.

Kleijn, D., Kohler, F., Báldi, A., Batáry, P., Concepción, E. D., Clough, Y., Díaz, M., Gabriel, D., Holzschuh, A., Knop, E., Kovács, A., Marshall, E. J. P., Tscharntke, T. & Verhulst, J. (2009) On the relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings of the Royal Society B: Biological Sciences,* **276,** 903-909.

Landolt, E., Bäumler, B., Erhardt, A., Hegg, O., Klölzli, F., Lämmler, W., Nobis, M., Rudmann-Maurer, K., Schweingruber, F. H., Theurillat, J., Urmi, E., Vust, M. & Wohlgemuth, T. (2010) *Flora indicativa. Ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen*.

Lessard-Therrien, M., Humbert, J.-Y., & Arlettaz, R. (2017). Experiment-based recommendations for biodiversity-friendly management of mountain hay meadows. *Applied Vegetation Science*, **20**, 352–362.

Müller, I. B., Buhk, C., Lange, D., Entling, M. H. & Schirmel, J. (2016) Contrasting effects of irrigation and fertilization on plant diversity in hay meadows. *Basic and Applied Ecology,* **17,** 576-585.

Onipchenko, V. G., Makarov, M. I., Akhmetzhanova, A. A., Soudzilovskaia, N. A., Aibazova, F. U., Elkanova, M. K., Stogova, A. V. & Cornelissen, J. H. C. (2012) Alpine plant functional group responses to fertiliser addition depend on abiotic regime and community composition. *Plant and Soil,* **357,** 103-115.

Rudmann-Maurer, K., Weyand, A., Fischer, M. & Stöcklin, J. (2008) The role of landuse and natural determinants for grassland vegetation composition in the Swiss Alps. *Basic and Applied Ecology,* **9,** 494-503.

Smith, R. S., Shiel, R. S., Bardgett, R. D., Millward, D., Corkhill, P., Rolph, G., Hobbs, P. J. & Peacock, S. (2003) Soil microbial community, fertility, vegetation and diversity as targets in the restoration management of a meadow grassland. *Journal of Applied Ecology,* **40,** 51-64.

Socher, S. A., Prati, D., Boch, S., Müller, J., Baumbach, H., Gockel, S., Hemp, A., Schöning, I., Wells, K., Buscot, F., Kalko, E. K. V., Linsenmair, K. E., Schulze, E.-D., Weisser, W. W. & Fischer, M. (2013) Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. *Basic and Applied Ecology,* **14,** 126-136.

Török, P., Brudvig, L. A., Kollmann, J., N. Price, J. & Tóthmérész, B. (2021) The present and future of grassland restoration. *Restoration Ecology,* **29,** e13378.

Török, P., Helm, A., Kiehl, K., Buisson, E. & Valkó, O. (2018) Beyond the species pool: modification of species dispersal, establishment, and assembly by habitat restoration. *Restoration Ecology,* **26,** S65-S72.

Zhang, P., Hefting, M. M., Soons, M. B., Kowalchuk, G. A., Rees, M., Hector, A., Turnbull, L. A., Zhou, X., Guo, Z., Chu, C., Du, G. & Hautier, Y. (2020) Fast and furious: early differences in growth rate drive short-term plant dominance and exclusion under eutrophication. *Ecology and Evolution,* **10,** 10116-10129.

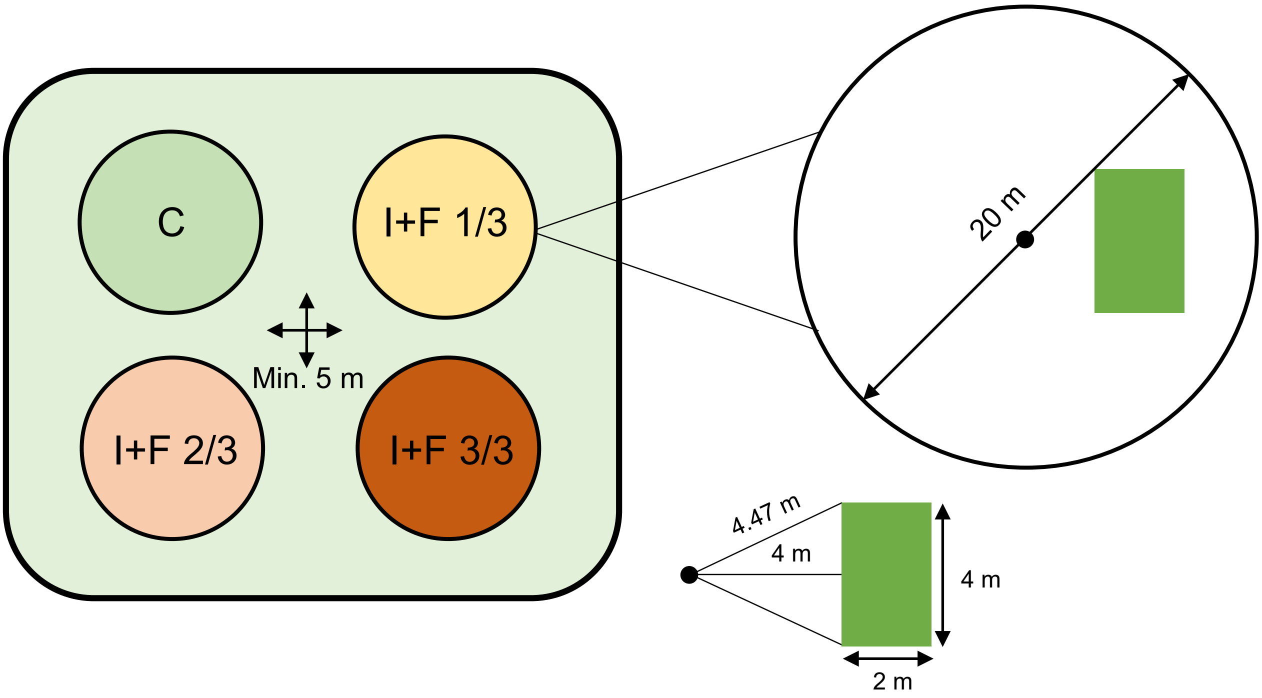

Figure S1. Experimental design. Coloured circles represent the four experimental plots spaced from each other by a minimum of 5 m distance. In each plot, we conducted vegetation relevés (4 m × 2 m L × l; dark green rectangle) within each plot.

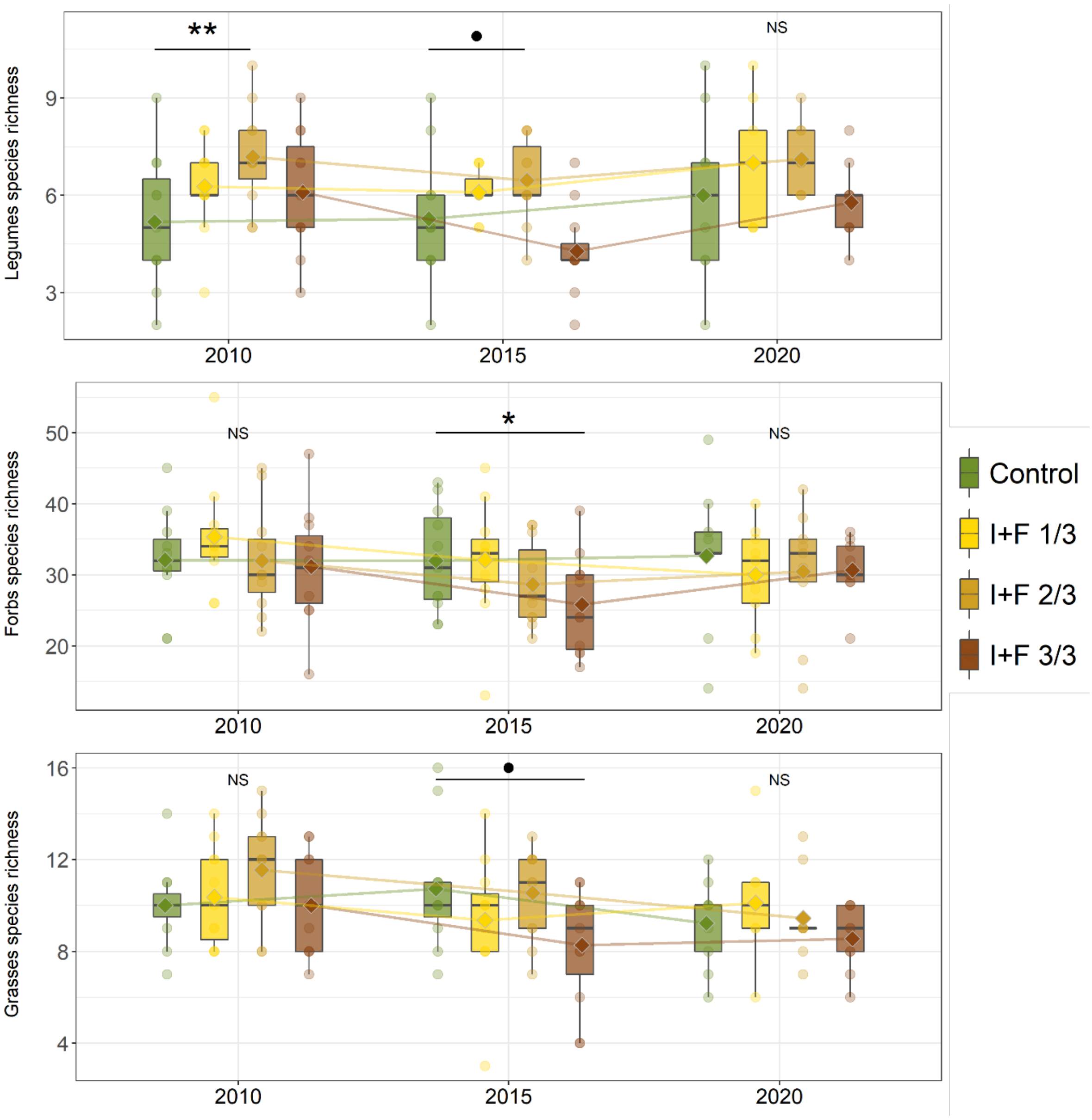

Figure S2. Effects of the different levels of combined irrigation and fertilization on the three functional groups species richness in 2010, 2015 and 2020 (see legend of Figure 1 for more details). Non-significant differences between treatments and control are indicated by the annotation “NS”, marginally non-significant effects are indicated by a point (● for $p < 0.1$) and significant effects are indicated by asterisks (* for $p < 0.05$; ** for $p < 0.01$).

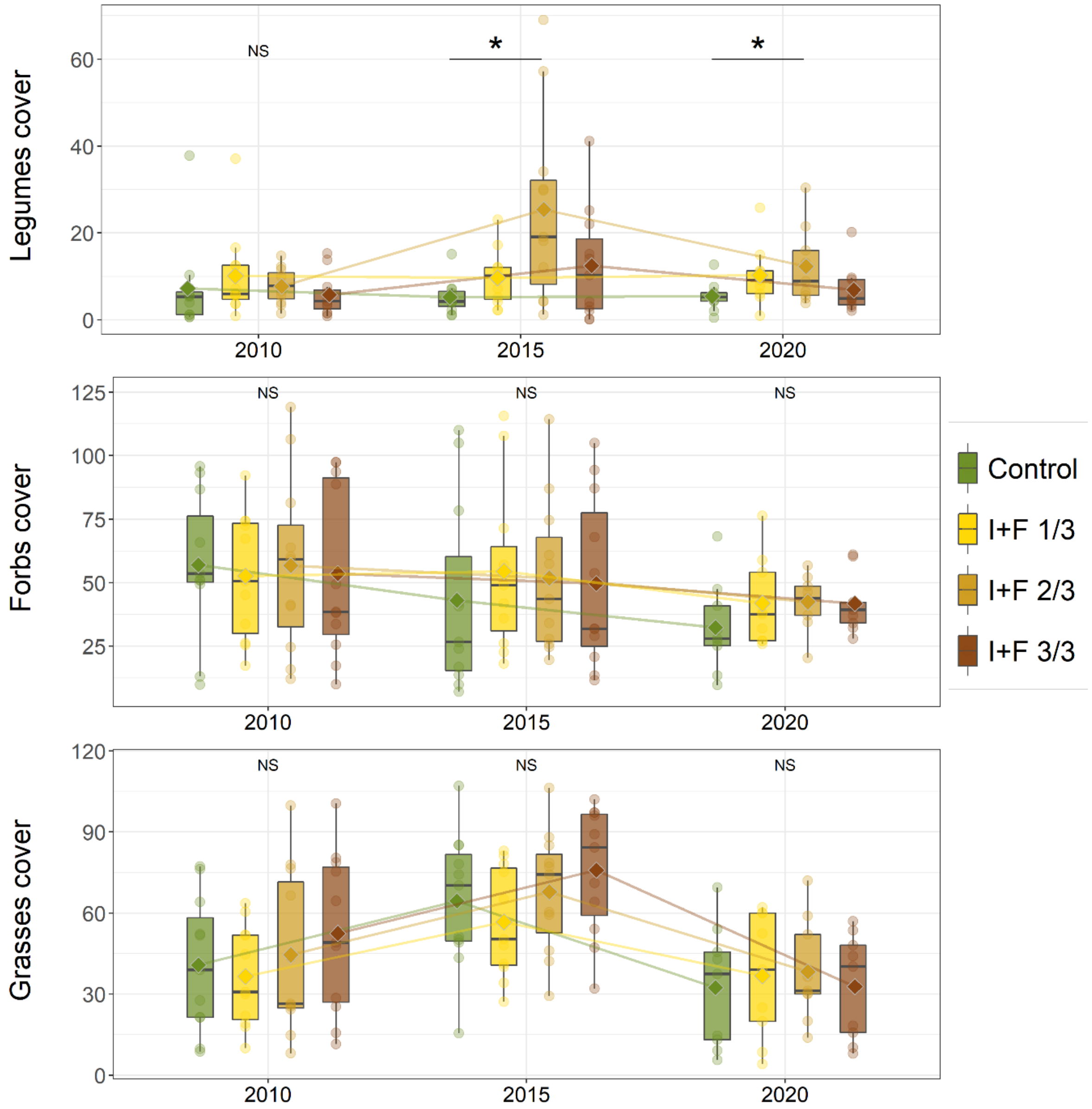

Figure S3. Effects of the different levels of combined irrigation and fertilization on the three functional groups covers in 2010, 2015 and 2020 (see legend of Figure 1 for more detail). Non-significant differences between treatments as well as between treatments and control are indicated by the annotation “NS” and significant effects are indicated by asterisks (* for $p < 0.05$).

Table S1. Productivity type (A: meadows situated at higher elevation with lower productivity; B: meadows situated around mid-elevation with intermediate productivity; C: meadows situated at lower elevation with higher productivity; Andrey et al. 2016, Lessard-Therrien et al. 2017), Coordinates and elevation (m above sea level) of the eleven meadows serving as spatial replicates in our experiment. Meadows with an asterisk have unfortunately been lost between 2015 and 2020 due to changes in land-use.

Meadows	Productivity type	Elevation [m a.s.l.]	Latitude	Longitude
Arbaz*	B	1270	46°16′42″N	7°22′47″E
Cordona	B	1153	46°19′45″N	7°33′8″E
Eison	A	1768	46°9′18″N	7°28′10″E
Euseigne	C	1028	46°10′9″N	7°25′27″E
Grimentz	A	1738	46°11′22″N	7°34′35″E
Icogne 1	B	1200	46°17′56″N	7°26′31″E
Icogne 2	C	880	46°16′42″N	7°26′10″E
La Garde	B	980	46°3′45″N	7°8′35″E
Orsières*	C	1022	46°1′44″N	7°9′8″E
Saint-Martin	A	1589	46°11′8″N	7°26′43″E
Vens	B	1373	46°5′7″N	7°7′24″E

Table S2. Number of cut per year, amount of fertiliser (kg ha^{-1} year^{-1}) and irrigation (mm week^{-1}) applied in each treatment between 2010 and 2015 (from Andrey et al. 2016 and Lessard-Therrien et al. 2017). See supplementary information in Andrey et al. 2016 for study site categorisation. Productivity types are described in Table S1.

		Amount of fertiliser									
		Productivity type A			Productivity type B			Productivity type C			
Treatment	Number of cut per year	N	P	K	N	P	K	N	P	K	Irrigation
C	1	0	0	0	0	0	0	0	0	0	0
I+F 1/3	2	13.3	4.8	36.9	20	7.3	55.4	26.7	9.7	73.8	10.0
I+F 2/3	2	26.7	9.7	73.8	40	14.5	110.6	53.3	19.4	147.5	20.0
I+F 3/3	2	40.0	14.5	110.6	60	21.8	166.0	80.0	29.1	221.4	30.0

Table S3. Mean and 95% confidence interval for the mean for each response variable with respect to degree of intensification and year.

	Total species				Functional groups species richness						Functional groups cover					
	Species richness		Simpson diversity		Legumes		Forbs		Grasses		Legumes		Forbs		Grasses	
	mean	95%[IC]	mean	95%[IC]	mean	95%[IC]	mean	95%[IC]	mean	95%[IC]	mean	95%[IC]	mean	95%[IC]	mean	95%[IC]
2010																
Control	47.455	[42.543,52.367]	0.819	[0.754,0.884]	5.182	[3.977,6.387]	32.091	[27.967,36.215]	10.000	[8.944,11.056]	7.226	[0.962,13.490]	56.966	[40.179,73.753]	40.808	[25.969,55.647]
I+F 1/3	52.182	[47.086,57.278]	0.850	[0.819,0.881]	6.273	[5.434,7.112]	35.364	[30.725,40.003]	10.364	[9.090,11.638]	10.151	[4.202,16.100]	52.628	[37.901,67.355]	36.502	[25.475,47.529]
I+F 2/3	50.818	[45.573,56.063]	0.798	[0.720,0.876]	7.182	[6.273,8.091]	32.000	[27.617,36.383]	11.545	[10.189,12.901]	7.742	[5.333,10.151]	56.773	[36.113,77.433]	44.480	[26.428,62.532]
I+F 3/3	47.455	[42.383,52.527]	0.788	[0.739,0.837]	6.091	[4.988,7.194]	31.182	[26.368,35.996]	10.000	[8.704,11.296]	5.796	[2.950,8.642]	53.609	[33.511,73.707]	52.487	[34.929,70.045]
2015																
Control	48.091	[42.470,53.712]	0.749	[0.645,0.853]	5.273	[4.119,6.427]	32.000	[27.625,36.375]	10.727	[9.139,12.315]	5.218	[2.905,7.531]	43.090	[20.856,65.324]	64.461	[49.443,79.479]
I+F 1/3	47.636	[41.585,53.687]	0.825	[0.752,0.898]	6.091	[5.677,6.505]	32.091	[27.171,37.011]	9.364	[7.708,11.020]	9.687	[5.871,13.503]	54.491	[35.305,73.677]	56.682	[44.591,68.773]
I+F 2/3	45.636	[41.990,49.282]	0.817	[0.774,0.860]	6.455	[5.691,7.219]	28.636	[25.177,32.095]	10.545	[9.383,11.707]	25.375	[12.451,38.299]	51.817	[33.914,69.720]	67.841	[54.393,81.289]
I+F 3/3	38.364	[33.003,43.725]	0.733	[0.629,0.837]	4.273	[3.475,5.071]	25.818	[21.718,29.918]	8.273	[6.752,9.794]	12.490	[4.887,20.093]	49.692	[29.678,69.706]	75.835	[61.815,89.855]
2020																
Control	47.889	[40.245,55.533]	0.847	[0.731,0.963]	6.000	[4.334,7.666]	32.667	[26.044,39.290]	9.222	[7.966,10.478]	5.428	[3.152,7.704]	32.367	[20.685,44.049]	32.402	[17.608,47.196]
I+F 1/3	47.222	[42.651,51.793]	0.891	[0.862,0.920]	7.000	[5.822,8.178]	30.000	[25.404,34.596]	10.111	[8.563,11.659]	10.262	[5.627,14.897]	41.900	[30.459,53.341]	36.764	[21.694,51.834]
I+F 2/3	47.000	[41.287,52.713]	0.919	[0.895,0.943]	7.111	[6.423,7.799]	30.444	[24.517,36.371]	9.444	[8.217,10.671]	12.306	[6.451,18.161]	42.432	[35.390,49.474]	38.281	[25.890,50.672]
I+F 3/3	45.000	[42.448,47.552]	0.915	[0.895,0.935]	5.778	[4.992,6.564]	30.667	[27.745,33.589]	8.556	[7.625,9.487]	6.906	[3.223,10.589]	41.788	[34.203,49.373]	32.766	[19.995,45.537]

Table S4. Linear mixed model outputs testing for an effect of the three levels of intensification on plants functional groups species richness fitted using a restricted maximum likelihood function. Transformation of the response variable is indicated in the estimate column and corresponding year. All the p-values presented are corrected for the false discovery rate. Significant effects ($p < 0.05$) are in bold and marginally non-significant effects ($0.05 < p < 0.1$) in italics. The difference between the AIC of the full model against the AIC of the null model (intercept only) is indicated in the ΔAIC column, a negative ΔAIC meaning that the full model better explains the data.

	Functional groups species richness														
	Legumes					Forbs					Grasses				
	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC
2010					-3.800	*Log*				1.300					1.200
Intercept (control)	5.182	0.523	32.052	**< 0.001**		3.446	0.072	21.362	**< 0.001**		10.000	0.638	36.923	**< 0.001**	
I+F 1/3	1.091	0.624	30.000	0.175		0.100	0.069	30.000	0.468		0.364	0.824	30.000	0.662	
I+F 2/3	2.000	0.624	30.000	**0.010**		-0.003	0.069	30.000	0.961		1.545	0.824	30.000	0.211	
I+F 3/3	0.909	0.624	30.000	0.234		-0.040	0.069	30.000	0.785		0.000	0.824	30.000	1.000	
2015					-10.400					-5.400					-1.800
Intercept (control)	5.273	0.421	36.640	**< 0.001**		32.000	2.166	21.574	**< 0.001**		10.727	0.762	37.209	**< 0.001**	
I+F 1/3	0.818	0.541	30.000	0.175		0.091	2.092	30.000	0.966		-1.364	0.989	30.000	0.472	
I+F 2/3	1.182	0.541	30.000	*0.055*		-3.364	2.092	30.000	0.355		-0.182	0.989	30.000	0.855	
I+F 3/3	-1.000	0.541	30.000	0.223		-6.182	2.092	30.000	**0.018**		-2.455	0.989	30.000	*0.057*	
2020					0.500	*Log*				4.700					2.500
Intercept (control)	6.000	0.585	26.877	**< 0.001**		3.433	0.100	12.209	**< 0.001**		9.222	0.643	31.193	**< 0.001**	
I+F 1/3	1.000	0.715	24.000	0.175		-0.058	0.073	24.000	0.647		0.889	0.865	24.000	0.472	
I+F 2/3	1.111	0.715	24.000	0.133		-0.068	0.073	24.000	0.542		0.222	0.865	24.000	0.855	
I+F 3/3	-0.222	0.715	24.000	0.759		-0.020	0.073	24.000	0.785		-0.667	0.865	24.000	0.673	

Table S5. Linear mixed model outputs testing for an effect of the three levels of intensification on plants functional groups species richness fitted using a restricted maximum likelihood function. Transformation of the response variable is indicated in the estimate column and corresponding year. All the p-values presented are corrected for the false discovery rate. Significant effects ($p < 0.05$) are in bold and marginally non-significant effects ($0.05 < p < 0.1$) in italics. The difference between the AIC of the full model against the AIC of the null model (intercept only) is indicated in the ΔAIC column, a negative ΔAIC meaning that the full model better explains the data.

	Functional groups species richness														
	Legumes					Forbs					Grasses				
	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC
Control	*Log*				3.800	*Log*				3.600					-0.100
Intercept (2010)	1.567	0.134	16.272	**< 0.001**		3.446	0.084	16.974	**< 0.001**		10.000	0.659	23.448	**< 0.001**	
2015	0.028	0.117	17.849	0.813		-0.004	0.076	17.974	0.957		0.727	0.766	18.034	0.355	
2020	0.054	0.125	18.234	0.805		-0.043	0.081	18.387	0.749		-0.953	0.817	18.812	0.344	
Intercept (2015)	1.567	0.134	16.272	**< 0.001**		3.446	0.084	16.974	**< 0.001**		10.000	0.659	23.448	**< 0.001**	
2020 vs 2015	0.028	0.117	17.849	0.813		-0.004	0.076	17.974	0.956		0.727	0.766	18.034	0.355	
I+F 1/3					0.900					-1.600	*Log*				2.500
Intercept (2010)	6.273	0.411	20.972	**< 0.001**		35.364	2.318	17.676	**< 0.001**		2.319	0.090	27.760	**< 0.001**	
2015	-0.182	0.446	17.498	0.689		-3.273	2.124	18.336	0.187		-0.140	0.122	18.562	0.355	
2020	0.607	0.477	18.168	0.220		-5.401	2.280	18.760	0.115		-0.034	0.130	19.717	0.794	
Intercept (2015)	6.273	0.411	20.972	**< 0.001**		35.364	2.318	17.676	**< 0.001**		2.319	0.090	27.760	**< 0.001**	
2020 vs 2015	-0.182	0.446	17.498	0.813		-3.273	2.124	18.336	0.187		-0.140	0.122	18.562	0.355	
I+F 2/3					-0.700					1.300					-1.300
Intercept (2010)	7.182	0.402	18.768	**< 0.001**		32.000	2.234	18.216	**< 0.001**		11.545	0.623	27.873	**< 0.001**	
2015	-0.727	0.398	17.833	0.168		-3.364	2.127	18.127	0.187		-1.000	0.858	18.963	0.355	
2020	0.107	0.426	18.355	0.805		-2.313	2.281	18.597	0.647		-2.096	0.906	20.118	0.126	
Intercept (2015)	7.182	0.402	18.768	**< 0.001**		32.000	2.234	18.216	**< 0.001**		11.545	0.623	27.873	**< 0.001**	
2020 vs 2015	-0.727	0.398	17.833	0.168		-3.364	2.127	18.127	0.187		-1.000	0.858	18.963	0.355	
I+F 3/3					-4.500					-0.900					-1.400
Intercept (2010)	6.091	0.458	28.000	**< 0.001**		31.182	2.067	25.157	**< 0.001**		10.000	0.649	25.044	**< 0.001**	
2015	-1.818	0.648	28.000	**0.036**		-5.364	2.568	17.039	0.187		-1.727	0.788	18.541	0.165	
2020	-0.313	0.683	28.000	0.805		-0.887	2.732	18.008	0.749		-1.503	0.839	19.391	0.178	
Intercept (2015)	6.091	0.458	28.000	**< 0.001**		31.182	2.067	25.157	**< 0.001**		10.000	0.649	25.044	**< 0.001**	
2020 vs 2015	-1.818	0.648	28.000	**0.036**		-5.364	2.568	17.039	0.187		-1.727	0.788	18.541	0.165	

Table S6. Linear mixed model outputs testing for an effect of the three levels of intensification on plants functional groups cover fitted using a restricted maximum likelihood function. Transformation of the response variable is indicated in the estimate column. All the p-values presented are corrected for the false discovery rate. Significant effects ($p < 0.05$) are in bold marginally non-significant effects ($0.05 < p < 0.1$) in italics. The difference between the AIC of the full model against the AIC of the null model (intercept only) is indicated in the ΔAIC column, a negative ΔAIC meaning that the full model better explains the data.

	Functional groups cover														
	Legumes					Forbs					Grasses				
	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC
2010	*Logit*				0.300					11.000					0.500
Intercept (control)	-3.265	0.319	28.419	**< 0.001**		0.570	0.093	17.808	**< 0.001**		0.408	0.080	19.097	**< 0.001**	
I+F 1/3	0.704	0.359	30.000	0.109		-0.043	0.078	30.000	0.362		-0.043	0.071	30.000	0.548	
I+F 2/3	0.620	0.359	30.000	*0.094*		-0.002	0.078	30.000	0.147		0.037	0.071	30.000	0.608	
I+F 3/3	0.139	0.359	30.000	0.701		-0.034	0.078	30.000	0.497		0.117	0.071	30.000	0.330	
2015	*Logit*				-3.000					3.700	*****				-1.100
Intercept (control)	-3.158	0.437	37.247	**< 0.001**		0.4309	0.101	16.900	**< 0.001**		0.627	0.198	1.045	**< 0.001**	
I+F 1/3	0.686	0.567	30.000	0.236		0.114	0.082	30.000	0.260		-0.042	0.047	29.516	0.521	
I+F 2/3	1.623	0.567	30.000	**0.023**		0.087	0.082	30.000	0.294		0.034	0.047	29.444	0.605	
I+F 3/3	0.233	0.567	30.000	0.701		0.066	0.082	30.000	0.497		0.078	0.047	29.516	0.639	
2020	*Logit*				-1.600	*Logit*				1.200					4.800
Intercept (control)	-3.131	0.297	27.215	**< 0.001**		-0.845	0.226	29.027	**< 0.001**		0.324	0.070	17.690	**< 0.001**	
I+F 1/3	0.686	0.365	24.000	0.109		0.503	0.289	24.000	0.260		0.044	0.069	24.000	0.548	
I+F 2/3	0.944	0.365	24.000	**0.024**		0.519	0.289	24.000	0.147		0.059	0.069	24.000	0.605	
I+F 3/3	0.297	0.365	24.000	0.701		0.502	0.289	24.000	0.285		0.004	0.069	24.000	0.958	

***** Since the residuals of this model had a bimodal distribution, we created a second categorical random variable (2 levels) with an arbitrary cut-off at 65% of grasses cover.

Table S7. Linear mixed model outputs testing for an effect of each year on plants functional groups cover. Transformation of the response variable is indicated in the estimate column. All the p-values presented are corrected for the false discovery rate. Significant effects ($p < 0.05$) are in bold and marginally non-significant effects ($0.05 < p < 0.1$) in italics. The difference between the AIC of the full model against the AIC of the null model (intercept only) is indicated in the ΔAIC column, a negative ΔAIC meaning that the full model better explains the d

	Functional groups cover														
	Legumes					Forbs					Grasses				
	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC	Estimate	SE	DF	P	ΔAIC
Control	*Logit*				3.900	*Gamma**				-2.000					-9.300
Intercept (2010)	-3.265	0.334	23.096	**< 0.001**		2.812	0.767		**< 0.001**		0.408	0.074	19.319	**< 0.001**	
2015	0.106	0.394	16.594	0.884		0.445	0.370		0.919		0.237	0.075	17.960	**0.030**	
2020	-0.011	0.420	17.452	0.979		1.239	0.515		0.064		-0.057	0.080	18.502	0.718	
Intercept (2015)	-3.158	0.334	23.096	**< 0.001**		3.257	0.780		**< 0.001**		0.645	0.074	19.319	**< 0.001**	
2020 vs 2015	-0.118	0.420	17.452	0.941		0.794	0.553		0.366		-0.294	0.080	18.502	**0.003**	
I+F 1/3					4.000					2.100	*Gamma**				-0.600
Intercept (2010)	0.102	0.024	26.541	**< 0.001**		0.526	0.079	25.791	**< 0.001**		2.816	0.476		**< 0.001**	
2015	-0.005	0.031	18.191	0.884		0.019	0.098	18.406	0.994		-0.944	0.510		*0.064*	
2020	-0.002	0.033	19.199	0.979		-0.116	0.105	19.327	0.623		-0.006	0.639		0.993	
Intercept (2015)	0.097	0.024	26.541	**< 0.001**		0.545	0.079	25.791	**< 0.001**		1.872	0.359		**< 0.001**	
2020 vs 2015	0.002	0.033	19.199	0.941		-0.135	0.105	19.327	0.366		0.939	0.547		*0.086*	
I+F 2/3	*Logit*				-1.600					2.200					-3.900
Intercept (2010)	-2.645	0.335	28.000	**< 0.001**		0.568	0.085	26.596	**< 0.001**		0.445	0.075	27.997	**< 0.001**	
2015	1.109	0.474	28.000	0.107		-0.050	0.110	17.871	0.994		0.234	0.106	16.642	**0.030**	
2020	0.458	0.500	28.000	0.979		-0.154	0.117	18.908	0.623		-0.061	0.112	18.020	0.993	
Intercept (2015)	-1.535	0.335	28.000	**< 0.001**		0.518	0.085	26.596	**< 0.001**		0.678	0.075	27.997	**< 0.001**	
2020 vs 2015	-0.651	0.500	28.000	0.813		-0.105	0.117	18.908	0.380		-0.295	0.112	18.020	**0.022**	
I+F 3/3	*Logit*				3.800					1.600					-12.900
Intercept (2010)	-3.126	0.439	27.325	**< 0.001**		0.536	0.089	20.484	**< 0.001**		0.525	0.076	20.511	**< 0.001**	
2015	0.201	0.587	16.922	0.884		-0.039	0.095	17.698	0.994		0.233	0.081	17.715	**0.026**	
2020	0.257	0.622	18.110	0.979		-0.153	0.101	18.325	0.623		-0.164	0.086	18.342	0.234	
Intercept (2015)	-2.925	0.439	27.325	**< 0.001**		0.497	0.089	20.484	**< 0.001**		0.758	0.076	20.511	**< 0.001**	
2020 vs 2015	-2.925	0.439	27.325	0.941		-0.114	0.101	18.325	0.366		-0.398	0.086	18.342	**0.001**	
*GLMM applied															

Table S8. Summary statistics of the analysis of multivariate homogeneity of group dispersions using the Bray-Curtis distance indices testing the effect of experimental management treatments on plant community variability. Presented are degrees of freedom, variance explained (R^2), F-values and p-values. P-values from the post-hoc tests of pairwise differences are presented using the Tukey Honest Significant Differences tests.

	DF	F-value	P
2010			
Treatment	3	0.167	0.918
Control vs. I+F 1/3			1.000
Control vs. I+F 2/3			1.000
Control vs. I+F 3/3			0.954
2015			
Treatment	3	0.113	0.952
Control vs. I+F 1/3			0.988
Control vs. I+F 2/3			0.946
Control vs. I+F 3/3			0.999
2020			
Treatment	3	0.506	0.681
Control vs. I+F 1/3			0.954
Control vs. I+F 2/3			0.825
Control vs. I+F 3/3			0.646

Table S9. Indicator species analysis in the four plots managed according to the four treatments. Stat column shows the association statistic of the permutational test from the indicator species analysis, higher stat value meaning that the species is more strongly associated with the treatment. All the p-values presented are corrected for the false discovery rate. Only species with a significant association with one of the four treatments are presented in the table.

Species	Treatment	Stat	P
2010			
Trifolium montanum	I+F 2/3	0.423	0.021
Festuca rubra aggr.	I+F 3/3	0.327	0.021
2015			
Bromus erectus	Control	0.506	0.008
Hippocrepis comosa	Control	0.468	0.035
Polygala vulgaris	Control	0.377	0.035
Laserpitium latifolium	Control	0.227	0.035
Salvia pratensis	I+F 1/3	0.287	0.019
Taraxacum officinale	I+F 3/3	0.394	0.019
Trisetum flavescens	I+F 3/3	0.379	0.035
2020			
Viola hirta	Control	0.371	0.043
Salvia pratensis	I+F 1/3	0.281	0.022
Festuca rubra aggr.	I+F 2/3	0.398	0.047
Trifolium repens	I+F 2/3	0.370	0.027
Rhinanthus alectorolophus	I+F 2/3	0.276	0.022
Tragopogon pratensis	I+F 3/3	0.442	0.022
Ranunculus acris	I+F 3/3	0.403	0.022
Heracleum sphondylium	I+F 3/3	0.279	0.047