Optimal balance between yield and biodiversity in montane and subalpine meadows

Inauguraldissertation

der Philosophisch-naturwissenschaftlichen Fakultät

der Universität Bern

vorgelegt von

Aline Hayoz-Andrey

von Val-de-Charmey (FR)

Leiter der Arbeit

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Der Dekan

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CONTENT

ABSTRACT

Montane and subalpine hay meadows are a hotspot for biodiversity as well as being of high conservation interest in Europe. Traditional and extensive management methods using solid manure applications and gravitationnal, terrestrial irrigation are currently shifting to the increased use of liquid manure (slurry) applications and aerial irrigation (sprinklers) for higher grass and hay production. The impacts these new practices have on biodiversity are poorly understood and call for quantitative assessments for the sustainable management of mountain hay meadows.

In order to meet this real conservation challenge, this thesis has first summarised current state of knowledge and identified scientific gaps through a meta-analysis that focused mainly on the effects of nitrogen application on mountain meadows (chapter 1). Then, an experimental study in a randomised block design was conducted to answer to the questions raised in this meta-analysis. The effects of different treatments corresponding to an increasing gradient of farming intensification were investigated on plants and arthropods. The aim was to identify an easily applicable management treatment for farmers that would be the best trade-off between productivity, plant diversity and arthropod abundance and biomass. The main hypothesis of the experimental study was based on the hump-shaped model : this stipulates that an intermediate level of intensification on extensively managed mountain meadows can be beneficial for hay productivity and plant species richness while increased plant-growth may, in turn, induce benefits for arthropod biomass and abundance.

The experimental study began in 2010 and was conducted in the inner European Alps (Valais, SW Switzerland) over 12 meadows. The experimental set up consisted of: control plots receiving neither fertiliser nor irrigation, experienced one cut a year (C-plots); plots that received only fertiliser (slurry), with two cuts a year (F-plots); plots that were only irrigated, with two cuts a year (I-plots); and plots that received low-, medium- and high-input of fertiliser and water, and experienced one (F+I 1/3-plots) or two cuts a year (F+I 2/3, F+I 3/3-plots). The immediate effects of some of these treatments (C, F, I and F+I 2/3-plots) on vegetation structure, hay productivity, plant species richness, arthropod biomass and abundance were investigated in 2011 (chapter 2) while short-term effects of all treatments were tested on Auchenorrhyncha in 2012 (chapter 3).

The results showed that although fertiliser applications on mountain meadows usually alter plant diversity (chapter 1), fertiliser treatment displayed positive effects on plant species richness, hay productivity and biomass of arthropods compared to C-plots. However, fertiliser applications did not affect vegetation structure, arthropod abundance or Auchenorrhyncha. Irrigation applications using sprinklers (I-plots) immediately increased plant species richness, vegetation structure, hay productivity and arthropod abundance and biomass compared to the control. Although our results showed that water was a limiting factor for the meadows studied, irrigation application affected neither positively nor negatively Auchenorrhnycha. The three treatments combining fertiliser and water immediately increased plant species richness, vegetation structure, hay productivity and arthropod abundance and biomass. Moreover, these treatments boosted Auchenorrhyncha abundance and biomass : in particular, treatments F+I 1/3 favoured specialist species (monophagous and oligophagous) while treatments F+I 2/3 and F+I 3/3 increased the generalist, eurytopic species.

Mountain hay meadows display high sensitivity to farming. Thanks to this fully-controlled experimental design able to minimise confounding effects that could influence the results, guidance for their management can be provided. Relying on the study findings, application of doses of fertiliser and water equivalent of a maximum of one third of the amount required to achieve the maximum theoretical hay yield locally can be recommended as it will ensure the maintenance of the specialised species of the indicator taxon studied.

GENERAL INTRODUCTION

European grasslands

Grasslands cover about 30% of agricultural land in Europe (Huyghe *et al.* 2014). They are defined as vegetated areas dominating by non-woody plants such as grasses, legumes and other forbs. In temperate regions, grasslands are generally considered to be anthropogenic, i.e. semi-natural, rather than natural (Wilkins, Hopkins & Hatch 2003) and their existence is therefore linked to a certain human-based farming activities that maintains the herbaceous communities dedicated to the production of forage. The resulting semi-natural grasslands can support high plant biodiversity with many rare and unique species linked to this type of open habitat (e.g. Pykälä *et al.* 2005). They are also inhabited by a large faunal community; from herbivorous to carnivorous animals, providing them food resources, shelter and reproduction sites (e.g Pärtel, Kalamees, *et al.*, 2005). In addition these semi-natural grasslands participate in many other ecosystem functions, such as, among others, sequestration and storage of carbon and other greenhouse gases and soil protection (Bugalho & Abreu 2008). They are, therefore, of high conservation interest (e.g. Poschlod & WallisDeVries 2002).

In the late 1940s, the area occupied by semi-natural grasslands in Europe dramatically fell (e.g. Strijker 2005) either through abandonment of agricultural practices or by converting semi-natural grasslands to other land-use types (e.g. croplands). This constituted a major break with the past in the countryside in developed countries. Another important component of the post-war agriculture changes was intensification. This corresponds to increased fertilisers, pesticides, equipment, resources (especially water) and energy inputs combined with progressive enlargement of field sizes and improved mechanisation. The intensification of the management practices within semi-natural grasslands leaded to a significant reduction in plant diversity and the loss of animal species (e.g. Erhardt & Thomas 1991; Knops *et al.* 1999; Isselstein, Jeangros & Pavlu 2005): typically, many specialised species were replaced by generalists. Altogether, this resulted in a profound simplification of the agricultural landscape and a massive biotic homogenization (Robinson & Sutherland 2002; Clavel, Julliard & Devictor 2010).

Mountain context

In mountainous regions the situation is somehow different than in the lowlands, as the complex topography and harder climatic conditions have in some way forced the farmers to hang on to the traditional low intensity management practices (Maurer *et al.* 2006; Kampmann *et al.* 2008). There, semi-natural grasslands are nutrient-poor and traditional management use little or no fertiliser (in the form solid organic manure) and in xeric places gravitational irrigation systems, allowing a single annual cut after flowering. In particular, mountain hay meadows – i.e. grasslands predominantly managed by mowing (Peeters *et al.* 2014) that are the focus of this PhD thesis – are home to many plants (Marini *et al.* 2007) and invertebrate species such as bees (Oertli, Mueller & Dorn 2005), butterflies and grasshoppers (Hohl 2006).

This said and despite their high ecological importance (Körner 2002; Baur *et al.* 2006), mountain hay meadows are currently under threat following rapid development in agricultural practices (Peter *et al.* 2009). It seems that we are currently repeating in mountain grasslands the same mistakes made decades ago when intensifying the lowland regions. Depending on their slope, elevation and accessibility (Kampmann, 2008), they are facing two dichotomous and challenging trends to the extent that they are causing ecological and economic problems (Tasser & Tappeiner 2002).

First, management practices are being abandoned in the most remote areas of low agro-economic value and on marginal and steep areas, causing vegetation encroachment and progressive return to forest (Meeus 1993; Mottet *et al.* 2006). Through the growth of successional plants and by the invasion of bushes and other woody species, plants requiring open habitats disappear and species diversity decreases (MacDonald *et al.* 2000).

Secondly, grasslands that are easily accessible and on terrain where mechanisation is possible are now intensified (Tasser & Tappeiner 2002). In mountainous areas, solid organic manure (farm manure mixed with straw) is gradually being replaced by a liquid organic alternative, which is easily and in higher amount being sprayed on fields. Increased fertiliser usually increases soil nitrification (Merino *et al.* 2001) and eurytopic plant species abundance (Crawley *et al.* 2005; Suding *et al.* 2005), which leads to a decrease in plant species richness (Humbert *et* *al.* 2015). In more arid areas like in many regions of the European Alps, irrigation is key to maintaining meadows and helping farmers ensure decent hay production (Leibundgut 2004). For centuries, complex networks of open conveyance channels ("bisses" in French) were built to conduct water from the headworks to the meadows to allow a distribution of water by gravity (Crook & Jones 1999). However, these are now being replaced by underground water pipe systems (sprinklers). These more efficient systems require much less maintenance allowing more frequent and higher irrigation applications that lead in turn to an increase in the growth of grass and hay productivity. Technical advances over the few last decades make it possible to manage larger surfaces over a much shorter time-period, thus allowing an increased number of cuts. Furthermore, the use of machines in mountain areas has also reduced labour inputs required to manage hay meadows.

Altogether, these new management practices are expected to gradually decrease species diversity partly due to predicted resource competition theory (Tilman 1988) and the exclusion of slow-growing species (Grime, 1973), that are competing with species adapted to high levels of disturbance (e.g. Hautier, Niklaus & Hector 2009; Riedener, Rusterholz & Baur 2013). Changes are not only visible on the plant community, but also in higher trophic levels of the food chain up to vertebrates. For example, bird species linked with traditionally managed hay meadows, such as the Alpine Whinchat, are now threatened as their food source (i.e. invertebrates) is being affected by intensification (Britschgi, Spaar & Arlettaz 2006). These changes are also harmful to all other ecosystem functions of these grasslands, as the valuable environment for recreation and tourism they provide (Bugalho & Abreu 2008).

The practices outlined above are mechanised and easily operated by farmers. However, there is currently no recommendation on volumes (i.e. kg of fertilisers and mm of water) that should be applied to mountain hay meadows to maintain an economically (decent yield) and environmentally-friendly agriculture. The main reason is a lack of scientific information about the effects of these new practices on the biodiversity.

Study aim and design

Understanding land use change and the effects of intensification through fertilisation and irrigation is important for conserving mountain hay meadows. It is commonly acknowledged that when intensification increases from low to high-input, hay production rises while biodiversity drops (e.g. Di Giulio, Edwards & Meister 2001; Humbert *et al.* 2015). However, the hump-shaped model of plant diversity (Grime 1973) stipulates that an intermediate level of management intensity, notably as low or moderate input of fertilisation an water intensification may support higher plant species richness than extensively or high-intensively managed meadows (Bowman *et al.* 2006; Peter *et al.* 2009). In turn, an increase in plant growth would benefit arthropods (e.g. Grime 1973; Haddad, Haarstad & Tilman 2000; Grandchamp *et al.* 2005).

The main aim of this thesis was to quantitatively define whether it exists an optimal trade-off, in term of management intensity, between hay production, plant diversity and arthropods (Fig. 1). Ultimately, the present manuscript will provide concrete guidelines to stakeholders for management of mountain hay meadows that sustains hay production while preserving the biodiversity. The research was carried out on mountain hay meadows (at montane and subalpine levels) scattered across Valais, an inner Alpine valley with xeric climatic conditions. One of the main features of the climate in this region is drought. Annual rainfall in Valais is approximately 600mm, while in the northern and southern Prealps it is about 2000 mm. Six management treatments mimicking agricultural practices for grassland management were investigated. The management treatments proposed had to be easy to apply for the farmers and in keeping with their practices. They consisted of applying different amounts of fertiliser and water, either separately or combined. In addition, they were adapted according to each site where they were applied in accordance with the site's theoretical hay productivity potential. The experimental set-up consisted of: a plot fertilised only at an intermediate level of intensity (representing an equivalent dose to two-thirds of the amount that would have been required for maximum local hay productivity) and mown twice a year; a plot irrigated at the same dose and mown twice a year; and three plots receiving low, medium and high-inputs of fertiliser and water, representing the equivalent of respectively one third, two thirds and the maximum dose required to achieve maximum hay productivity and mown respectively one, two and two times a year. These treatments were compared to a control plot where no fertilisation or irrigation was applied and which was mown once a year. These plots were applied on 20 m diameter plots and replicated on 12 meadows covering a wide altitudinal gradient (range 790m - 1740m). Following a randomized block design format, treatments were randomly assigned to each circular plot. This thesis studied the short-term effects of treatments on plants and arthropods during the first three years (from 2010 to 2012 included).

A clear advantage of the design used is the ability to minimise confounding effects that could influence the results such as surrounding environmental variables and local abiotic conditions. In order to overcome the conventional gap that exists between science, policy and practice (Arlettaz et al. 2010), so as to favour the communication and implementation of the future management recommendations, a group of experts encompassing different environment and agriculture organisations was created in 2010 and involved in the whole project. This group included, in particular, representatives of the Department of Agriculture for the Canton of Valais and other Swiss Cantons, the Federal Office for the Environment (FOEN), the Federal Office for Agriculture (FOAG), the AGRIDEA Centre which represents the link between science and farming, and scientists from University of Bern and Agroscope, the Swiss Federal Centre for Agricultural Research.

Thesis overview

The first objective of this thesis was to review the literature to synthesize current knowledge and identify research gaps. In particular, we realised early on that no experimental study assessed the effect of different irrigation doses on the biodiversity of mountain meadows. The first chapter, which was the subject of a publication (Humbert *et al*. 2015) presents a meta-analysis of 42 studies that seeks to examine the effects of the addition of nitrogen on the biodiversity of mountain meadows. Although this meta-analysis was published only in 2015, it identified several gaps that have been addressed by this thesis, including the fact that very few experimental studies have assessed the effect of fertilisation on grassland invertebrate populations.

The second objective was to understand the immediate effects of fertilisation and irrigation, separately or combined, on plants and arthropods in mountain hay meadows. In chapter two, which was also the subject of a publication (Andrey *et al.* 2014) the experimental approach described in the above section was used to analyse the short-term effects of four of the six management treatments. The treatments analysed were the control, the fertilised and irrigated-only plots and the plot combining both at a dose of two-thirds to that for maximum hay productivity. The effects of these treatments on hay productivity, plant diversity, vegetation structure and invertebrate abundance and biomass were analysed.

The third objective of this thesis was to investigate the effects of all experimental management treatments on Auchenorrhyncha communities (chapter 3). This taxon is an excellent bioindicator of land-use change due to its high diversity and abundance in grasslands (Biedermann *et al.* 2005).

Finally, a general conclusion sums up and discusses the outcomes of the various chapters and draw management recommendations for stakeholders based on the first three years of the project (short and mid-term effects) while areas for future research are also defined.

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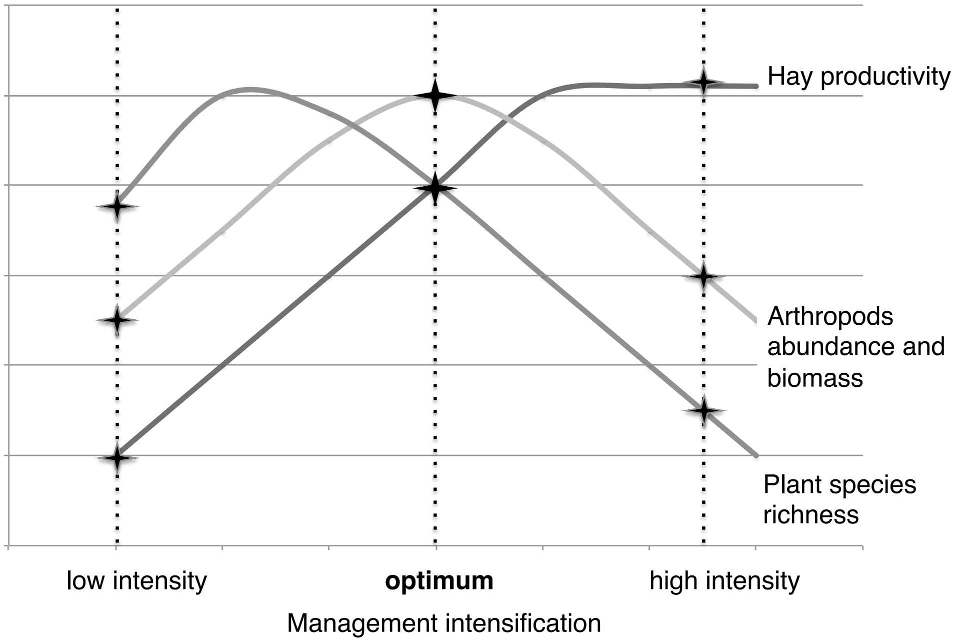

Figure 1: The hypothetical relationship between management intensity, hay productivity, plant species richness and arthropods abundance and biomass on mountain hay meadows. Self-developed hypothetical model but based on the hump-shaped model.

CHAPTER 1

Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review

Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review

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Abstract

Although the influence of nitrogen (N) addition on grassland plant communities has been widely studied, it is still unclear whether observed patterns and underlying mechanisms are constant across biomes. In this systematic review, we use meta-analysis and meta-regression to investigate the influence of N addition (here referring mostly to fertilisation) upon the biodiversity of temperate mountain grasslands (including montane, subalpine and alpine zones). Forty-two studies met our criteria of inclusion, resulting in 134 measures of effect size. The main general responses of mountain grasslands to N addition were increases in phytomass and reductions in plant species richness, as observed in lowland grasslands. More specifically, the analysis reveals that negative effects on species richness were exacerbated by dose (ha^{-1} $year^{-1}$) and duration of N application (years) in an additive manner. Thus, sustained application of low to moderate levels of N over time had effects similar to short term application of high N doses. The climatic context also played an important role: the overall effects of N addition on plant species richness and diversity (Shannon index) were less pronounced in mountain grasslands experiencing cool rather than warm summers. Furthermore, the relative negative effect of N addition on species richness was more pronounced in managed communities, and was strongly negatively related to N-induced increases in phytomass, i.e. the greater the phytomass response to N addition, the greater the decline in richness. Altogether, this review not only establishes that plant biodiversity of mountain grasslands is negatively affected by N addition, it also demonstrates that several local management and abiotic factors interact with N addition to drive plant community changes. This synthesis yields essential information for a more sustainable management of mountain grasslands, emphasizing the importance of preserving and restoring grasslands with both low agricultural N application and limited exposure to N atmospheric deposition.

Introduction

Reactive nitrogen (N) addition to terrestrial ecosystems through agricultural fertilisation or atmospheric deposition has increased substantially in recent decades and is today considered to be one of the most widespread drivers of global change

(Galloway *et al.* 2008). This dramatic increase concerns both the rate of N application or deposition, and its spatial extent (Galloway *et al.* 2004; Erisman *et al.* 2008). Although N addition to terrestrial ecosystems has recently levelled off in some areas of the globe, it is predicted to increase further on a global scale (Dentener *et al.* 2006; Erisman *et al.* 2008). Among terrestrial ecosystems, grasslands, especially in the lowlands, have received considerable research attention. Several empirical studies and reviews have demonstrated the general response of grassland plant communities to N addition, notably in terms of decreases in species richness and resulting increases in phytomass productivity (e.g. Bobbink *et al.* 2010; Maskell *et al.* 2010; De Schrijver *et al.* 2011; Borer *et al.* 2014). These results have raised several conservation concerns and policy responses, like the critical load policy concept (a policy tool for the control of air pollution, see Payne *et al.* 2013; Roth *et al.* 2013). Some studies, however, have shown that responses can differ among plant communities as well as along large environmental gradients, with considerable variation in the magnitude of the responses (e.g. Clark *et al.* 2007).

The composition of the original plant community, including the relative proportion of functional groups (i.e. grasses, forbs, legumes and sedges), can influence the direction and magnitude of the changes to N addition (e.g. Tilman *et al.* 2001; Bassin *et al.* 2007; Marquard *et al.* 2009; Bai *et al.* 2010; Onipchenko *et al.* 2012). Grasses are generally favoured by N addition, while legumes are not, and forb responses tend to be species specific (Theodose & Bowman 1997; Leto *et al.* 2008; Niu *et al.* 2008; Duprè *et al.* 2010).

Regional environmental conditions such as climate and local soil characteristics also influence the response of the original plant community to N addition. Climate may influence responses to N addition by controlling important aspects of energy supply that contribute to plant productivity and diversity maintenance (Hawkins *et al.* 2003; Cross *et al.* 2015), or by influencing secondary stress impacts such as frost damage in cold climates (e.g. Clark *et al.* 2007), and heat and water constrains in hot climates (Rustad *et al.* 2001; Ren *et al.* 2010). It has been shown that relative productivity responses to N addition increase with latitude (LeBauer & Treseder 2008). At very local scales, responses can further differ due to different original soil nutrient limitation and/or soil moisture (Theodose & Bowman 1997; but see Seastedt & Vaccaro 2001). Relevant to these finding, resource ratio theory (Tilman 1982) predicts that responses to N addition may be contingent on the supply of other limiting resources such as phosphorus or potassium (Ren *et al.* 2010; Harpole *et al.* 2011). Soil pH may also alter responses to N supply by influencing soil microbial activity and rates of N and carbon cycling (Kemmitt *et al.* 2006; Duprè *et al.* 2010).

In addition to environmental conditions, plant community responses may vary according to the form (oxidized vs reduced) and type (ammonium nitrate, urea, etc.) of N addition (Gaudnik *et al.* 2011). Nitrogen fertiliser origin, i.e. mineral or organic, also appears to play a role, but we lack quantitative evidence about its effects (but see Pacurar *et al.* 2012; Kirkham *et al.* 2014). Finally, biomass removal via grazing and mowing, especially within semi-natural agricultural grasslands, also influences grassland community diversity and composition (e.g. Marriott *et al.* 2009; Humbert *et al.* 2012), and responses to N addition (e.g. Kampmann *et al.* 2008; Lanta *et al.* 2009; Pavlu, Schellberg & Hejcman 2011; Borer *et al.* 2014).

Overall, research to date tends to show that local biotic and abiotic conditions play an important role in moderating plant response to N addition. As the vast majority of studies were carried out in lowland grasslands, conclusions drawn from experiments at low altitude are not readily transferable to other types of ecosystems, notably to mountain grasslands (Sebastia 2007). Biome-specific systematic syntheses are therefore required before we can generalise (Pullin 2012). This systematic review thus focuses on temperate mountain ecosystems including montane, subalpine and alpine grasslands. It aims at assessing the available evidence regarding the effects of N addition upon biodiversity and productivity of temperate zone mountain grasslands. In particular, it investigates how abiotic factors that potentially interact with N addition drive the variable plant community responses that are commonly observed in nature. The term N addition refers here to N from anthropogenic origin, either in the form of agricultural fertilisation (sometimes in combination with other nutrients) or atmospheric deposition. The present review not only provides a basis for sound predictions about community changes but also informs land managers and policy makers about the conservation threats potentially affecting mountain grasslands and remedies for biodiversity more friendly management (Maurer *et al.* 2006; Maskell *et al.* 2010; Bobbink & Hettelingh 2011).

Methods

We followed the review methodology of the Collaboration for Environmental Evidence partnership (Pullin & Stewart 2006) and published an *a priori* protocol that was peer-reviewed (Appendix S1 Dwyer, Humbert & Arlettaz 2010)

Search strategy

The following web databases were searched for documents: ISI Web of Science, Science Direct, JSTOR, Google (100 first hits), Google Scholar (100 first hits). A high-sensitivity and low-specificity approach was used to ensure that all important relevant information was found (Pullin & Stewart 2006). The databases searches were carried out between September 2012 and January 2013 (see Appendix S2 for exact term lists and dates). Any apparently relevant citations or links were followed one step away from the original hit. In addition, national and international experts on the subject were asked for any related literature and unpublished data.

Study inclusion criteria

All references retrieved from the web search (2285) were scanned at the title, abstract and full text filter levels by a first reviewer. From the 2285 initial references, 20% were randomly selected and rescanned by a second reviewer in order to check for inclusion consistency. The following inclusion criteria were used:

- Relevant subjects: natural or semi-natural grasslands in temperate mountain zones. Grasslands were defined as generally treeless, dominated by graminoid and forb species (>50% graminoid and herbaceous cover prior to interventions), which excluded heath or other shrub dominated lands. Mountain grasslands were defined as those occurring on mountain ranges within temperate regions that experience winter snow cover. Temperate regions were defined as those: (1) within temperate latitudes; and (2) classified in the Köppen-Geiger climate classification system as: Cfb, Cfc, Cwb, Cwc, Dfb, Dfc, Dfd, Dwb, Dwc, Dwd and Et (Kottek *et al.* 2006). The use of these eleven categories excluded Mediterranean, subtropical and arid climates that occur within the temperate latitudes.

- Types of intervention: addition of nitrogen (alone or in combination with other nutrients).
- Types of comparator: non-fertilised control plots (experimental studies) or suitable reference areas that have not been fertilised (observational studies). Control plots had to be managed in the same way as treatment plots with the exception of fertiliser addition.
- Types of outcome: species richness or Shannon index of diversity (*H'*) of at least one taxonomic group. Also changes in biomass production or absolute abundance of functional groups were recorded if provided.

Manipulative micro- and mesocosm experiments were not included as they cannot be considered ‘semi-natural’ grasslands. While the definition of what is or what is not a mountain grassland is difficult to state, the majority of the authors defined their study sites as mountain, montane, subalpine or alpine grasslands. Importantly, study sites had to be located on the slopes or on the top of a recognized mountain range. Elevation *per se* was not a criterion, as high plateaus like the Xilin River Basin, Inner Mongolia, were not considered mountain sites (e.g. Bai *et al.* 2010, study site at 1250 m). Included taxonomic groups were restricted to aboveground communities, which excluded soil microbial, fauna and fungi communities as well as seed banks. Inclusion consistency was checked with kappa statistics, and agreement between the reviewers was satisfactory (k = 0.81) (Pullin & Stewart 2006).

Data extraction

Many studies reported more than one treatment (different amounts or types of fertiliser added), and some studies reported the results of the same experiment replicated in different habitats [e.g. Theodose & Bowman (1997) duplicated their experiment in dry and wet meadows]. In these cases, all comparisons were recorded as independent data points, and this is why there are more data points (units of analysis) than studies (Pullin & Knight 2003; Humbert *et al.* 2012). The majority of studies (40 out of 42) that respected inclusion criteria were on vascular plants, with only two studies on either bryophytes (Bergamini & Pauli 2001) or Coleoptera (Majzlan & Gajdoš 2007). We therefore decided to limit this review to vascular plants.

The following information was extracted for each relevant treatment from the selected studies: (1) species richness and/or *H'*; (2) total vascular plant biomass and biomass per functional group (i.e. grass, sedge, legume or forb); (3) number of replicates per treatment; (4) study duration in years; (5) Köppen-Geiger climate (hereafter call "K-G climate"); (6) country where the study was carried out; (7) precipitation per year in mm; (8) mean monthly temperature; (9) altitude; (10) latitude; (11) soil pH before the experiment started; (12) nitrogen fertiliser origin, classified as mineral or organic; (13) dose of N [$kg \cdot ha^{-1} \cdot year^{-1}$] applied; (14) dose [same units] of phosphorus (P) and potassium (K) if applied with N; (15) fertilisation frequency per year; and finally (16) management type of the plots during the experiment, classified as unmanaged, grazed, mown, or grazed and mown.

For studies with multiple replicates per treatment, standard deviations (SD) were retrieved from published standard errors (SE) or variances. If no estimate of variance was provided, we requested it from the original authors. If original authors could not provide estimate of variance, or sample size was equal to one (i.e. no variance), the corresponding study was included only in the unweighted analyses (see statistical analysis section below). K-G climates were rarely provided in the publications, but could be inferred from study site averaged monthly precipitations and temperatures. These values were either found in the original publication, provided by the authors, or found on webpages linked to the corresponding publication (e.g. from the Niwot Ridge Long-Term Ecological Research Site at http://niwot.colorado.edu/index.html). In some cases we relied on the data from the nearest available weather station (e.g. for Swiss studies at: http://www.meteosuisse.admin.ch/) or highest resolution (30 arc-seconds latitude, i.e. ca 1 km) WorldClim global climate data (www.worldclim.org). Values for soil pH could not always be extracted from the publication or obtained by the authors and therefore some values were missing.

Additional potential sources of heterogeneity were also extracted such as exact fertiliser form (e.g. ammonium nitrate or urea), type of geological substrate, habitat type (e.g. dry, wet or mesophilous), plot size of vegetation relevés, original plant community, and former management. However, these factors could not be sourced for all studies and were later disregarded from analyses due to insufficient data.

Statistical analysis

Meta-analyses (MAs) were conducted on three response variables: 1) species richness; 2) *H'*; and 3) biomass. Studies lasted up to 65 years, and if multiple time-points were available along the time series, only the data for the last year (longest time period) were considered.

The response ratio statistic was used to estimate effect sizes. The response ratio (*lr*) for a given comparison is the difference between the mean ln-transformed treatment value and the mean ln-transformed control value (Hedges, Gurevitch & Curtis 1999):

$$lr = \ln(\overline{\mathrm{X}}_{\mathrm{F}}) - \ln(\overline{\mathrm{X}}_{\mathrm{C}})$$

Where $\overline{\mathrm{X}}_{\mathrm{F}}$ and $\overline{\mathrm{X}}_{\mathrm{C}}$ are the means of the fertilised and control (unfertilised) groups. The *lr* is symmetric around 0 and negative values indicate a negative effect of N addition on the response variable. The *lr* and its variance (see Hedges, Gurevitch & Curtis 1999, eq. 1) was calculated using the function *escalc* of the R package *metafor* (Viechtbauer 2010).

Random- and mixed-effects meta-analytical models were used (Gurevitch, Curtis & Jones 2001). Under random- and mixed-effects models, the true effect size, i.e. the effect size as if there were no sampling error, can vary from study to study, but is assumed to do so under a normal distribution (Perera 2009; Viechtbauer 2010). Here the *Q* test and I^2 statistic were used to assess heterogeneity among studies. The *Q* test is the test of significance, and the I^2 statistic estimates how much of the total variability in the mean effect size (composed of heterogeneity and sampling error) can be attributed to heterogeneity among the true effect sizes (Borenstein *et al.* 2009; Perera 2009) .

Following Johnson & Omland (2004), a set of candidate models was generated including moderator(s) related to potential biotic and abiotic processes than can be biologically or agronomically interpreted. Candidate models comprised all models including one of the following moderators: study duration in years; K-G climate; mean summer temperature (i.e. mean monthly averages of May-August); number of month(s) with mean temperature ≥ +10 °C; fertiliser origin (organic vs mineral); fertiliser type (i.e. N, NP, NK or NPK); fertilisation doses of N, P, and K; management type (nominal variable with four classes: unmanaged, grazed, mown, or grazed and mown); and management occurrence (binary variable with two classes: managed or unmanaged, with managed including grazed, mown, and grazed and mown). Candidate models also consisted of all models that included N dose applied plus one of the above mentioned moderators, and the following more complex model: N dose + P dose + K dose. Influences of mean yearly precipitation, altitude and latitude were not tested independently as they are all encompassed in the K-G climate variable. The set of candidate models were ranked based on AIC values (Akaike Information Criterion) and on the level of significance of the estimates (Johnson & Omland 2004; Borenstein *et al.* 2009). Influences of soil pH and productivity ratio (phytomass production in fertilised plots / phytomass production in control plots) were also investigated, but could not be included in the model selection process because of missing values for several studies. Publication bias was assessed using funnel plots, by applying a regression test for funnel plot asymmetry (Borenstein *et al.* 2009; Viechtbauer 2010). Normal quantile-quantile (Q-Q) plots were inspected to assess normality of the residuals. Only the results where corresponding Q-Q and funnel plots were satisfactory are presented here.

In addition to the weighted MAs that utilised variances among replicates, unweighted meta-analyses were applied to a larger dataset that included effect sizes without associated variances. Unweighted MAs were limited only to null models (i.e. without moderators) to assess overall effects of N addition. Bootstrapping was used to calculate 95% confidence interval (CI) of the estimated effect size; if CIs overlapped zero, the effect size was considered to be non-significant. All statistics were performed using R version 3.1.*2* (R Core Team 2014).

Results

2285 references were retrieved from the web. Only 43 articles matched inclusion criteria, i.e. dealt with the influence of N addition on grassland biodiversity (Appendix S3). Among them, eight articles were excluded due to paper content duplication. This was apparent when two distinct references stemming from the same experiment presented the same data to address different questions or presented results over different time periods. Seven additional articles were found in the bibliography sections of the retained papers or obtained after contacting experts, which resulted in a total of 42 suitable studies. In some studies more than one treatment or more than one habitat type were investigated, resulting in a total of 134 treatment – control comparisons (i.e. data points, Table 1).

From these 134 data points, 98 reported results on plant species richness, 60 on Shannon index (*H'*) for plants (in some cases the original reference did not report results on *H'*, but the corresponding author provided the raw data for its calculation), and 103 on changes in phytomass production. In 43 cases (twelve for plant species richness, 20 for *H'* and 11 for phytomass), the study did not report SD, or replication per treatment was one. Consequently, these data could only be included in the unweighted MA. All studies were experimental, except one which was observational (Jeangros & Troxler 2008) but of sufficient quality to be included (quality category II-2 of Pullin & Knight 2003). Most observational studies were excluded because they did not report the quantity of nitrogen applied (e.g. Spiegelberger *et al.* 2006). A list of all studies considered and of all treatment – control comparisons is provided in Appendix S4. Appendix S5 lists the articles excluded after full text filtering, mentioning reasons for exclusion.

We present the results of the different MAs as follows: 1) all null models (models without moderators) for plant species richness, *H'*, and biomass productions; 2) the best-supported models for species richness and *H'*; and 3) several single-moderator models that did not emerge as 'best' models, but included moderators of high agronomical or biological relevance.

Null models

Species richness was reduced by N addition in weighted and unweighted MAs (Fig. 1). Regarding *H'* (Shannon index) weighted MA indicated no effect of N addition, while the unweighted MA indicated a significant decrease of *H'* in response to N addition. Overall, vascular plant and grass biomass were higher in fertilised compared to unfertilised plots. Sedge and legume biomass did not change following N addition, while forb biomass exhibited a significant increase with weighted MA and no change with unweighted MA (Fig. 1; see Appendix S6 for detailed model outputs).

Best-supported models

The model with best-support explaining changes in species richness was the bivariate model including N dose and study duration as moderators (Fig. 2 and Appendix S7). Both moderators had a significant negative effect on plant species richness effect size (N dose: *lr* = −0.0007, z = 0.0003, *P* = 0.026; study duration: *lr* = −0.0041, z = 0.0014, *P* = 0.003). Heterogeneity among studies was significant (Q = 605.28, d.f. = 83, *P* < 0.001), indicating that other moderators likely influence responses to N addition.

The best model explaining *H'* was the single moderator model including the number of months with mean temperature ≥ +10°C (Fig. 3b and Appendix S7), which indicated reduced *H'* in warmer regions. However, the resulting funnel plot showed significant asymmetry due to an outlying data point ('Kassioumi 2003 Site.T ii', from Kassioumi 2003 unpublished PhD Thesis). Excluding this data point improved model diagnostics, but did not alter conclusions (number of months with mean temperature ≥ +10°C: *lr* = −0.044, z = 0.008, *P* < 0.001) or selection of the "best" model. As for the richness models, heterogeneity among studies was significant, indicating that other moderators likely influence effect sizes.

Other single-moderator models

The negative effect of N addition on species richness was more pronounced in warmer than in cooler regions (Fig. 3a) and where phytomass responses to N addition were largest (higher productivity ratios; Fig. 4a). In contrast, there was no significant relationship between *H'* effect size and the productivity ratio (Fig. 4b). The negative effect of N addition on species richness was significantly more pronounced where experimental plots were managed (*lr* = -0.1070, z = -2.5717, *P* = 0.0101; Fig. 5). Species richness effect sizes were also moderated by fertiliser type (i.e. N, NP, NK or NPK): there was a stronger negative effect on species richness when NPK was applied compared to N alone (Appendix S8). Initial soil pH did not influence the effect of N addition on species richness (Appendix S8). Unsatisfactory funnel plots prevented further meta-regression on *H'*.

Discussion

Overall, this systematic review indicates that N addition generally reduces plant species diversity and increases biomass in temperate mountain grassland systems, but these responses are also strongly influenced by N dose, application duration and management practice. Our results also suggest that plant community responses to N addition are modulated by mean summer monthly temperatures.

Both weighted and unweighted MAs showed that N addition typically induced a decrease in plant species richness. This is in agreement with the general negative pattern found in broad-scale studies of lowland grassland fertilisation (e.g. De Schrijver *et al.* 2011; Van den Berg *et al.* 2011). Shannon index (*H'*) also decreased with N addition but to a lesser extent and the decrease was significant only when using unweighted MA. Vascular plant biomass increased with N addition, and this pattern was mostly driven by an increase in grass biomass. Biomass of sedges and legumes did not change with N addition while forb biomass exhibited a small significant increase, but only when weighted MA was applied. Mean effect sizes for legume biomass were clearly negative but the variances of both weighted and unweighted MAs were large with the 95% CI overlapping 0 (i.e. no effect). It suggests that adding N has the tendency to decrease legume biomass, though other factors, such as the addition of P with N, can have interactive effects (e.g. Willems *et al.* 1993; Ren *et al.* 2010).

Best-supported models

The model with best support for explaining changes in species richness following N addition was the bivariate model including N dose and study duration (years of N application, which indicates cumulative N; see Fig. 2). Both moderators had pronounced additive negative effects on species richness, revealing that low N doses applied for long periods lead to similar richness declines as high N doses applied for short periods. This additive effect has been recurrently presumed in the literature about grasslands. To the best of our knowledge, it is here demonstrated for the first time (but see Clark & Tilman 2008). Because the effect sizes are on a log scale, significant negative linear relationships with N dose and study duration indicate that the steepest richness declines occur at low N doses and in the first few years of

N application, respectively. These results are consistent with common grassland models that predict the steepest species declines occurring as N supply initially increases (Kleijn *et al.* 2009), and with findings from longer-term fertilisation and N atmospheric deposition studies (Clark *et al.* 2007; Clark & Tilman 2008; Duprè *et al.* 2010; De Schrijver *et al.* 2011).

For both species richness and *H'* the negative effect of N addition was weaker in cooler regions (regions with fewer months with ≥ +10°C mean monthly ambient temperature) than in warmer regions. This is consistent with some findings from alpine and artic tundra regions (DiTommaso & Aarssen 1989; but see Seastedt & Vaccaro 2001; Bowman *et al.* 2006), but contrasts with findings from multiple sites across North America where the greatest species losses were observed in colder regions (Clark *et al.* 2007). It is likely that colder growing season temperatures limit the extent to which plant species can respond to increased N supply (Cross *et al.* 2015). Slower growth rates in cold adapted species have been widely reported and attributed to a trade-off between freezing tolerance and growth rate (Savage & Cavender-Bares 2013). It has also been shown in cold ecosystems that warming alone can enhance plant productivity (Rustad *et al.* 2001), mostly because warming increases nitrogen and phosphorus uptake capacity by plants (Jonasson *et al.* 1999).

Influences of management, productivity ratio, and initial soil pH

Further analyses of data showed that responses varied according to management occurrence (presence or absence of management). The relative negative effect of N addition on species richness was significantly more pronounced in managed communities (grazed, mown, or grazed and mown). This result reflects our choice of control-treatment comparisons – we always compared the effects of N addition between experimental communities that experienced the same management. Control plots in managed communities generally had higher richness than control plots in unmanaged communities, and this richness was maintained through time in longitudinal studies (e.g. Krajčovič *et al.* 1990; Kohler *et al.* 2004; Bonanomi, Caporaso & Allegrezza 2009). As such, these managed communities had more species to "lose" after N addition. In unmanaged communities, it is likely that successional processes had already reduced diversity in control plots (Pavlu *et al.* 2012; Gaisler *et al.* 2013), and so further losses due to N addition were not as pronounced in relative terms. In the subset of studies that applied combinations of fertilisation and management treatments to the same community (Kohler *et al.* 2004; Kohler *et al.* 2005; Bonanomi, Caporaso & Allegrezza 2009; Lanta *et al.* 2009), fertilised-managed plots displayed higher species richness than fertilised-unmanaged plots in all cases, indicating that mowing and grazing actually maintains a higher level of species richness following fertilisation compared with no biomass removal. This corroborates the findings of Borer *et al.* (2014) that grazing can rescue richness losses in fertilised plots by allowing more light to reach ground level and by preventing competitive exclusion via intense light competition.

Species richness, but not *H'*, was strongly negatively related to N-induced increases in phytomass, i.e. the greater the phytomass response to N addition, the greater the decline in species richness (Fig. 4). Clark *et al.* (2007) found a similar pattern, suggesting that changes in productivity plays a key ecological role regarding species richness responses to N addition.

It is known that fertilisation often decreases soil pH and that subsequent soil acidification has negative effects on plant communities (e.g. Duprè *et al.* 2010; Van den Berg *et al.* 2011; Liu *et al.* 2012), however the influence of initial soil pH on plant community responses to N addition is still unclear (De Schrijver *et al.* 2011; Veresoglou *et al.* 2011). Although Clark *et al.* (2007) found greatest species losses following N addition in plant communities with lower soil cation exchange capacity, i.e. in soils most prone to acidification, we did not find evidence of such a link with soil pH.

Mechanistic link to species loss

There are several ecological mechanisms that can drive grassland plant community changes following N addition. First, it has been demonstrated that fertilisation can negatively impact species richness by reducing the number of available limiting resources, which diminishes trade-off opportunities that allow species coexistence (Levine & HilleRisLambers 2009; Harpole *et al.* 2011). This increases the biomass of exploitative species which deter smaller species with low growth rates through intensified light competition (Hautier, Niklaus & Hector 2009). Accordingly, species losses would be expected to be smaller in sites with smaller relative biomass responses to N, because small changes in foliage quantity would induce only small changes in overall light availability for the whole plant community (Ren *et al.* 2010; Borer *et al.* 2014). This mechanism is supported by the data at hand. In line with the concept of limiting resources reduction, stronger negative effects on plant species richness were found when P and K were jointly added to N, compared to N alone, suggesting nutrient co-limitation [Appendix S8, see also Ren *et al.* (2010) and Harpole *et al.* (2011)] Fertilisation can also increase belowground root competition, causing additional competitive exclusion among species (Rajaniemi 2002; but see Hautier, Niklaus & Hector 2009; Dickson & Foster 2011).

Limitations and research gaps

Other factors that have been shown to influence plant community responses to N addition include: the form of N input (i.e. ammonium, nitrate, ..., see Song *et al.* 2012), the scale (i.e. size of the experimental plot, see Spiegelberger *et al.* 2006; Gross *et al.* 2009), habitat (e.g. dry or wet, see Theodose & Bowman 1997) and grassland type (e.g. Wang *et al.* 2010). Unfortunately, the effects of these factors could not be investigated in this MA due to incomplete data. We have also to recognise that changes in management from pre-experimental conditions may also generate confounding effects (Kralovec *et al.* 2009); such changes could not be investigated as information about pre-experimental conditions were rarely provided.

The main research gap identified by this systematic review is certainly the lack of studies on invertebrate responses to grassland N addition. All studies that met inclusion criteria were on vascular plants, except two: one on bryophytes (Bergamini & Pauli 2001) and one on Coleoptera (Majzlan & Gajdoš 2007). While there are few observational studies on invertebrate responses to fertilisation (e.g. Grandchamp *et al.* 2005; Boschi & Baur 2008), these did not meet our inclusion criteria. Given that insect herbivory has been demonstrated to influence both nutrient cycling and plant production (Blumer & Diemer 1996; Belovsky & Slade 2000), the dearth of information about the role of herbivory in grassland responses to abiotic change is a serious issue (Scherber *et al.* 2010; Borer *et al.* 2014). We need more comprehensive experimental research on the influence of nutrient addition on both plant and invertebrate communities, and interactions in between, to better appraise the functional ecology of grasslands (Scherber *et al.* 2010; Littlewood, Stewart & Woodcock 2012; Andrey *et al.* 2014).

Conclusions

Plant species losses and biomass increases following N addition appear to be a universal pattern across grassland systems. Here, we further establish that effects on species richness are negatively and additively influenced by the dose of N applied and duration of application. This finding has important conservation implications; it implies that sustained addition of relatively small N doses will ultimately reduce plant diversity in the long-term. Consequently, it is important to protect grasslands not only where N application is limited if not null, but also which are not affected by N deposition (Payne *et al.* 2013). In addition, refined estimates of exposure thresholds below which no harmful effects can be detected (e.g. in the critical load concept, Bobbink & Hettelingh 2011) have to be defined from the dual point of view of quantity and time, this given their additive effects (see also Clark & Tilman 2008).

We also found that the effects of N addition on species diversity are less pronounced in cool summer mountain areas than in warm summer mountain areas (see also DiTommaso & Aarssen 1989). These two areas closely match the segregation between subalpine and alpine grasslands. We even observed a positive effect of N addition on *H’* in the coldest summer mountain areas, i.e. where less than 4 months have a mean ambient temperature ≥ +10°C. In the face of global warming, this finding indicates that the magnitude of the effects of N addition upon mountain plant communities might increase as summer temperatures increase (Rustad *et al.* 2001). Given that climate warming is more pronounced in mountain ranges and in boreal regions (Nogues-Bravo *et al.* 2007; Engler *et al.* 2011), it is in subalpine and boreo-alpine grassland ecosystems that we might expect major changes in vegetation.

Of course, it remains to be seen how subtle changes in the species diversity and productivity of these mountain systems may alter their functioning and resilience to further environmental change. Anthropogenic N enrichment is likely to become more widespread in the future, touching remote mountain regions that have so far remained unaffected. Research is urgently needed to predict its impacts on ecosystems and their services so as to take appropriate conservation action (Bobbink & Hettelingh 2011; Manning 2012).

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Table 1: List of studies included in the meta-analysis, with study area and number of extracted data point(s). Appendix S4 provides a more comprehensive overview of variables considered for each data point.

Source (study reference)	Country	Number of data points
(Bassin *et al.* 2007)	Switzerland	4
(Bassin *et al.* 2012)	Switzerland	4
(Baumberger *et al.* 1996)	Switzerland	2
(Bergamini & Pauli 2001)	Switzerland	2
(Bonanomi, Caporaso & Allegrezza 2009)	Italy	3
(Bowman *et al.* 2006)	USA, Colorado	3
(Bowman *et al.* 2012)	USA, Colorado	3
(Brinkmann & Reif 2006)	Romania	12
(Britanak *et al.* 2007)	Slovakia	2
(Chytrý *et al.* 2009)	Germany	4
(Delpech 1984)	France	1
(Elisseou, Veresoglou & Mamolos 1995)	Greece	9
(Fahnestock & Detling 1999)	USA, Wyoming	2
(Gross *et al.* 2009)	France	2
(Jeangros & Troxler 2008)	Switzerland	2
(Kassioumi 2003)	Greece	4
(Kohler *et al.* 2004)	Switzerland	4
(Kohler *et al.* 2005)	Switzerland	4
(Krajčovič *et al.* 1990)	Slovakia	3
(Kralovec *et al.* 2009)	Czech Republic	4
(Lanta *et al.* 2009)	Czech Republic	3
(Leto *et al.* 2008)	Croatia	2
(Li *et al.* 2010)	China, Tibet	1
(Majzlan & Gajdoš 2007)	Slovakia	3
(Mamolos, Vasilikos & Veresoglou 2005)	Greece	4
(Mudrak & Leps 2010)	Czech Republic	1
(Niu *et al.* 2012)	China, Tibet	2
(Olofsson & Shams 2007)	Sweden	2
(Onipchenko *et al.* 2012)	Russia	4
(Pauli, Peintinger & Schmid 2002)	Switzerland	2
(Pavlu, Schellberg & Hejcman 2011)	Germany	4
(Pavlu *et al.* 2012)	Czech Republic	2
(Ren *et al.* 2010)	China, Tibet	4
(Rixen *et al.* 2008)	Switzerland	1
(Seastedt & Vaccaro 2001)	USA, Colorado	6
(Sebastia 2007)	Spain, Pyrenees	2
(Song *et al.* 2012)	China, Tibet	3
(Suding *et al.* 2008)	USA, Colorado	1

(Tenz *et al.* 2010)	Switzerland	1
(Theodose & Bowman 1997)	USA, Colorado	4
(Veresoglou *et al.* 2011)	Greece	4
(Wang *et al.* 2010)	China, Tibet	4

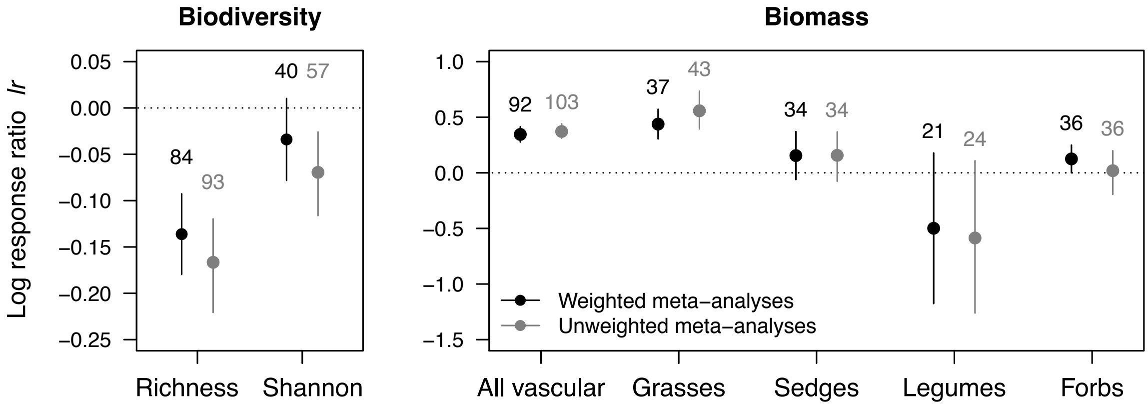


Figure 1. Influence of N addition on plant species richness, diversity (Shannon index) and biomass production. Effect sizes are response ratios (*lr*), with negative values meaning a negative effect of N addition. Points represent means and error bars ± 95% CI. Sample size (numbers of data points) are given above each bar.

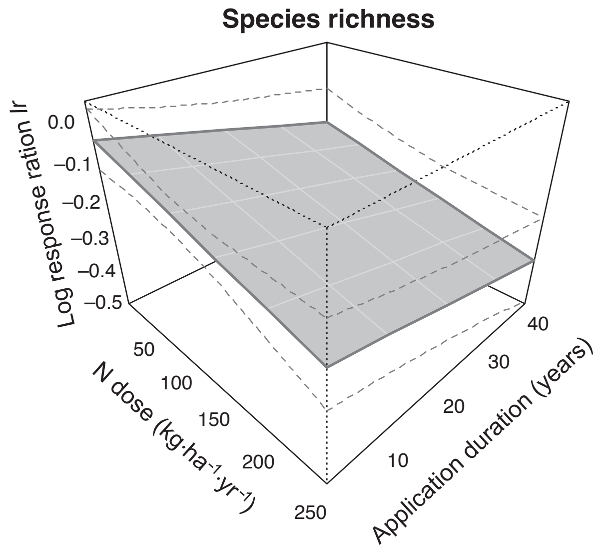


Figure 2. 3D perspective plot of species richness response ratio (*lr*) versus the amount of nitrogen applied per year and application (study) duration. The grey plane represents the mean response and dashed lines are corresponding 95% CI.

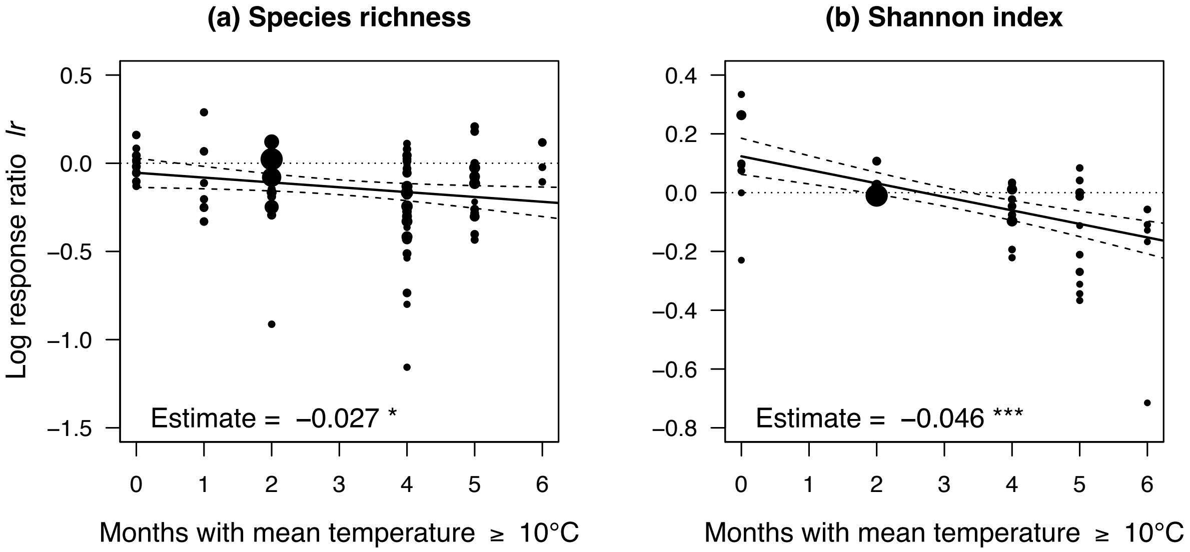


Figure 3. Species richness and Shannon index response ratios (*lr*) versus the number of months with mean temperature ≥ +10°C. Significance levels for slope estimates are given for each regression line (* $P < 0.05$; *** $P < 0.001$). Symbol size depicts study weight.

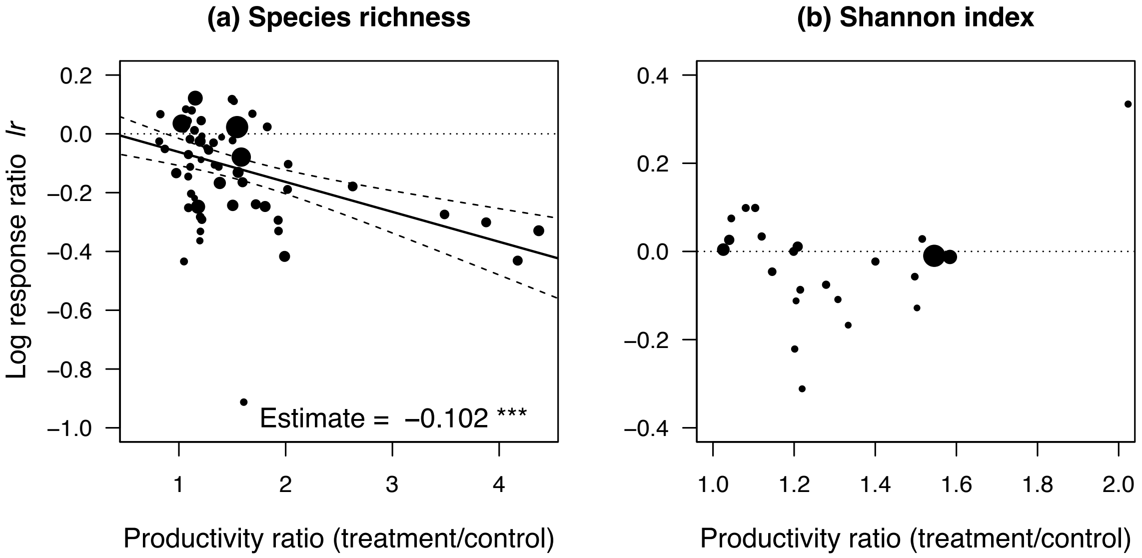


Figure 4. Species richness and Shannon index (*H'*) response ratios (*lr*) versus phytomass productivity ratio (treatment/control). Significance level for slope estimate is given (*** $P < 0.001$). Symbol size depicts study weight.

Figure 5. Boxplot presentation of species richness response ratio (*lr*) with respect to management occurrence. Managed control and fertilised plots were grazed, mown, or grazed and mown. Bold transversal bars represent medians; grey crosses means; box boundaries of the first and last quartiles; whiskers the inter-quartile distance multiplied by 1.5; and open circles the outliers. The asterisk indicates a significant difference between managed and unmanaged plots at $P < 0.05$.

Appendices

Appendix S1. Systematic review protocol published in 2010.

Dwyer, J. 2010. Does fertilisation decrease biodiversity in temperate montane grasslands? CEE protocol 09-022 (SR83). Collaboration for Environmental Evidence: www.environmentalevidence.org/SR83.html.

Appendix S2. Search terms used and dates when literature searches were conducted.

Search terms

For ISI Web of Science, Science Direct and JSTOR, the following logical search terms were used:

(oligotrophic OR mesotrophic OR "nutrient limited" OR "nutrient poor" OR seminatural OR semi-natural OR "semi natural" OR "traditionally managed" OR unimproved OR "extensively managed" OR alpine OR subalpine OR montane OR submontane OR sub-montane OR mountain* OR upland OR highland) AND (grassland OR meadow OR fen OR tundra OR moor*) AND (fertilis* OR fertiliz* OR nitrogen OR ammonium OR nitrate OR manur* OR intensifi* OR eutrophi* OR "land use change" OR "nutrient addition") AND (diversity OR richness OR assemblag* OR "functional type" OR "functional group" OR guild OR "growth form" OR "species number" OR "species density" OR "botanical composition" OR "species composition" OR "number of species" OR "floristic composition" OR "community composition")

Quotation marks indicates exact quote search while asterisks indicate possible word truncation.

For Google and Google Scholar, searches were conducted in English, French and German using a shortened list of the above search terms due to specific word and character limits applied by these search engines.

Google and Google Scholar English search:

(subalpine OR alpine OR mountain OR montane OR upland OR highland) AND (grassland OR meadow OR fen OR moor) AND (fertilisation OR fertilization OR nitrogen OR nitrate OR ammonium OR manure OR intensification OR eutrophication OR "nutrient addition")

Google and Google Scholar French search:

(subalpin OR subalpine OR alpin OR alpine OR montagne OR "haut plateau" AND (prairie OR pâturage OR pré OR herbage OR marais) AND (fertilisation OR azote OR nitrate OR ammonium OR lisier OR fumier OR engrais OR intensification OR eutrophisation)

Google and Google Scholar German search:

(subalpin OR alpin OR Alpen OR Berg OR Berggebiet OR montan OR Hochland) AND (Grasland OR Grünland OR Wiese OR Marschland OR Moor) AND (Düngung OR Dünger OR Stickstoff OR Nitrat OR Ammonium OR Mist OR Gülle OR Intensivierung OR Eutrophierung)

Search dates

ISI Web of Science (http://apps.isiknowledge.com)

Searched conducted on 28 September 2012. Search terms in “Topic”. 978 hits.

Science Direct (http://www.sciencedirect.com)

Searched conducted on 28 September 2012. Search terms in “Abstract, Title and Keywords”. 1648 hits.

JSTOR (http://www.jstor.org)

Searched conducted on 28 September 2012. Search terms in “Abstract”. 66 hits.

Google Scholar (http://www.scholar.google.com)

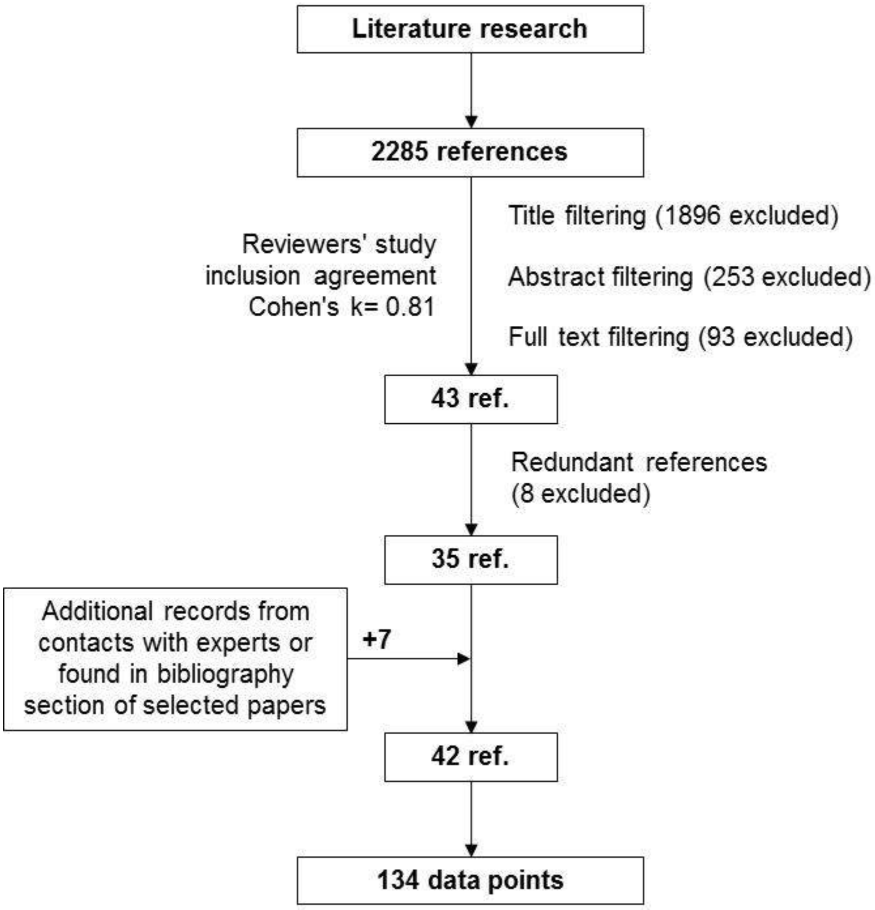
Searched conducted on 28 September 2012. 100 first hits of English, German and French searches.

Google (http://www.google.com)

Searched conducted on 16 January 2013. 100 first hits of English, German and French searches.

In Google Scholar and Google, any apparently relevant links were followed one step away from the original hit. Each relevant document was included in an EndNote X database.

Appendix S3. Flow diagram reporting the number of references identified, excluded and added during the literature screening process. Appendix S5 provides a list of the articles that have been excluded after content filtering, indicating reasons for exclusion.



Appendix S4. Lists of all data points included in the review with details on extracted variables. * Mean summer temperature, with summer defined as 1 May to 31 August ; ** Köppen-Geiger climate category, according to site description (see Kottek et al. 2006) ; *** in control plot or before the experiment started ; **** N = nitrogen; P = phosphorus; K = potassium

Appendix is available online at :

http://api.onlinelibrary.wiley.com/asset/v1/doi/10.1111%2Fgcb.12986/asset/supinfo%2Fgcb12986-sup-0004-AppendixS4.xlsx?l=j6%2BNsqLlmq%2FUMxNKl%2FfD%2BQ9QhhWsh7gjA%2BGAQu7qxgcdTljroq85gGeGGrT3OTJMuHbD8b%2B%2Fa83s%0AQ98bRiadrPNeFiZ2jwMW

Appendix S5. List of all studies excluded after abstract or full text filtering and reasons for exclusion.

Appendix is available online at:

http://api.onlinelibrary.wiley.com/asset/v1/doi/10.1111%2Fgcb.12986/asset/supinfo%2Fgcb12986-sup-0005-AppendixS5.xlsx?l=j6%2BNsqLlmq%2FUMxNKl%2FfD%2BQ9QhhWsh7gjA%2BGAQu7qxgcdTljroq85gOc1BMWhyT7oLbNxEjgzthjd%0A5xaDtRs0eE%2FFsLOw7oGY

Appendix S6. Influence of N addition on plant species richness, Shannon index and biomass production. The statistical outputs of all null models are presented (related to Figure 2).

						95% CI	
Metric	**n**	**Estimate**	**SE**	***z* value**	***P*-value**	**Lower**	**Upper**
Weighted meta-analyses							
Species richness	84	-0.136	0.022	-6.142	<0.001	-0.180	-0.093
Shannon index	40	-0.034	0.023	-1.509	0.131	-0.078	0.010
Biomass all vascular	92	0.346	0.035	9.771	<0.001	0.276	0.415
Biomass grasses	37	0.438	0.068	6.448	<0.001	0.305	0.571
Biomass sedges	34	0.155	0.110	1.408	0.159	-0.061	0.370
Biomass legumes	21	-0.499	0.346	-1.443	0.149	-1.176	0.179
Biomass forbs	36	0.125	0.063	1.971	0.049	0.001	0.249
Unweighted meta-analyses							
Species richness	93	-0.168				-0.219	-0.120
Shannon index	57	-0.070				-0.116	-0.025
Biomass all vascular	103	0.373				0.309	0.439
Biomass grasses	43	0.559				0.392	0.729
Biomass sedges	34	0.157				-0.076	0.373
Biomass legumes	24	-0.586				-1.268	0.099
Biomass forbs	36	0.014				-0.194	0.202

Metric	**Tau^2**	**SE of Tau^2**	***I*^{2}**	**H^2**	***Q***	***Q* df**	***P*-value of *Q***
Species richness	0.030	0.006	91.14	11.28	697.69	83	<0.001
Shannon index	0.016	0.004	95.44	21.95	335.60	39	<0.001
Biomass all vascular	0.086	0.016	97.03	33.69	2463.70	91	<0.001
Biomass grasses	0.124	0.039	94.14	17.06	729.27	36	<0.001
Biomass sedges	0.294	0.097	99.74	385.95	230.10	33	<0.001
Biomass legumes	2.086	0.780	96.35	27.43	197.36	20	<0.001
Biomass forbs	0.090	0.032	90.96	11.06	180.73	35	<0.001

Appendix S7. Model selection processes and detailed statistical outputs of the most parsimonious models about the influence of N addition on plant species richness and Shannon index.

Appendix available online at:

http://onlinelibrary.wiley.com/store/10.1111/gcb.12986/asset/supinfo/gcb12986-sup-0007-AppendixS7.pdf?v=1&s=21bc06315ec2d389c70f1e55bbd67ee447efbb47

Appendix S8. Model outputs on the influence of fertiliser type or soil pH on species richness.

Influence of fertiliser type

The impact of N addition on species richness was moderated by the fertiliser type applied (i.e. N, NP, NK or NPK); there was a stronger negative effect on species richness when NPK was applied compared to N alone (Fig. S8.1; Estimate = -0.155, SE = 0.043, *P*-value < 0.001). This difference was still significant when N dose application [kg N·ha^{-1}·year^{-1}] was considered in the model (Estimate = -0.150, SE = 0.043, *P*-value < 0.001). Note that there was only one study that applied NK, removing it did not change level of significance of estimated parameters.

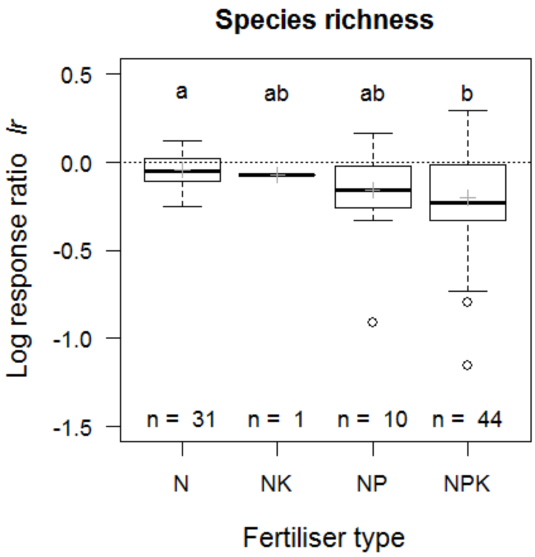


Fig. S8.1. Species richness response ratios (*lr*) with respect to fertiliser type applied (median: bold line; mean: cross; first and third quartiles: box borders; interquartile distance multiplied by 1.5: whiskers; and open circles: outliers). Different letters indicate significant differences at an alpha rejection level of 0.05. N = nitrogen, P = phosphorus and K = potassium.

Influence of soil pH

Initial soil pH did not influence the effect of N addition on species richness (Fig. S8.2; Estimate = 0.043, SE = 0.023, *P*-value = 0.061).

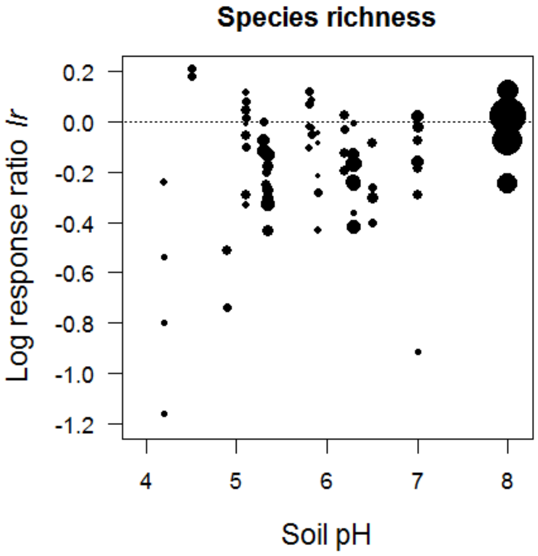


Fig. S8.2. Species richness response ratios (*lr*) versus initial soil pH. Symbol size depicts study weight.

CHAPTER 2

Experimental evidence for the immediate impact of fertilisation and irrigation upon the plant and invertebrate communities of mountain grasslands

Experimental evidence for the immediate impact of fertilisation and irrigation upon the plant and invertebrate communities of mountain grasslands

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Manuscript:
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doi: 10.1002/ece3.1118

Abstract

The response of montane and subalpine hay meadow plant and arthropod communities to the application of liquid manure and aerial irrigation – two novel, rapidly spreading management practices – remains poorly understood which hampers the formulation of best-practice management recommendations for both hay production and biodiversity preservation. In these nutrient-poor mountain grasslands a moderate management regime is likely to be beneficial. This study experimentally assessed, at the site scale, among low-input montane and subalpine meadows, the short-term effects (one year) of a moderate intensification (slurry fertilisation: 26.7–53.3 kg N ha^{-1} $year^{-1}$; irrigation with sprinklers: 20 mm $week^{-1}$; singly or combined together) on plant species richness, vegetation structure, hay production, and arthropod abundance and biomass in the inner European Alps (Valais, SW Switzerland). Results show that: 1) montane and subalpine hay meadow ecological communities respond very rapidly to an intensification of management practices; 2) on a short-term basis, a moderate intensification of very low-input hay meadows has positive effects on plant species richness, vegetation structure, hay production, and arthropod abundance and biomass; 3) vegetation structure is likely to be the key factor limiting arthropod abundance and biomass. Our ongoing experiments will in the longer term identify which level of management intensity achieves an optimal balance between biodiversity and hay production.

Keywords: Agriculture; grassland management; liquid manure; arthropods; vegetation heterogeneity.

Introduction

Numerous studies have documented that grassland management intensification alters biodiversity, leading to decline of plant and arthropod species richness and modifying plant traits as well as community structure (e.g. Marini *et al.* 2008; Riedener, Rusterholz & Baur 2013; Niu *et al.* 2014). Similarly, but on the other extreme of the grassland management intensity gradient, abandonment occurring in steep and less accessible mountain regions leads to forest encroachment and the disappearance of many open-habitat species (MacDonald *et al.* 2000; Tasser *et al.* 2007). However, alternatives to this dichotomous trend (agriculture intensification versus abandonment) exist in the form of an intermediate intensity of management in terms of mowing regime (e.g. Tonn & Briemle 2010; Bernhardt-Romermann *et al.* 2011), irrigation (Jeangros & Bertola 2000) and fertilisation (e.g. Pauli, Peintinger & Schmid 2002; Bowman *et al.* 2006). This moderate management is likely to have conjugated positive effects on plant and invertebrate diversity, hay production and forage nutritional quality. Different theories and factors can explain why an intermediate or moderate management intensity is likely to benefit grassland flora and fauna communities. For example, based on the hump-shaped species diversity curve of Grime (1973; Mittelbach *et al.* 2001), a moderate addition of resources should enhance plant species growth and richness. This phenomenon is especially expected in nutrient-poor montane and subalpine grasslands (Peter *et al.* 2009). Corollary, an increase in plant growth will provide more food, space and shelters for arthropods, boosting their abundances (e.g. Haddad, Haarstad & Tilman 2000; Perner *et al.* 2005; Dittrich & Helden 2011; Buri, Arlettaz & Humbert 2013). Higher plant species richness not only provides more potential host plants for herbivores, but also greater horizontal and vertical vegetation structure complexity which seems to be crucial to support higher diversity and abundance of arthropods (e.g. Brown, Gibson & Kathirithamby 1992; Morris 2000; Woodcock *et al.* 2009; Dittrich & Helden 2011). A more abundant arthropod community will promote higher trophic levels up to vertebrates through a cascading process (Hunter & Price 1992; Britschgi, Spaar & Arlettaz 2006). In semi-natural mountain meadows, the exact management practices that would permit decent hay production without degrading the functional integrity of the system remains unknown, thus meriting further investigation.

We launched a two-way factorial experiment on the short-, mid- and long-term effects of fertilisation and irrigation on plant and arthropod communities of montane and subalpine hay meadows of the inner European Alps (Valais, SW Switzerland). The main objective of this paper is to document the short-term changes that occurred just one year after the onset of differential experimental management treatments. While end user management recommendations will be based on the longer-term outputs of the study, thoroughly assessing the short-term effects clarifies the ecological mechanisms at play during the temporal process of grassland intensification. More specifically, we addressed two questions: 1) what are the short-term effects of fertilisation and irrigation, considered separately and in combination, on plant species richness, vegetation structure, hay production and arthropod abundance and biomass? and 2) what is the relationship between vegetation and arthropod parameters?

Plants and arthropods were hypothesised to respond differently to the fertilisation and irrigation treatments in the short-term, i.e. after just one year of experimental manipulation, partly because plants typically have a slower reaction time than animals to changes in environmental conditions (Mortimer, Hollier & Brown 1998; Cole *et al.* 2010). More specifically, we expected slight positive effects of fertilisation on plant species richness and hay production (Grime 1973). An increase in plant growth and richness was expected to increase vegetation structure, which would in turn promote arthropod populations (Woodcock *et al.* 2009). On the other hand, we predicted that irrigation would have no effect on plant species richness (Riedener, Rusterholz & Baur 2013), but still positive effects on arthropod abundance through an increased phytomass productivity and protection against dessication (Nielsen 1955). Fertilisation was also predicted to increase herbivorous arthropod abundances, owing to an increase of plant tissue nitrogen content (Haddad, Haarstad & Tilman 2000; Dittrich & Helden 2011). However, due to a highly diverse plant species pool among all our meadows (given that they have been extensively managed over the past decades), a high ecological stability and resistance against the experimental treatments were expected in the short-term (Tilman & Downing 1994), therefore translating into few contrasted effects.

Material and methods

Study sites

In 2010, twelve extensively managed montane and subalpine hay meadows were selected according to their management history. The meadows had to be managed extensively for at least the last 10 years with no or very low levels of fertilisation (with solid manure only) and irrigation (terrestrial only), and only a single cut per year. Their homogeneous topography and their size were also considered (> 4000 m^2). The study sites were situated in the inner Alps (Valais, SW Switzerland) between 790 and 1740 m above sea level, encompassing a wide gradient of altitudes and ambient temperatures (Table 1). This region experiences a continental climate with cold and wet winters, and dry and hot summers.

Design

A two-way full factorial design was applied in our experiments. At each study site, i.e. in each meadow, four circular plots of 20 m in diameter were established with at least 5 m between plot boundaries. The different management treatments were randomly assigned to the four plots within a given meadow. A first plot served as a control (C-plot: neither irrigation nor fertilisation). A second plot was only irrigated (I-plot) at regular time intervals with sprinklers. A third plot was only fertilised (F-plot) with liquid manure, and a fourth plot was irrigated and fertilised (I+F-plot). C-plots were cut once a year, which corresponds to local standards for extensively managed meadows, while I, F and I+F plots were cut twice a year. Although this discrepancy deviated the design from a purely speaking two-way full factorial design, it made agronomical sense; local farmers would not irrigate or fertilise their field without doing a second cut. Treatments I and I+F were irrigated weekly from mid-May to the beginning of September, except when heavy rainfall occurred (> 20 mm over the previous week). Weekly sprinkler irrigation amounted to 20 mm of water column. The fertiliser consisted of organic dried manure NPK pellets (MEOC SA, 1906 Charrat, Switzerland) and mineral potassium oxide (K_2O) dissolved in water to reach the equivalent of standard-farm liquid manure (Sinaj *et al.* 2009), consisting namely of 2.4 kg of usable nitrogen, 2 kg of phosphate (P_2O_5) and 8 kg of potassium oxide (K_2O) per m^3 of solution. 174, 262 or 349 l of liquid manure per plot, corresponding to respectively 26.7, 40.0 or 53.3 kg N ha^{-1} $year^{-1}$, were applied three times in August 2010, May 2011 and August 2011 (Table 1). The exact amount of manure applied at each site depended on the theoretical local hay production potential calculated using pre-experimental hay yield (when extensively managed) and site elevation and it matched the local mid-intensive management norm recommended in (Sinaj *et al.* 2009). In each plot, a 4 x 2 m permanent rectangle subplot was established at a distance of 4 m from plot centre, randomly placed along the slope axis on the right or the left side of the plot. In each subplot, we measured plant species richness, vegetation structure, hay production and abundance and biomass of arthropods (Fig. 1).

Vegetation sampling

In 2011, plant species richness, vegetation structure and hay production were assessed twice: once just before the first cut (from mid-June to end of July, at a similar vegetation stage, depending on altitude; hereafter referred to as July samples) and once just before the second cut (from August to September; hereafter August samples). Vegetation surveys were performed using the point quadrat method in order to obtain information on the vertical distribution of each plant species (Stampfli 1991). For that purpose, we developed an *ad hoc* device that consisted of a 4.10 m long steel bar (supported by two tripods) that contained 41 holes distant of 10 cm (Appendix S1). Graduated metal sticks of 5 mm in diameter were inserted vertically into the holes. Each plant species touching the stick was recorded and the height at which the plant touched the stick was noted. If the same species touched more than once a single stick, the maximal height was retained. The sampling device was positioned along each long side of the permanent rectangular subplot, first 10 cm and then 25 cm from the long edge (Fig. 1). We recorded contacts between plants and sticks at 20 and 41 holes (points) when the device was positioned at 10 cm and 25 cm from the edge, respectively. Altogether we thus recorded 122 points in each plot. A modified Shannon-Wiener diversity index (Woodcock *et al.* 2009) was used to define the structure of the vegetation:

$$Struct = \sum_{i=1}^{n} p_i \log_e p_i$$

Where *Struct* is the index for vegetation structure and p_i the proportion of the number of contacts with the stick at each height *i*, in each subplot, at each sampling session. Thus, greater structural complexity of the vegetation results in a higher value.

Just before each grass cut hay production was estimated by clipping two strips of grass with an area of 0.2 x 4 m along each long edge of the permanent subplot at 6 cm above the ground, exactly where the vegetation relevés had been performed (Fig. 1). The two samples from the same subplot were then pooled together. The collected plant material was dried at 105°C during 72 h and then weighed (± 0.1 g) in order to quantify hay production.

Arthropod sampling

Arthropods were sampled using a suction sampler (Stihl SH 86 D; Stihl) equipped with a gauze sampling sack fixed inside the nozzle to collect arthropod items. This technique has been proved to be efficient for grassland vegetation-dwelling arthropods (Sanders & Entling 2011). All plots were sampled twice during the vegetation season, once before each grass cut. At each sampling session three subsamples were collected at three regularly spaced locations in the middle of each permanent subplot (Fig. 1). Subsamples consisted of the vacuumed content of a metallic cylinder of 50 cm height and 50.5 cm diameter (0.2 m^2 area) that was placed directly on the ground. The content of the gauze sampling sack was transferred into a sealed plastic bag stored at low temperature in an ice-cooled box. Sampling was undertaken between 11:00 and 17:00, only under dry vegetation conditions and with low or moderate wind. Arthropod specimens were then stored in the laboratory at -20°C before being classified in six main taxonomic groups: spiders, Auchenorrhyncha (i.e. plant- and leafhoppers), weevils, leaf beetles, ants and others (other arthropods not belonging to the previous groups). The number of specimens was counted prior to drying the arthropods at 60° during 72 h. Finally, all arthropod groups stemming from one subsample were weighed (± 0.1 mg). For statistical analyses, the three subsamples per plot were summed. Ants had to be discarded because suction trapping proved to be inefficient for sampling this group due to their massive local colonial aggregations.

Statistical analysis

Treatment effects were analysed with linear mixed effects models (LMMs) using the lmer function from the lme4 package for R (Bates, Maechler & Bolker 2011). *P*-values and confidence intervals (CI) were computed with the pvals.fnc function from the languageR package using 100’000 Markov chain Monte Carlo iterations (Baayen 2011). Response variables were log-transformed plant species richness, vegetation structure, hay production, log-transformed arthropod abundance and log-transformed arthropod biomass. As grass (Poaceae), legume (Fabaceae) and forb species may respond differently to the management treatments (e.g. Li *et al.* 2010), additional models on the relative cover of each functional group were run. Note that not all variables needed log-transformation prior to analysis to achieve normal distribution of residuals. The fixed effects were the treatments (C, I, F, or I+F) and the sampling sessions (July or August) which was added as a factor to take in account the fact that two measures were made per plot. For hay production, analyses were performed on the sum of the July and August (pooled samples). Thus, for this variable, fixed effects were limited to the treatments. The study sites (geographic replicates) were designated as a random effect. To better appraise differences between treatments, post-hoc tests were performed using the function relevel of R to change the first (reference) level of the factor 'treatment'.

In order to further understand the relationship between the vegetation and arthropod parameters, simple linear regressions were performed using the lm function (Crawley 2007). The log transformed abundance and biomass of arthropods were fitted against plant species richness, vegetation structure (index *Struct*) and hay production. Finally, to test whether the variance in arthropod abundance and biomass (variance of the non-transformed raw data) changes with respect to vegetation structure, an homoscedasticity test (Bartlett's test) was conducted between the values obtained from the first and the third quantiles of *Struct* (Crawley 2007). Thus, a significant *P*-value would indicate that with low vegetation structure there are only few arthropods while with a higher vegetation structure it is possible to have few or more arthropods (see Fig. 4). In other words, this value indicates whether vegetation structure limits arthropod abundance and/or biomass. All statistical tests were performed using R version 2.15.3 (R Core Team 2013).

Results

Effects of irrigation and fertilisation on the vegetation

In total, 194 plant species belonging to 34 families were identified during the two sampling sessions across all meadows (see Appendix S2 for a complete list of the plant species recorded). F-plots, I-plots and I+F-plots harboured significantly more plant species than C-plots (Fig. 2; and Table S3.1 in Appendix S3 for related model outputs). Moreover, irrigated plots (I and I+F) had significantly higher species richness than F-plots, but treatment I+F was not different from I. Irrigated plots exhibited a higher vegetation structure (index *Struct*) than C-plots and F-plots, while treatment F did not differ from C. The greatest vegetation structure was measured in July and the lowest in August; this pattern was consistent across all treatments.

Annual hay production (sum of both sampling sessions) ranged from 96.5 to 1’111 g m^{-2} across all plots. It was approximately three times higher in the irrigated plots compared to C-plots but I+F treatment did not differ from I-treatment. Fertilisation (F) had a lower effect compared to irrigation but still gave a significantly higher hay production than C.

Relative cover of grasses decreased in I, F and I+F plots compared to the control plots, while legumes increased their cover (Fig. 3). Relative changes were all significant at a $P < 0.01$ level (see Table S3.2 in Appendix S3 for exact values of models outputs). Forb species cover did not differ among treatments except I+F that had significantly less cover than C ($P = 0.011$).

Effects of irrigation and fertilisation on the arthropods

In total, 7’198 arthropods (ants excluded) were collected across all replicates (3’923 in July and 3’275 in August). The samples included *n* individuals of the following taxa: 629 spiders (Araneae), 1’869 plant- and leafhoppers (Hemiptera: Auchenorrhyncha), 562 weevils (Coleoptera: Curculionidae), 587 leaf beetles (Coleoptera: Chrysomelidae) and 3’551 others. Abundance of arthropods in I-plots and I+F-plots were significantly higher than in C-plots and F-plots (Fig. 2; and Table S3.1 in Appendix S3 for related model outputs). F-treatment did not deliver a higher abundance of arthropods compared to C-treatment. The only significant differences within a single arthropod group were for plant- and leafhoppers where in I+F-plots there were more individuals compared to C-plots (MCMC mean = 0.890, 95% CI = 0.281 – 1.511, *P* MCMC = 0.005) and to F-plots (MCMC mean = 0.766, 95% CI = 0.161 – 1.385, *P* MCMC = 0.015). For spiders, abundance in I+F-plots was marginally significantly higher than in C-plots (MCMC mean = 0.375, 95% CI = -0.021 – 0.759, *P* MCMC = 0.060), while no differences were detected between I-plots and F-plots, on one side, and C-plots, on the other side.

In total, 26.92 g dry weight of arthropods was collected across all replicates (17.13 g in July and 9.79 g in August). The samples (excluding ants) included the following taxa: 1.856 g of spiders, 2.705 g of plant- and leafhoppers, 0.766 g of weevils, 0.458 g of leaf beetles and 21.130 g for others. All treatments affected positively the biomass of arthropods (Fig. 2; and Table S3.1 in Appendix S3). The biomass of plant- and leafhoppers was significantly higher in I+F-plots than in the C-plots (MCMC mean = 0.019, 95% CI = 0.001 – 0.037, *P* MCMC = 0.038), while there were no significant biomass differences among treatments and controls in another arthropod taxonomic group.

Relationships between arthropods and vegetation

The total abundance of arthropods was positively linked to hay production (Estimate = 2.60 10E-03, $t = 4.767$, $P < 0.001$; adjusted $R^2 = 0.186$, i.e. 18.6% explained variance), plant species richness (Estimate = 6.79 10E-02, $t = 6.696$; $P < 0.001$, $R^2 = 0.316$) and vegetation structure (Estimate = 0.572, $t = 2.752$, $P = 0.007$, $R^2 = 0.065$). The variance in arthropod biomass was explained in about the same order of magnitude by hay production (Estimate = 2.905 10E-03, $t = 5.085$, $P < 0.001$, $R^2 = 0.207$), plant species richness (Estimate = 5.580 10E-02, $t = 4.747$, $P < 0.001$, $R^2 = 0.185$) and vegetation structure (Estimate = 1.049, $t = 5.182$, $P < 0.001$, adjusted $R^2 = 0.214$). Note that estimates are on the log scale. Regarding the analyses to see if vegetation structure limits arthropods, for both arthropod abundance (Bartlett's $K^2 = 6.933$, df = 1, $P = 0.008$) and biomass (Bartlett's $K^2 = 23.145$, df = 1, $P < 0.001$), Bartlett’s test showed a greater variance at the third than at the first quantile of vegetation structure (Fig. 4).

Discussion

This study shows that among low-input montane and subalpine hay meadows plant species richness, vegetation structure, hay production as well as arthropod abundance and biomass all immediately and positively react to moderate experimental fertilisation and irrigation. It should be noted, however, that the starting conditions in our study meadows were typical of the traditional, extremely extensive management practices that have been prevailing for centuries in the inner Alps, with very low fertiliser application and limited terrestrial irrigation. It is thus not totally surprising that our experimental treatments improved both biodiversity and hay yield in the very short-term. These traditional grasslands typically are poor in nitrophilous species with specialised taxa additionally present due to a very constraining edaphic context and watering regime (Peter *et al.* 2009). The speed at which these changes operated in response to intensification was however unexpectedly rapid. A powerful advantage of our full block design approach is certainly that it allows a direct comparison of the effects of both irrigation and fertilisation, which were either separated or conjugated, upon meadowland ecological communities regardless of other potentially confounding abiotic factors such as altitude, exposition or soil properties.

Effects of fertilisation and irrigation on the vegetation

Fertilising with liquid manure and watering with sprinklers are two modern, currently spreading management practices, even in remote areas of the Alps (Riedener, Rusterholz & Baur 2013). Our treatments thus mimic the trends of modern agriculture in these areas. Although we had predicted slower effects on plant species richness, basing our predictions on the dynamics observed in most long-term studies in alpine and arctic regions (e.g. Carlen, Darbellay & Gex 1998; Yang, van Ruijven & Du 2011), our findings are in accordance with the predictions of the hump-shaped model of plant diversity (Grime 1973; Mittelbach *et al.* 2001) . This model stipulates that an intermediate level of intensification must support a higher plant species richness than low or high input systems. Yet, we cannot exclude, given that we measured effects just one year after the onset of the experimental treatments, that abiotic factors, such as interannual weather variation, might have interacted with the treatment effects, amplifying the signal (Walker *et al.* 1994). What is certain, however, is that no plant community would ever reach an equilibrium after just one year of this management (Yang, van Ruijven & Du 2011). Hence, a short-term, moderate intensification as applied here may indeed promote high plant species richness because it rapidly offers favourable conditions to nitrophilous and mesophilous species that are normally absent on nutrient-poor and dry soils. Some of the original plant species pool consisting of heliophilous species, tolerant to reduced nutrients and water supply but particularly intolerant to intensification and shade, may actually have persisted in the community merely because they were already extant. This suggests the possibility of a short-term coexistence of plants with different life history traits and varied ecological requirements (Bowman *et al.* 2006). In the mid- and long-run, however, one would expect that interspecific competition for resources such as light will especially increase among some species. Species exhibiting characteristics such as low growth rate could become progressively disadvantaged and possibly decline to local extinction (Rajaniemi 2002; Hautier, Niklaus & Hector 2009).

Irrigating and fertilising increased the relative cover of legumes, which appears to be mostly at the expense of the cover of grasses. While this seems in contradiction with most grassland fertilisation studies that found the reverse pattern regarding their biomasses (e.g. DiTommaso & Aarssen 1989; Carlen, Darbellay & Gex 1998; Li *et al.* 2010), it must be stressed that relative cover does not necessarily correlate with biomass, especially when comparing grasses that grow tall and thin with legumes that tend to grow wider. In addition, fertilisation studies that found positive effects of intensification on grasses and negative effects on legumes usually applied mineral fertilisers, while the application of organic fertilisers is known to have slightly different influences, typically favouring legume species (e.g. Vintu *et al.* 2011).

In contradiction to our prediction that fertilisation would have a positive short-term effect on all vegetation parameters, addition of liquid manure alone did not increase vegetation structure, while the combination of fertilisation and irrigation did not elicit a greater response from vegetation parameters than did irrigation alone. This indicates that in the short-term, application of fertiliser (only) might enhance the sensitivity of the vegetation to water stress (Huston 1997) or that our meadows were more likely to be limited by water supply than nitrogen supply. Indeed, the climatic context in the inner Alps is characterised by its dryness (Central Valais, around Sion-Visp, is the pole of xericity in the whole Alpine massif, with ca 500 mm annual precipitation), with even April-June 2011 slightly drier than interannual average (94 mm versus 136 mm mean rainfall during 2006 to 2010 in Sion; MeteoSwiss). Plant nutrient uptake may also have been improved by water addition thus enhancing plant growth (Davis, Grime & Thompson 2000). Future vegetation surveys in the same study meadows will enable disentangling climatic from agronomic effects, while characterising mid- and longer-term changes in plant communities.

Effects of fertilisation and irrigation on arthropod communities

Irrigation in turn had a positive effect on arthropod species richness, as predicted. This indicates that water might be a limiting factor for arthropods (e.g. intolerance to desiccation; (Nielsen 1955), or that there is an indirect effect mediated via plants onto arthropods. In contrast, fertilisation *per se* led to no discernible effect on arthropods, corroborating previous findings in comparable montane ecosystems (Grandchamp *et al.* 2005). The less complex vegetation structure achieved via fertilisation alone compared to irrigation means that the offer of microhabitats and the resulting ecological niche opportunities are less favourable when only fertilisation is augmented (Reid & Hochuli 2007). Irrigation and fertilisation were also expected to increase the rate of herbivory, i.e. the abundance of plant- and leafhoppers, and as a result increase the abundance of their predators such as spiders (Kirchner 1977). However, only plant- and leafhoppers showed a numeric response to irrigation and fertilisation suggesting that a steady state had not been achieved with no discernible effects being propagated to the upper trophic levels along the food chain at this stage. It is also important to note that a much smaller sample size for predator taxa than for prey taxa could have blurred the pattern due to lower statistical power.

Relationships between arthropods and vegetation

Vegetation parameters such as plant species richness, plant biomass and vegetation structure, all influence arthropod community to some extent (Knops *et al.* 1999; Haddad, Haarstad & Tilman 2000). There is still an ongoing debate about which factor has the greatest impact on arthropods (Perner *et al.* 2005), but recent studies have pointed out that vegetation structure might be the crux (Woodcock *et al.* 2009; Dittrich & Helden 2011). Our analyses show that all vegetation parameters influence arthropods to a certain degree. However, neither plant species richness (31.6% of explained variance for abundance/18.5% for biomass) nor hay production (18.6%/20.7%) or vegetation structure (6.5%/21.4%) individually accurately predicted arthropod abundance and biomass. This seems to contradict the view that vegetation structure is a key factor. However, there is evidence that vegetation structure did profoundly influence the number of arthropods in our meadows (Fig. 4), yet vegetation structure is more likely to act as a limiting than a predictive factor. Indeed, at low vegetation structure, low arthropod abundance and biomass always prevail, whereas at high vegetation structural diversity, arthropod abundance and biomass can either be low or high. This pattern is in line with the predictions of the habitat heterogeneity hypothesis (Brown, Gibson & Kathirithamby 1992). A higher entanglement of plant above-ground parts can increase the mobility of grass-dwelling arthropods (Randlkofer *et al.* 2009) through better vertical and horizontal connectivity while it offers a broader palette of ecological niches (Duffey 1962). Thus, if complex vegetation structure is a *sine qua non* condition for high arthropod abundance and biomass, it does not guarantee it. It is likely that source populations must exist in the surrounding matrix to colonise any newly emerging, highly structured vegetation patches. Moreover, new detrimental factors generated by high vegetation structure might also obliterate the ability of arthropod populations to develop, such as microclimatic conditions that adversely affect some taxa (increase moisture or shade) or altered diffusion of plant volatiles that hampers resource location (e.g. Van Wingerden, van Kreveld & Bongers 1992; Finch & Collier 2000; Després, David & Gallet 2007).

Conclusions

Although plant community stability was likely not achieved after just one year of experimental fertilisation and irrigation, our findings demonstrate that on a short-term basis a moderate level of intensification positively affects biodiversity and hay production of low-input, extensively managed montane and subalpine meadows. Tremendous land use changes steadily affect mountainous regions, leading either to abandonment of marginal grasslands or to intensification of fields accessible to machinery (Tasser *et al.* 2007). This rather dichotomous trend should be reversed, which calls for more intermediate management practices if one wants to concomitantly promote grassland biodiversity and acceptable agricultural revenue. Although this short-term study only provides insights into the mechanism of intensification within upland grasslands, the continuation of our experiments will deliver detailed prescriptions in the medium-term for optimising slurry fertilisation and aerial irrigation so as to achieve the best possible compromise between hay production, biodiversity preservation and ecosystem functioning among montane and subalpine hay meadows.

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Table 1: Description of the twelve study sites with altitude, exact coordinates and quantity of fertiliser, i.e. nitrogen (N), phosphorus (P), and potassium (K), applied per hectare per year. The fertiliser consisted of organic NPK pellets and mineral K_2O dissolved in water to reach the equivalent of standard-farm liquid manure.

Site	Name	Altitude [m]	Coordinates		Fertiliser applied [kg ha^{-1} yr^{-1}]		
			Latitude	Longitude	N	P	K
1	La Garde	980	46°3’45”N	7°8’35”E	40.0	33.3	133.3
2	Sembrancher	798	46°4’24”N	7°8’36”E	53.3	44.4	177.7
3	Orsières	1022	46°1’44”N	7°9’8”E	53.3	44.4	177.7
4	Vens	1373	46°5’7”N	7°7’24”E	40.0	33.3	133.3
5	Euseigne	1028	46°10’9”N	7°25’27”E	53.3	44.4	177.7
6	Eison	1768	46°9’18”N	7°28’10”E	26.7	22.3	89.0
7	St-Martin	1589	46°11’8”N	7°26’43”E	26.7	22.3	89.0
8	Grimentz	1738	46°11’22”N	7°34’35”E	26.7	22.3	89.0
9	Arbaz	1270	46°16’42”N	7°22’47”E	40.0	33.3	133.3
10	Icogne1	1200	46°17’56”N	7°26’31”E	40.0	33.3	133.3
11	Icogne2	880	46°17’6”N	7°26’10”E	53.3	44.4	177.7
12	Cordona	1153	46°19’45”N	7°33’8”E	40.0	33.3	133.3

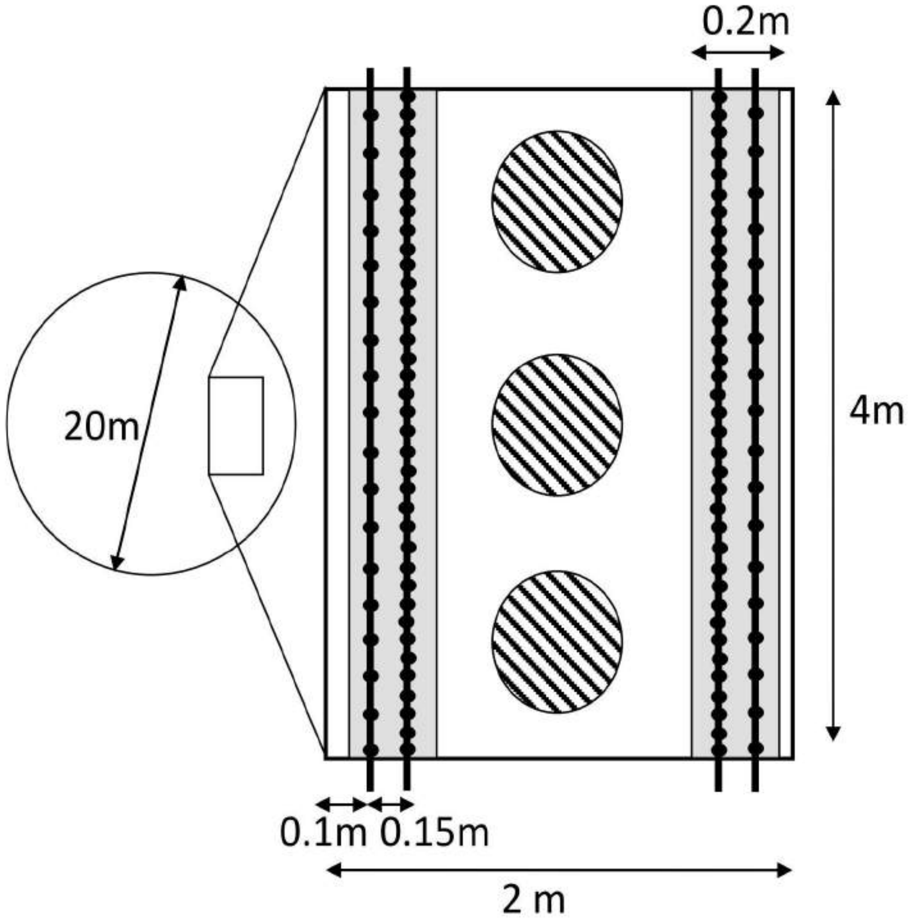


Figure 1. Experimental design. Four management treatments were applied at random onto 20 m diameter circles delineated on each meadow. In each circle (excerpt), vegetation (*n* = 122 records per circle, black dots), hay production (grey strips) and arthropods (three dashed circles of 0.2 m^2) were sampled.

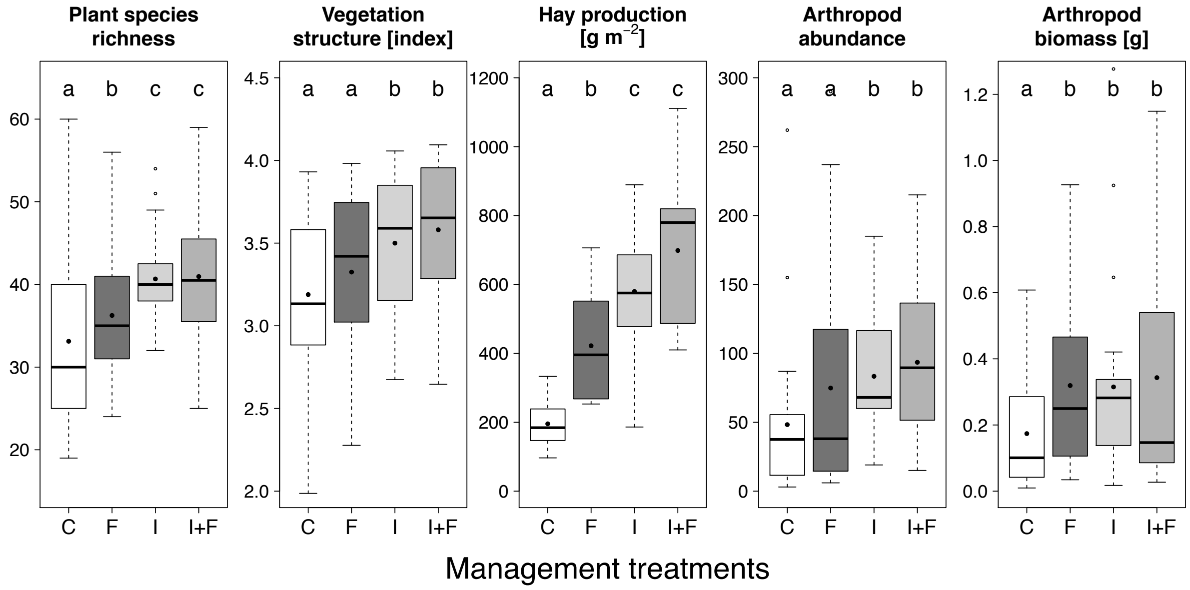


Figure 2. Responses of the vegetation (plant species richness, vegetation structure and hay production) and arthropod (abundance and dry biomass) variables to the different management treatments. Bold lines represent medians, solid points the means; boxes the first and third quantiles. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Treatments abbreviations are: (C) control; (I) irrigated, (F) fertilised; and (I+F) irrigated and fertilised.

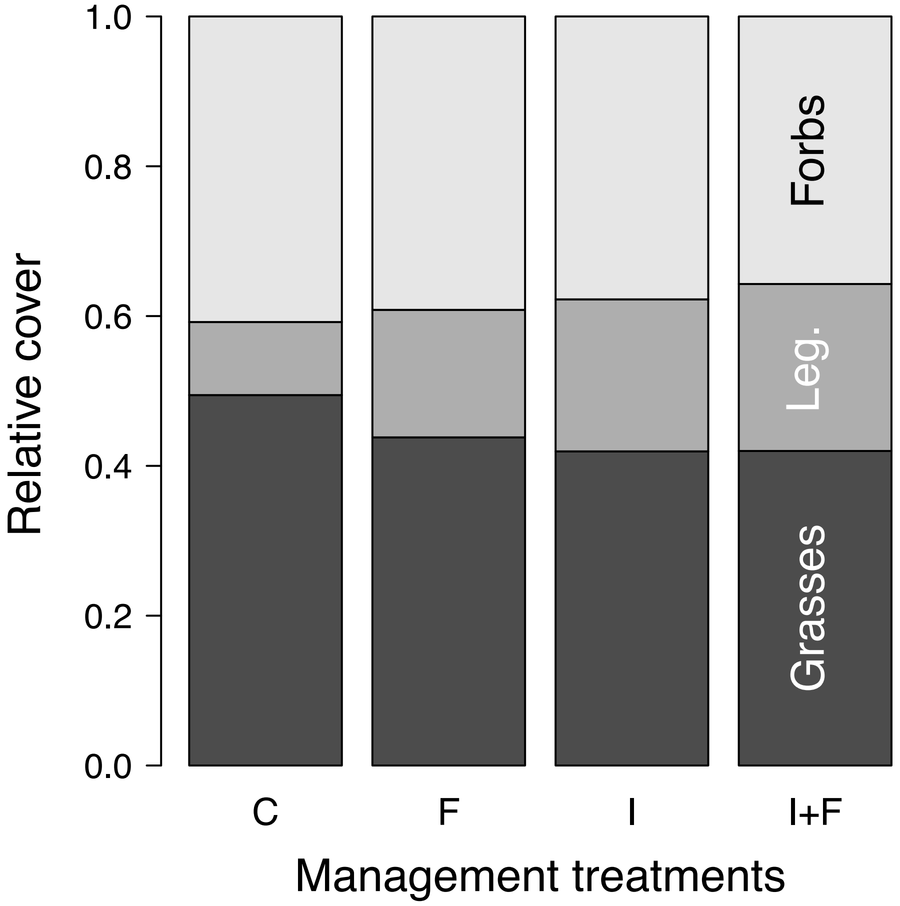


Figure 3. Responses of on the relative cover of grass (dark-grey), legume (mid-grey) and forb (ligh-grey) species to the different management treatments. Model outputs (including estimates, CIs and *P*-values) are provided in Table S3.2 in Appendix S3. For treatment abbreviations, see legend of Fig. 2.

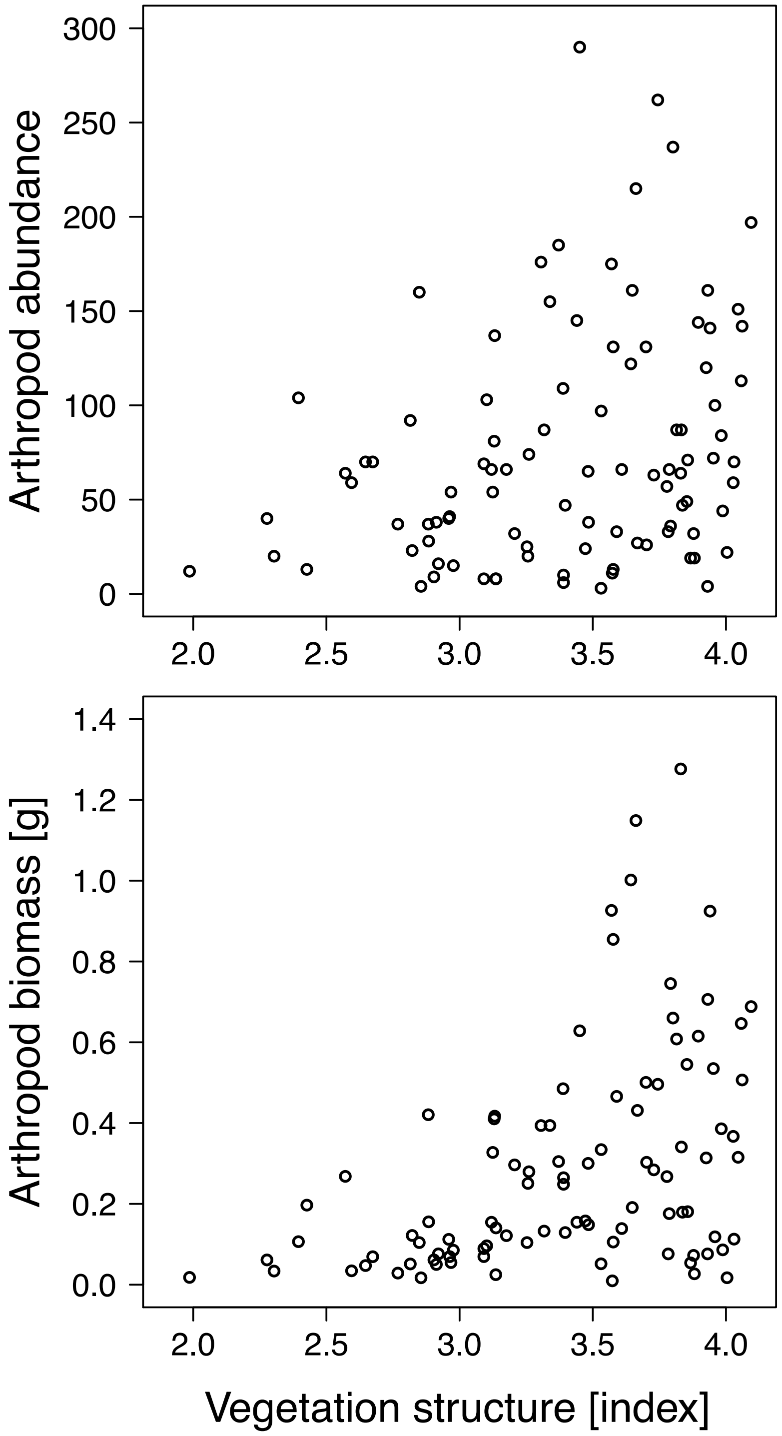
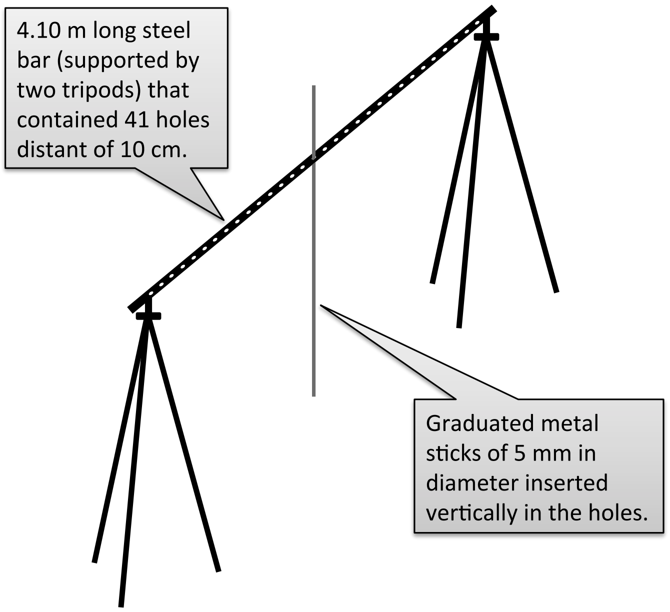


Figure 4. Relationships between arthropod abundance and biomass versus vegetation structure (index *Struct*). Greater the structure of the vegetation is, higher the *Struct* index is.

Supporting information

Appendix S1. Drawing of the ad hoc device used to sample the vegetation (point quadrat method).



Appendix S2. A complete list of the plant species identified during the two sampling sessions across all treatments in all meadows.

Table S2.1. In total, 194 plant species belonging to 34 families were identified during the two sampling sessions across all meadows.

Plant species name	Family	Plant species name	Family
Achillea millefolium l.	Asteraceae	*linaria vulgaris Mill.*	Scrophulaceae
Acinos alpinus (l.) Moench	Lamiaceae	*linum catharticum l.*	Linaceae
Agrimonia eupatoria l.	Rosaceae	*Listera ovata (l.) r. Br.*	Orchidaceae
Agrostis capillaris l.	Poaceae	*Lolium perenne l.*	Poaceae
Agrostis stolonifera l.	Poaceae	*Lotus corniculatus l.*	Fabaceae
Ajuga pyramidalis l.	Lamiaceae	*Luzula campestris (l.) DC.*	Joncaceae
Ajuga reptans l.	Lamiaceae	*Luzula nivea (l.) DC.*	Joncaceae
Alchemilla vulgaris aggr.	Rosaceae	*Luzula sylvatica aggr.*	Joncaceae
Allium oleraceum l.	Liliaceae	*Medicago lupulina l.*	Fabaceae
Anthericum ramosum l.	Liliaceae	*Molinia arundinacea schrank*	Poaceae
Anthoxanthum odoratum l.	Poaceae	*Molinia caerulea (l.) Moench*	Poaceae
Anthriscus sylvestris (l.) Hoffm.	Apiaceae	*Myosotis arvensis Hill.*	Boraginaceae
Anthyllis vulneraria l.	Fabaceae	*Myosotis sylvatica Hoffm.*	Boraginaceae
Arabis ciliata Clairv.	Brassicaceae	*Nardus stricta l.*	Poaceae
Arabis hirsuta (l.) scop.	Brassicaceae	*Onobrychis viciifolia scop.*	Fabaceae
Arenaria serpyllifolia l.	Caryophyllaceae	*Ononis repens l.*	Fabaceae
Arrhenatherum elatius (l.)	Poaceae	*Ononis spinosa l.*	Fabaceae
Asperula cynanchica l.	Rubiaceae	*Paradisea liliastrum (l.) Bertol.*	Liliaceae
Avenella flexuosa (l.) Drejer	Poaceae	*Pastinaca sativa l.*	Apiaceae
Botrychium lunaria (l.) sw.	Ophiolglossaceae	*Peucedanum oreoselinum (l.)*	Apiaceae
Brachypodium pinnatum (l.)	Poaceae	*Phleum alpinum l.*	Poaceae
Briza media l.	Poaceae	*Phleum pratense l.*	Poaceae
Bromus erectus Huds.	Poaceae	*Phyteuma betonicifolium Vill.*	Campanulaceae
Bunium bulbocastanum l.	Apiaceae	*Phyteuma orbiculare l.*	Campanulaceae
Campanula glomerata l.	Campanulaceae	*Phyteuma spicatum l.*	Campanulaceae
Campanula rhomboidalis l.	Campanulaceae	*Picris hieracioides l.*	Asteraceae
Campanula rotundifolia l.	Campanulaceae	*Pimpinella saxifraga l.*	Apiaceae
Campanula scheuchzeri Vill.	Campanulaceae	*Plantago atrata Hoppe*	Plantaginaceae
Cardamina hirsuta	Brassicaceae	*Plantago lanceolata l.*	Plantaginaceae
Carex caryophyllea latourr.	Cyperaceae	*Plantago media l.*	Plantaginaceae
Carex flacca schreb.	Cyperaceae	*Poa alpina l.*	Poaceae
Carex montana l.	Cyperaceae	*Poa bulbosa l.*	Poaceae
Carex ornithopoda Willd.	Cyperaceae	*Poa pratensis l.*	Poaceae
Carex pallescens l.	Cyperaceae	*Poa trivialis l.*	Poaceae

Carex sempervirens Vill.	Cyperaceae
Carlina acaulis l.	Asteraceae
Carum carvi l.	Apiaceae
Centaurea jacea l.	Asteraceae
Centaurea scabiosa l.	Asteraceae
Cerastium arvense l.	Caryophyllaceae
Cerastium fontanum	Caryophyllaceae
Chaerophyllum hirsutum l.	Apiaceae
Cirsium acaule scop.	Asteraceae
Cirsium arvense (l.) scop.	Asteraceae
Clinopodium vulgare l.	Lamiaceae
Colchicum alpinum DC.	Liliaceae
Colchicum autumnale l.	Liliaceae
Crepis aurea (l.) Cass.	Asteraceae
Crepis biennis l.	Asteraceae
Crepis conyzifolia (Gouan)	Asteraceae
Crepis pyrenaica (l.) Greuter	Asteraceae
Crocus albiflorus Kit.	Iridaceae
Cynosurus cristatus l.	Poaceae
Dactylis glomerata l.	Poaceae
Dactylorhiza fuchsii (Druce) soó	Orchidaceae
Descampsia sp	Poaceae
Elymus repens (l.) Gould.	Poaceae
Erucastrum nastrurtiifolium	Brassicaceae
Euphorbia cyparissias l.	Euphorbiaceae
Euphorbia verrucosa l.	Euphorbiaceae
Euphrasia rostkoviana aggr.	Scrophulaceae
Festuca arundinacea schreb.	Poaceae
Festuca ovina l.	Poaceae
Festuca pratensis Huds.	Poaceae
Festuca rubra l.	Poaceae
Festuca valesiaca Gaudin	Poaceae
Filipendula vulgaris Moench	Rosaceae
Galium anisophyllum Vill.	Rubiaceae
Galium boreale l.	Rubiaceae
Galium mollugo aggr.	Rubiaceae
Galium pumilum Murray	Rubiaceae
Galium verum l.	Rubiaceae
Gentiana acaulis l.	Gentianacees
Gentiana campestris l.	Gentianacees
Gentiana verna l.	Gentianacees
Geranium sanguineum l.	Geraniaceae
Geranium sylvaticum l.	Geraniaceae
Geum montanum l.	Rosaceae
Gymnadenia conopsea (l.) r. Br.	Orchidaceae
Helianthemum nummularium (l.) Mill.	Cistaceae
Polygala alpestris rchb.	Polygalceae
Polygala chamaebuxus l.	Polygalceae
Polygala comosa schkuhr	Polygalceae
Polygala sp.	Polygalceae
Polygala vulgaris l.	Polygalceae
Polygonatum odoratum	Liliaceae
Polygonum viviparum l.	Polygonaceae
Potentilla aurea l.	Rosaceae
Potentilla crantzii fritsch	Rosaceae
Potentilla erecta (l.) raeusch.	Rosaceae
Potentilla pusilla Hostr	Rosaceae
Potentilla rupestris l.	Rosaceae
Potentilla thuringiaca link	Rosaceae
Primula veris l.	Primulaceae
Prunella grandiflora (l.) scholler	Lamiaceae
Prunella vulgaris l.	Lamiaceae
Pulmonaria australis (Murr)	Lamiaceae
Pulsatilla alpina (l.) Delarbre	Renonculaceae
Ranunculus acris l.	Renonculaceae
Ranunculus bulbosus l.	Renonculaceae
Ranunculus montanus aggr.	Renonculaceae
Ranunculus tuberosus lapeyr.	Renonculaceae
Rhinanthus alectorolophus (scop.)	Scrophulaceae
Rosa pendulina l.	Rosaceae
Rubus caesius l.	Rosaceae
Rumex acetosa l.	Polygonaceae
Salvia pratensis l.	Lamiaceae
Sanguisorba minor scop.	Rosaceae
Sanguisorba officinalis l.	Rosaceae
Scabiosa columbaria l.	Dipsacaceae
Securigera varia (l.) lassen	Fabaceae
Selaginella selaginoides (l.)	Selaginellaceae
Sesleria caerulea (l.) Ard.	Poaceae
Silene nutans l.	Caryophyllaceae
Silene vulgaris (Moench) Garcke	Caryophyllaceae
Soldanella alpina l.	Primulaceae
Stachys recta l.	Lamiaceae
Taraxacum officinale aggr.	Asteraceae
Thalictrum minus aggr.	Renonculaceae
Thesium alpinum l.	Santalaceae
Thesium pyrenaicum Pourr.	Santalaceae
Thymus serpyllum aggr.	Lamiaceae
Tragopogon pratensis l.	Asteraceae
Trifolium alpestre l.	Fabaceae
Trifolium badium schreb.	Fabaceae
Trifolium dubium sibth.	Fabaceae

Helictotrichon pubescens (Huds.) Pilg.	Poaceae	*Trifolium medium l.*	Fabaceae
Hepatica nobilis schreb.	Renonculaceae	*Trifolium montanum l.*	Fabaceae
Heracleum sphondylium l.	Apiaceae	*Trifolium pratense l.*	Fabaceae
Hieracium murorum aggr.	Asteraceae	*Trifolium repens l.*	Fabaceae
Hieracium piloselloides Vill.	Asteraceae	*Trisetum flavescens (l.) P. Beauv.*	Poaceae
Hippocrepis comosa l.	Fabaceae	*Trollius europaeus l.*	Renonculaceae
Hypericum perforatum l.	HypEricaceae	*Vaccinium myrtillus l.*	Ericaceae
Hypochoeris maculata l.	Asteraceae	*Verbascum nigrum l.*	Scrophulaceae
Inula salicina l.	Asteraceae	*Veronica arvensis l.*	Scrophulaceae
Knautia arvensis (l.) Coult.	Dipsacaceae	*Veronica chamaedrys l.*	Scrophulaceae
Knautia dipsacifolia Kreutzer	Dipsacaceae	*Veronica teucrium l.*	Scrophulaceae
Koeleria pyramidata (lam.) P. Beauv.	Poaceae	*Vicia cracca l.*	Fabaceae
Laserpitium latifolium l.	Apiaceae	*Vicia sativa l.*	Fabaceae
Laserpitium siler l.	Apiaceae	*Vicia sepium l.*	Fabaceae
Lathyrus pratensis l.	Fabaceae	*Viola hirta l.*	Violaceae
Leontodon hispidus l.	Asteraceae	*Viola rupestris f. W. schmidt*	Violaceae
Leucanthemum vulgare aggr.r	Asteraceae	*Viola tricolor l.*	Violaceae

Appendix S3. Outputs of the linear mixed effects models (LMMs) carried out on: 1) the effects of fertilisation and irrigation on plant species richness, vegetation structure, hay production, arthropod abundance and biomass; and 2) the effects of fertilisation and irrigation on the relative cover of grass, legume and forb species. Table A3.1 refers to figure 2 and Table A3.2 refers to figure 3.

Table S3.1. Results of the linear mixed effects models (LMMs) carried out on the effects of fertilisation and irrigation on plant species richness, vegetation structure, hay production, arthropod abundance and biomass. Table refers to figure 2 in the article. The fixed factors were the experimental treatments (with four levels: C = control plots; F = fertilised; I = irrigated; I+F = irrigation and fertilisation combined) and the sampling sessions (two levels: July and August). Random factors were the experimental study sites. *P*-values and 95% confidence intervals (CI) were computed with 100’000 Markov chain Monte Carlo (MCMC) iterations. MCMC mean parameter estimates (differences between expected mean densities) are given for the paired treatments comparisons and significant contrasts are highlighted in bold.

Response variable and comparison	MCMC mean	Lower 95% CI	Upper 95% CI	MCMC *P*-value
		Plant species richness (log scale)		
F vs. C	0.109	0.016	0.205	**0.023**
I vs. C	0.24	0.145	0.333	**<0.001**
I+F vs. C	0.236	0.144	0.331	**<0.001**
I vs. F	0.13	0.035	0.223	**0.007**
I+F vs. F	0.127	0.033	0.221	**0.009**
I+F vs. I	-0.003	-0.097	0.092	0.947
		Structure of vegetation [index]		
F vs. C	0.136	-0.001	0.272	0.051
I vs. C	0.311	0.176	0.45	**<0.001**
I+F vs. C	0.392	0.255	0.529	**<0.001**
I vs. F	0.175	0.039	0.311	**0.012**
I+F vs. F	0.256	0.121	0.395	**0.001**
I+F vs. I	0.081	-0.054	0.219	0.247
		Hay production [g m^{-2}]		
F vs. C	226.8	101.1	352.5	**0.001**
I vs. C	384.4	262.6	514.1	**<0.001**
I+F vs. C	503.2	379.7	630.8	**<0.001**
I vs. F	157.6	29	280.2	**0.015**
I+F vs. F	276.7	150.2	400.7	**<0.001**
I+F vs. I	118.8	-7.2	245.6	0.065

	Arthropod abundance (log scale)			
F vs. C	0.403	-0.039	0.845	0.072
I vs. C	0.935	0.497	1.378	**<0.001**
I+F vs. C	1.014	0.579	1.452	**<0.001**
I vs. F	0.534	0.087	0.966	**0.018**
I+F vs. F	0.612	0.164	1.044	**0.006**
I+F vs. I	0.077	-0.365	0.514	0.73
	Arthropod biomass [g] (log scale)			
F vs. C	0.829	0.327	1.303	**0.001**
I vs. C	0.824	0.325	1.306	**0.001**
I+F vs. C	0.734	0.237	1.219	**0.004**
I vs. F	-0.005	-0.501	0.477	0.983
I+F vs. F	-0.094	-0.579	0.397	0.706
I+F vs. I	-0.091	-0.587	0.389	0.716

Table S3.2. Results of the linear mixed effects models (LMMs) carried out on the effects of fertilisation and irrigation on the relative cover of grass, legume and forb species. Table refers to figure 3 in the article. The fixed factors were the experimental treatments (with four levels: C = control plots; F = fertilised; I = irrigated; I+F = irrigation and fertilisation combined) and the sampling sessions (two levels: July and August). Random factors were the experimental study sites. *P*-values and 95% confidence intervals (CI) were computed with 100'000 Markov chain Monte Carlo (MCMC) iterations. MCMC mean parameter estimates (differences between expected mean densities) are given for the paired treatments comparisons and significant contrasts are highlighted in bold.

Response variable and comparison	MCMC mean	Lower 95% CI	Upper 95% CI	MCMC *P*-value
Grasses (Poaceae)				
F vs. C	-0.056	-0.099	-0.014	**0.009**
I vs. C	-0.075	-0.117	-0.033	**0.001**
I+F vs. C	-0.075	-0.116	-0.032	**0.001**
I vs. F	-0.019	-0.061	0.023	0.38
I+F vs. F	-0.018	-0.059	0.024	0.39
I+F vs. I	0.001	-0.042	0.043	0.974
Legumes (Fabaceae)				
F vs. C	0.073	0.037	0.108	**<0.001**
I vs. C	0.105	0.07	0.14	**<0.001**
I+F vs. C	0.125	0.091	0.162	**<0.001**
I vs. F	0.033	-0.003	0.068	0.07
I+F vs. F	0.053	0.018	0.088	**0.004**
I+F vs. I	0.02	-0.015	0.055	0.261
Forbs				
F vs. C	-0.016	-0.055	0.024	0.415
I vs. C	-0.03	-0.07	0.009	0.131
I+F vs. C	-0.051	-0.09	-0.012	**0.011**
I vs. F	-0.014	-0.054	0.025	0.479
I+F vs. F	-0.035	-0.074	0.005	0.083
I+F vs. I	-0.021	-0.059	0.02	0.302

CHAPTER 3

Leaf-, frog- and plant-hoppers’ response to modern fertilisation and irrigation of hay meadows: lessons for sustainable grassland management

Leaf-, frog- and plant-hoppers' response to modern fertilisation and irrigation of hay meadows: lessons for sustainable grassland management

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Abstract

Traditional land-use of montane and subalpine meadows – i.e. extensively managed grasslands with solid manure application and gravitational, terrestrial irrigation via water channels – is currently shifting towards application of slurry and aerial irrigation, with the main objective of increasing hay production. The impact of these new practices upon biodiversity remains poorly understood and calls for quantitative assessments of their effects, as well as the framing of prescriptions for sustainable, biodiversity-friendly management of mountain hay meadows.

Relying on a full block design, we tested the effects of six management treatments corresponding to an increasing gradient of farming intensification (fertilisation with slurry and/or irrigation with sprinklers) on Auchenorrhyncha (Hemiptera) communities occurring in the inner European Alps (Valais, SW Switzerland). The experimental set up consisted of: control plots (no fertiliser, no irrigation, one cut a year; hereafter C-plots); plots that received only slurry, with two cuts a year (F-plots); plots that were only irrigated, with two cuts a year (I-plots); and plots that received low-, medium- and high-input of fertiliser and water, and experienced 1-2 cuts a year (F+I 1/3-plots; F+I 2/3, F+I 3/3-plots).

After two years of experimental treatment (2012), F and I-plots showed no change in the population sizes of Auchenorrhyncha, while F+I 1/3, F+I 2/3 and F+I 3/3-plots harboured significantly greater abundances (1.9, 1.5 and 1.4 times greater, respectively) of this taxon, as well as a greater biomass (1.8, 1.6 and 1.8 times greater, respectively) than C-plots. Species richness also increased in F+I 1/3, F+I 2/3 and F+I 3/3-plots, but the difference was significant only between F+I 2/3 and C-plots. Monophagous and oligophagous species, which are generally specialists, were favoured by the F+I 1/3 treatment while the F+I 2/3 and F+I 3/3 treatments increased the number of the generalist, eurytopic species.

Synthesis and applications – Auchenorrhyncha show a high sensitivity to farming intensification, with only low-input management (F+I 1/3; corresponding to one third of the level of fertilisation that would be necessary for achieving maximum theoretical hay yield locally) enabling the maintenance of the more specialised species. This provides guidance for the management of biodiversity-rich mountain hay meadows.

Keywords: Arthropods; Auchenorrhyncha; farming intensification; Hemiptera; invertebrates, montane meadows; slurry; species richness; sprinkler.

Introduction

In the past 50 years, land-use in montane and subalpine meadows has shifted from traditional management practices with no or low input of solid animal manure to more mechanized practices with higher input of fertilisers, essentially in the form of liquid manure (Strijker 2005). In addition, in the drier inner valleys of mountain massifs such as the European Alps, hay meadows are also irrigated. For centuries, gravitational, terrestrial irrigation systems conducting water from the main tributaries to the cultivated slopes have been used. Yet, this traditional system has been progressively abandoned and replaced by underground water pipe networks conducting water to aerial sprinklers, which require far less maintenance (Crook & Jones 1999). These marked changes of farming practices have negatively impacted the biodiversity of plants, arthropods and vertebrates found in traditionally managed mountain meadows (e.g. Britschgi, Spaar & Arlettaz 2006; Marini *et al.* 2008; Riedener, Rusterholz & Baur 2013)

Farming intensification in mountain grasslands results from increasing fertiliser and/or water inputs, which enhances phytomass production but induces a sheer reduction of plant species richness (Dietschi *et al.* 2007; Homburger & Hofer 2012; Humbert *et al.* 2015). The resulting alteration of vegetation structure (Andrey *et al.* 2014) and microclimate can in turn positively or negatively affect arthropod abundance, biomass and species richness, with the direction of response depending on the taxon (e.g. Grandchamp *et al.* 2005; Delley 2014). Several studies have suggested that an intermediate level of grassland management intensity, notably a low or moderate input of fertiliser and/or water, may indeed benefit productivity and fodder nutritional quality, as well as plant species richness (Jeangros & Bertola 2000; Bowman *et al.* 2006; Peter *et al.* 2009). Although this might in turn boost resources for herbivorous arthropods (Haddad, Haarstad & Tilman 2000; Grandchamp *et al.* 2005; Andrey *et al.* 2014), a recent meta-analysis by Humbert *et al.* (2015) pointed out the lack of experimental studies of the effects of intensification on grassland arthropod communities.

To fill this knowledge gap, we launched a series of controlled experiments with the objective to quantitatively define whether an optimal trade-off exists in terms of the degree of grassland management intensity, looking in particular at hay production versus maintenance of biodiversity and related ecosystem functions. The research was conducted at 12 replicated sites in the SW Swiss Alps, among traditionally managed montane and subalpine hay meadows that fulfilled the criteria of biodiversity promoting areas (as defined by Swiss agri-environment scheme). Six different experimental treatments were applied to the study plots from 2010 onwards according to a block design approach, as follows: control plots that received no fertilizer or additional water input; plots that were only fertilised with a medium input of slurry (liquid manure); plots that were only irrigated, with a medium water input provided by aerial sprinklers; and three plots that received low, medium or high inputs of fertiliser and water, respectively. The study plots were sufficiently large (20 m diameter; 314 m^2) to allow investigating the responses of plant and arthropods populations to experimental manipulation of management type and intensity. As study models, we selected Auchenorrhyncha (Hemiptera: Fulgoromorpha and Cicadomorpha), also known as plant-, frog- and leafhoppers. This taxon is highly diverse and fairly abundant in grasslands, and is considered as an excellent bioindicator, notably of land-use change (reviewed in Biedermann *et al.* 2005). In addition, these primary consumers play an important role both as prey for upper trophic levels in the food chain (Moreby & Stoate 2001) and in nutrient cycling (Andrzejewska 1979). Finally, their diversity of ecological traits, notably in terms of trophic specialisation (mono-, oligo- and polyphagous) and species-specific habitat associations (steno- and eurytopic) provides opportunities for mechanistic investigations of ecological functionalities.

The plots had all been very extensively managed (solid manure, terrestrial irrigation) for years before the onset of our experiments. We thus expected a marked response of Auchenorrhyncha (abundance, biomass and species richness) to the intensity gradient of our experimental manipulations. More specifically, we predicted an increase of food resources and niches availability for non-graminoid feeders (according to Andrey *et al.* 2014) and generalist Auchenorrhyncha (polyphagous and eurytopic species), both of which should be reflected in abundance, as well as an increase in species richness (Di Giulio, Edwards & Meister 2001). Conversely, a negative impact of intensification was expected upon specialists (monophagous and stenotopic species), which are known for their higher sensitivity to even slight modifications of their habitat (Nickel & Hildebrandt 2003). From this research we shall draw recommendations for biodiversity-friendly management of mountain hay meadows, notably as concerns slurry application and sprinkler irrigation.

Material and methods

Study sites

Twelve traditionally managed montane and subalpine hay meadows (790–1740 m above sea level) were selected within the inner Alps (Central Valais, SW Switzerland) in 2010, primarily based to their management history: they had to be managed extensively from at least the year 2000, with no or very low levels of fertilisation and irrigation and only a single cut per year (Tables 1 & 2). In reality, most study meadows had probably been managed traditionally for decades if not centuries. Meadow topography and area (> 4000 m^2) were additional selection criteria. Central Valais is characterised by a continental climate, with cold winters, and dry and hot summers.

Design

Within each of the 12 meadows, six circular plots of 20 m diameter were delineated, with at least 5 m distance between the boundaries of adjacent plots (Fig. 1). One out of six different management treatments were randomly assigned to each of the six plots in a given meadow. The first plot served as a control (C-plot: neither fertilisation nor irrigation); the second plot received only fertilisation with slurry (F-plot); the third plot received only regular aerial irrigation from a sprinkler (I-plot); the three other plots received a combination of fertilisation and aerial irrigation at respectively 1/3, 2/3 or 3/3 of a quantity that was estimated to allow maximum hay yield (F+I 1/3, F+I 2/3, F+I 3/3-plots). C-plots and F+I 1/3-plots were mown once a year, which corresponds to local standards for extensively managed meadows, while F, I, F+I 2/3 and F+I 3/3-plots were mown twice a year. I, F+I 1/3, F+I 2/3 and F+I 3/3-plots were irrigated weekly from mid-May to the beginning of September, except under heavy rainfall (>20 mm water column during the previous week). Weekly sprinkler irrigation amounted to 10 mm in F+I 1/3-plots, 20 mm in I and F+I 2/3-plots or 30 mm in F+I 3/3-plots.

The fertiliser consisted of dried organic manure NPK pellets (MEOC SA, 1906 Charrat, Switzerland) and mineral potassium oxide (K_2O) dissolved into water so as to reach the viscosity of standard farm slurry (Sinaj *et al.* 2009). One m^3 of this solution contained 2.4 kg of monopolizable nitrogen, 2 kg of phosphate (P_2O_5) and 8 kg of potassium oxide (K_2O). Liquid manure was applied four times during our experiment (2010-2012) (each time with half of the corresponding yearly dose), in August 2010, May 2011, August 2011 and May 2012. The exact amount of manure applied per plot depended on the theoretical local hay production potential, calculated from pre-experimental hay yield and site elevation (see Appendix S1 in Supporting Information). As a reference base, our F+I 3/3-plots would correspond to the criteria of mid-intensive management described by Sinaj *et al.* (2009), with a maximum fertilisation of 80 $kg \cdot ha^{-1} \cdot year^{-1}$ nitrogen. In other words, the chosen design created a site-adapted management intensification gradient ranging from extensive (C treatment) to mid-intensive (F+I 3/3 treatment) management (Table 2).

Auchenorrhyncha sampling

Auchenorrhyncha were collected using a suction sampler (Stihl SH 86 D; Stihl) equipped with a gauze in its nozzle to retain arthropod items (Sanders & Entling 2011). Each plot was sampled twice during the vegetation period (2012), before the two grass mowing events. At each sampling session, five subsamples were collected: four at 3 m distance from plot centre along the slope gradient, with 90° in between, and one at 6 m distance from plot centre, positioned along the slope axis (Fig. 1). Subsamples were vacuumed from the standing vegetation present within an open metallic cylinder of 50 cm height and 50.5 cm diameter that was placed on the ground (0.2 m^2 area). Sampling was conducted between 11:00 and 17:00, only under dry vegetation conditions and with low or moderate wind. Nymphs and adults were deep-frozen at -20°C before being visually identified under the microscope to the species level according to the key by Biedermann and Niedringhaus (2009). The number of individuals was counted prior to drying the material in an oven at 60°C during 72 h, which allowed estimating biomass (± 0.1 mg). For statistical analyses, all the subsamples from a plot in a given sampling session were grouped together, therefore providing information about abundance, biomass and species richness over an area of 2 m^2. Auchenorrhyncha were further categorized according to:

1) feeding guild: graminoid-feeders (including grasses, sedges and rushes in their diet) versus non-graminoid-feeders (including all vascular plants that are not graminoids, such as legumes);

2) trophic specialisation, in function of the host-plants: monophagous species (feeding on a single plant species or genus: 1st or 2nd degree monophagy); oligophagous species (feeding on one or two plant families, or exploiting less than five plant species from less than five families (Nickel & Remane 2002; Nickel & Hildebrandt 2003); and polyphagous species (foraging upon a broad spectrum of plant species and genera);

3) habitat specialisation: eurytopic species (broadly adapted species that can occur in a wide spectrum of habitats, which are usually oligophagous or polyphagous) and stenotopic species (tolerating only a small range of habitat conditions), which mainly comprise of monophagous species (Rombach 2000; Nickel 2003; Maczey 2004) (see Appendix S2).

Vegetation sampling

In each plot, a 4 x 2 m permanent subplot was established at a distance of 4 m from the plot centre, randomly placed along the axis of the meadow slope (Fig. 1). In 2012, just before the first mowing, we assessed plant species richness and coverage (%) of three different functional groups (Fabaceae, Poaceae and other families). Before the first cut (for all plots) and just before the second cut (for I, F, F+I 2/3 and F+I 3/3-plots; C and F+I 1/3 not considered as they did not undergo a second mowing), hay production was estimated by clipping two strips (1 m x 1.6 m) of grass at 6 cm above the ground (total area per plot: 3.2 m^2) at random locations at 2 m of the centre but not overlapping with the other sampling points (Fig. 1). The collected plant material was dried in an oven at 105°C during 72 h and then weighed to ± 0.1 g. Measures such as plant species richness and hay production served as co-variables in the analyses.

Statistical analysis

Treatment effects were analysed with linear mixed-effects models (LMMs) using the *lmer* function from the *lmerTest* library (Kuznetsova, Brockhoff & Christensen 2014) implemented in R 3.1.2 (R Core Team 2014). Response variables were: 1) Auchenorrhyncha abundance (log-transformed); 2) Auchenorrhyncha biomass (no transformation); 3) Auchenorrhyncha species richness (log-transformed); 4) abundance of non-graminoid- and graminoid-feeders (log-transformed + 1); 5) abundance of monophagous, oligophagous and polyphagous species (log-transformed + 1); 6) species richness of eurytopic and stenotopic species (log-transformed); 7) abundance of dominant Auchenorrhyncha species (we chose species that occurred in at least six meadows) (log-transformed + 1).

Following Johnson and Omland (2004), six candidate models were generated. A first model included only the six different treatments as fixed effect (C, F, I, or F+I 1/3, F+I 2/3 and F+I 3/3-plots), while the five other models alternately included in addtition one of the measured covariables (plant species richness; hay production; coverage of Fabaceae, Poaceae and other families, respectively) that could influence Auchenorrhyncha responses to the treatments. The model with the lowest Akaike's Information Criterion (AIC) value, considered as the best-fit modelling, was retained (Bozdogan 1987). In order to assess to which extent management treatments differed in their effects, planned orthogonal comparisons were additionally conducted by successfully removing: 1) the control (C-plots); 2) the C and F-plots; 3) the C, F and I-plots; 4) the C, F, I and F+I 1/3-plots. Models always fulfilled modelling assumptions. In particular, all dataset followed a Gaussian distribution. The study sites (twelve meadows) were designated as a random effect.

Results

Overall, 5'570 Auchenorrhyncha (3'752 adults and 1'818 nymphs) were collected: 4'552 individuals could be identified to the species (n = 73), genus (n=717), sub-family (n=244) or family (n=57) level. All identified individuals belonged to the three families Cicadellidae, Aphrophoridae or Delphacidae. The species *Philaenus spumarius* (Linnaeus, 1758) (12.4% of all individuals), *Ditropsis flavipes* (Signoret, 1865) (9.8%), *Evacanthus interruptus* (Linnaeus, 1758) (6.3%) and *Eupteryx heydenii* (Kirschbaum, 1868) (6.2%) were most abundant (see Appendix S2).

Forty-five species (2’245 individuals) were allocated to the guild of graminoid-feeders, 18 to non-graminoid-feeders (1’490 individuals) and four (783 individuals) to non-graminoid- and graminoid-feeders (Nickel 2003). In terms of trophic specialisation, 27 species (1’415 individuals) were monophagous, 30 oligophagous (1’572 individuals) and 14 polyphagous (1’563 individuals). With respect to habitat specialisation, 40 species (2’425 individuals) were classified as stenotopic and 30 as eurytopic (2’107 individuals) (for more details see Appendix S2). In any statistical analysis of all six response variables, the best-fit models always included only treatment as fixed effect, i.e. vegetation co-variables were never included in best-fit models.

Auchenorrhyncha abundance, biomass and species richness

Mean abundance and biomass were significantly higher in the three treatments combining fertilisation and irrigation than in the C-plots (see Fig. 2, Tables 3 & 4 for details). In relative terms, this represents increases in abundance and biomass of, respectively, 86% and 77% (F+I 1/3-plots), 45% and 63% (F+I 2/3-plots) and 41% and 81% (F+I 3/3-plots). In contrast, the treatments including either fertilisation or irrigation (F and I-plots) did not differ from the controls (C-plots), both for abundance and biomass. Similarly, there was not difference either in abundance or biomass among the three levels of combined fertilisation and irrigation. Beside these general patterns there were, however, further pairwise differences between treatments. As regards abundance, fertilisation alone did not differ from the combined treatment F+I 2/3 and F+I 3/3, while F+I 1/3 did. In terms of biomass, the combined treatment F+I 2/3 did not differ from irrigation treatment alone. For species richness, there were only two differences: F+I 2/3-plots harboured, on average, more species than C (increase by 27%) and F-plots (increase by 25%).

Auchenorrhyncha feeding guilds, trophic and habitat specialisation, and dominant species

The influence of experimental treatment on feeding guilds, trophic specialisation and habitat specialisation is presented in details in Appendix S3. The mean abundance of non-graminoid-feeders differed only between F+I 1/3 and I-plots (they were more numerous in the former), contrary to graminoid-feeders, which were more abundant in F+I 1/3, F+I 2/3 and F+I 3/3 than in both C and I-plots. Abundance of graminoid-feeders was also greater in F+I 1/3-plots than in F-plots.

Trophic specialisation analyses showed a greater abundance of monophagous and oligophagous species in F+I 1/3-plots compared to C, F and I-plots. F+I 2/3-plots also showed a higher abundance of monophagous species compared to I-plots, while a higher abundance of polyphagous species was observed in F+I 3/3-plots compared to C-plots.

Finally, when considering habitat specialisation, only eurytopic Auchenorrhyncha had a greater species richness in F+I 2/3 and F+I 3/3-plots compared to C and F-plots.

Regarding dominant species, only 20 matched our selection criterion (occurrence in at least six meadows) (see Appendix S4). For three species only, some combinations of fertilisation and irrigation positively affected abundance, in comparison to C-plots: *Adarrus multinotatus* (Boheman, 1847), *Agallia brachyptera* (Boheman, 1847) and *Megadelphax sordidula* (Stål, 1853) (see Appendix S5).

Discussion

This research establishes that a moderate fertilisation (slurry) and irrigation (sprinklers) of traditional, very low-input montane and subalpine hay meadows is beneficial, at least in the very short term (2 years), for the abundance, biomass and species richness of Auchenorrhyncha. Most previous investigations of the impact of fertilisation and irrigation on the biodiversity of mountain hay meadows have been observational (e.g. Grandchamp *et al.* 2005; Riedener, Rusterholz & Baur 2013). A first originality of the present study thus resides in the reliance upon a fully randomized block design approach, which minimizes any biases due to possible confounding factors, thereby providing conclusive evidence. Second, we applied different doses of fertiliser and water, whose quantities were adjusted to the potential productivity of a given study site so as to mimic real farming practices. This means that the conclusions drawn from this study can provide concrete guidance for sustainable, i.e. more biodiversity-friendly, management of mountain hay meadows.

Auchenorrhyncha abundance, biomass and species richness

Auchenorrhyncha abundance and biomass were significantly increased in plots combining fertilisation and irrigation (+41%-86%, depending on F+I level), whereas mean species richness was higher (+27%) only in plots with a medium level of intensification. In comparison, fertilisation and irrigation alone had no effect on either of the three above metrics.

Based on previous studies (Prestidge 1982; Sedlacek, Barrett & Shaw 1988), we expected that fertiliser application alone would have a positive effect on Auchenorrhyncha. Fertilisation is likely to boost the nutritional quality of plants, increasing the survival or the reproductive performance of Auchenorrhyncha, or overall plant biomass, theoretically providing them with a greater diversity of feeding, oviposition and refuge opportunities (Sedlacek, Barrett & Shaw 1988). However, the absence of such an effect in our experiment rather indicates that fertilisation alone is not sufficient to induce these benefits for Auchenorrhyncha. Interestingly, Körösi *et al.* (2012) claimed that vegetation structural complexity is the main determinant of Auchenorrhyncha communities because it offers a greater palette of micro-climates (Whittaker 1963). This result (drawn from the same experimental set up as here) would indeed be in line with our finding that fertilisation does not enhance vegetation structure, at least in the short term (Andrey *et al.* 2014). However, vegetation structure is unlikely to be the only factor influencing Auchenorrhyncha. As a matter of fact, even irrigation, which alone has demonstrated to increase vegetation structure (Andrey *et al.* 2014), did not promote Auchenorrhyncha in this study. In conclusion, the fact that fertilisation and irrigation in isolation did not have any effect on Auchenorrhyncha, whereas their combination did, confirms that it is the interaction between these two factors which, via agricultural intensification, induces changes on grassland biodiversity (see also Gaujour *et al.* 2012; Riedener, Rusterholz & Baur 2013; Andrey *et al.* 2014).

Auchenorrhyncha feeding guilds, trophic and habitat specialisation, and dominant species

The species collected belonged mainly to the guild of graminoid-feeders that were ca 1.5 times more abundant than the non-graminoid-feeders. In exact opposition to our prediction, we only observed an effect of the experimental treatments on the former guild: graminoid-feeders were +61%-135% (depending on F+I level) more abundant in plots combining fertilisation and irrigation. This is all the more intriguing since grasses in our experiment were generally negatively affected by slurry application, contrary to legumes – which belong to forbs – that normally greatly benefit from it (Andrey *et al.*, 2014; see also Carlen, Gex & Rölliker 1998). Auchenorrhyncha have been described as being especially sensitive to the nitrogen content of their food sources, rendering them exceptional bioindicators. Their reproductive output, for instance, is often much increased around a rather narrow, optimal nitrogen content of the host plants (Prestidge 1982; Prestidge & McNeill 1983). The distinct responses of our two foraging guilds would thus indirectly support the view that farming intensification affects the nitrogen content of grasses and forbs in different ways: Turner and Knapp (1996) found that under similar levels of nitrogen addition, the nitrogen concentration in forb tissues is, on average, higher than in grasses. However, the observation that number of graminoid-feeders in our experiment was increased by intensification while non-graminoid-feeders showed no response at all remains puzzling. As graminoid-feeders were more abundant under all combinations of fertilisation and irrigation than under irrigation alone (note that a similar pattern occurs in non-graminoid-feeders for F+I 1/3-plots versus I-plots), while their abundance did not differ mostly from F-plots (but see F+I 1/3), we conclude that fertiliser input is the ultimate factor at play. Among our 27 monophagous, trophic specialists, a majority of Auchenorrhyncha species are typically associated with common host plants. In our plots, *Festuca rubra* aggregate, for instance, hosted *Acanthodelphax spinosa* (Fieber, 1866), *Dicranotropis divergens* (Kirschbaum, 1868) and *Rhopalopyx adumbrata* (Sahlberg, 1842). This plant has a broad ecological tolerance, frequently occurring in intensively managed grasslands (Pavlů *et al.* 2012). As *F. rubra* aggregate, most plant species hosting monophagous species did actually not disappear from plots subjected to increased fertilisation and irrigation. In contrast, only three species of Auchenorrhyncha exhibited very narrow trophic niches: *Batracomorphus irrotatus* (Lewis, 1834) (43 individuals, host plant: *Helianthemum nummularium* (L.) Miller *sensu lato* (1768), *Eupelix cuspidata* (Fabricius, 1775) (one individual, host plant: *Festuca ovina* aggregate) and *Kelisia haupti* (Wagner, 1939) (one individual, host plant: *Carex humilis* Leyss.) (Lauber *et al.* 2001). The observation that both monophagous and oligophagous species' abundance was greater where intensification was shallow (F+I 1/3) compared to control plots and to plots submitted to fertilisation and irrigation in isolation, and that it also tended to be greater at F+I 1/3 than in the more intensive treatments, might be indicative of an optimal trade-off between host plant diversity and plant nutritional quality. Oligophagous Auchenorrhyncha, in contrast, tended to be more abundant at higher levels of intensification, which supports the view that they benefitted more from an increase in overall phytomass. Yet, the short duration of our study (two years) and the relatively moderate quantities of fertiliser and water applied, even at the higher intensity levels, may have enabled nitrophilous, but also more demanding host-plant specialists' cohabitation, as a result of the availability of a broad palette of trophic niches.

Overall, habitat specialists, i.e. stenotopic Auchenorrhyncha (44% of total abundance), were slightly more abundant than eurytopic species (38%; while 18% of species remained undefined). The progressive increase of eurytopic species richness with increased fertilisation (+38% and +44% in F+I 2/3 and F+I 3/3-plots, respectively, compared to controls) is in line with the observation that stenotopic Auchenorrhyncha usually predominate among low-input mountain meadows (Nickel & Achtziger 2005). As our experiment progresses (ongoing) we expect eurytopic species to further increase in more intensified plots in the near future (Nickel 2003; Nickel & Hildebrandt 2003; Nickel & Achtziger 2005).

Regarding dominant Auchenorrhyncha species, fertilisation and irrigation increased the abundance of *A. multinotatus* (Boheman, 1847) and *M. sordidula* (Stål, 1853). This was expected as both species tightly depend on *Brachypodium pinnatum* and *Arrhenaterum elatius* (L.) P.Beauv. ex J. Presl & C. Presl, (1819), respectively, two grasses known to profit from fertilisation (Bobbink, Bik & Willems 1988; Liancourt, Viard-Crétat & Michalet 2009). The abundance of *Agallia brachyptera* (Boheman, 1847), a ground living Auchenorrhyncha of the litter layer (Andrzejewska 1965) that requires open conditions (Kirby 1992) and plays the role of an umbrella species (Maczey 2004), was promoted by shallow intensity treatment conditions (F+I 1/3), whereas it did less well in F+I 2/3-plots.

Finally, some remarkable species were collected during this study. *Arboridia simillima* (Wagner, 1939), *Bobacella corvina* (Horvath, 1903) and *Athysanus quadrum* (Boheman, 1845) were recorded for the first time in Switzerland, while

Emeljanovianus medius (Mulsant & Rey, 1855) was collected for the second time (Trivellone *et al.* 2015). Moreover, four species that are considered to be of conservation concern in Germany were collected (there is no Swiss red list for Auchenorrhyncha): *A. simillima* (Wagner, 1939), *A. quadrum, D. flavipes* (Signoret, 1865) (all endangered), and *E. heydenii* (Kirschbaum, 1868) *(*vulnerable) (Nickel, Witsack & Remane 1999).

Conclusions and management recommendations

Shallow fertilisation with slurry and irrigation with sprinklers promptly enhanced conditions for Auchenhorryncha in very low-input mountain hay meadows in the short term, notably by increasing the abundance of monophagous and oligophagous species and, possibly, the richness of stenotopic species. A greater application of fertiliser and water still enhanced abundance and species richness of Auchenorrhyncha, but exclusively through an increase of trophic and habitat generalists. We predict that prolonging applied experimental treatments in our field experiment is likely to negatively affect plant species richness due to the continuous addition of fertiliser (Humbert *et al.* 2015). This should irremediably lead to an impoverishment of the palette of host-plants for Auchenorrhyncha, which will in turn affect monophagous and stenotopic species.

In many mountainous regions of the European Alps, the management of hay meadows on flat and accessible terrain is currently undergoing massive intensification, whereas meadows on steep, less accessible slopes are progressively being abandoned (Tasser & Tappeiner 2002). This dichotomous, negative trend should be reversed. Based on this research on a key indicator taxon of mountain biocenoses, we can already recommend applying doses of fertiliser and water roughly equivalent to one third of the amount that would be necessary to achieve the maximum theoretical local hay yield (our figures for F+I 1/3 can serve as reference). Future results of our ongoing experiment will certainly help fine-tune these prescriptions. Finally, studies should also investigate to which extent organisms situated higher up along the food chain (predatory arthropods, vertebrates) might benefit from the management recommendations that promote emblematic taxa such as Auchenorrhyncha, especially given that they constitute a staple food for higher trophic levels, all the way up to vertebrates.

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Table 1: Description of the twelve study sites with altitude and exact geographical coordinates.

Sites	Name	Altitude [m]	Coordinates	
			Latitude	Longitude
1	Sembrancher	798	46°4′24″N	7°8′36″E
2	Orsières	1022	46°1′44″N	7°9′8″E
3	Euseigne	1028	46°10′9″N	7°25′27″E
4	Icogne 2	880	46°16′42″N	7°26′10″E
5	La Garde	980	46°3′45″N	7°8′35″E
6	Vens	1373	46°5′7″N	7°7′24″E
7	Arbaz	1270	46°16′42″N	7°22′47″E
8	Icogne 1	1200	46°17′56″N	7°26′31″E
9	Cordona	1153	46°19′45″N	7°33′8″E
10	Eison	1768	46°9′18″N	7°28′10″E
11	Saint-Martin	1589	46°11′8″N	7°26′43″E
12	Grimentz	1738	46°11′22″N	7°34′35″E

Table 2: Management intensities applied on each study site (see Table 1 for a list of sites) according to initial conditions. Abbreviations for experimental treatments: C = control; F = fertilised; I = irrigated; F+I 1/3, F+I 2/3 and F+I 3/3 = fertilised and irrigated at respectively 1/3, 2/3 or 3/3 of the dose that would be necessary to achieve the maximum theoretical local hay yield. For each treatment indications are provided for: quantity of nitrogen (N), phosphorus (P) and potassium (K) fertiliser applied per hectare and year; water column applied via sprinkler irrigation per week; and number of grass cuts per year. The fertiliser consisted of organic NPK pellets and mineral K_2O dissolved in water to reach the equivalent of standard-farm liquid manure. Cuts were done at 6 cm above the ground.

Sites 1 to 4

Treatments	Fertiliser applied [kg·ha^{-1}·year^{-1}]			Water irrigation [mm/week]	Number of cuts per year
	N	P	K		
C	0	0	0	0	1
F	53.3	44.4	177.7	0	2
I	0	0	0	20	2
F+I 1/3	26.7	22.2	88.9	10	1
F+I 2/3	53.3	44.4	177.7	20	2
F+I 3/3	80	66.6	266.6	30	2

Sites 5 to 9

Treatments	Fertiliser applied [kg·ha^{-1}·year^{-1}]			Water irrigation [mm/week]	Number of cuts per year
	N	P	K		
C	0	0	0	0	1
F	40	33.3	133.3	0	2
I	0	0	0	20	2
F+I 1/3	20	16.7	66.7	10	1
F+I 2/3	40	33.3	133.3	20	2
F+I 3/3	60	50	200	30	2

Sites 10 to 12

Treatments	Fertiliser applied [kg·ha^{-1}·year^{-1}]			Water irrigation [mm/week]	Number of cuts per year
	N	P	K		
C	0	0	0	0	1
F	26.7	22.3	89	0	2
I	0	0	0	20	2
F+I 1/3	13.4	11.2	44.5	10	1
F+I 2/3	26.7	22.3	89	20	2
F+I 3/3	40.2	33.5	133.5	30	2

Table 3: Outputs of the linear mixed-effects models used to measure the impact of experimental treatments on Auchenorrhyncha abundance, biomass and species richness. For treatment abbreviations, see legend of Table 2. Estimate, standard error (SE), t-value and *P*-value (*P*). Significant *P*-values are highlighted in bold. The fixed factor is the management treatment while the random factor is the study site.

Auchenorrhyncha	**Abundance (log scale)**				**Biomass**				**Species richness (log scale)**			
	Estimate	SE	t-value	*P*	Estimate	SE	t-value	*P*	Estimate	SE	t-value	*P*
F vs C	0.193	0.185	1.046	0.300	0.017	0.016	1.052	0.297	-0.005	0.127	-0.037	0.971
I vs C	0.039	0.185	0.213	0.832	0.027	0.016	1.629	0.109	0.020	0.127	0.158	0.875
F+I 1/3 vs C	0.683	0.185	3.694	**0.001**	0.056	0.016	3.409	**0.001**	0.242	0.127	1.903	0.062
F+I 2/3 vs C	0.454	0.185	2.455	**0.017**	0.046	0.016	2.802	**0.007**	0.282	0.127	2.219	**0.031**
F+I 3/3 vs C	0.520	0.185	2.815	**0.007**	0.059	0.016	3.585	**0.001**	0.237	0.127	1.865	0.068
I vs F	-0.154	0.192	-0.801	0.428	0.009	0.018	0.533	0.597	0.025	0.129	0.192	0.849
F+I 1/3 vs F	0.490	0.192	2.546	**0.015**	0.039	0.018	2.176	**0.035**	0.246	0.129	1.910	0.063
F+I 2/3 vs F	0.260	0.192	1.355	0.182	0.029	0.018	1.616	0.113	0.286	0.129	2.221	**0.032**
F+I 3/3 vs F	0.327	0.192	1.701	0.096	0.042	0.018	2.339	**0.024**	0.242	0.129	1.873	0.068
F+I 1/3 vs I	0.643	0.203	3.170	**0.003**	0.029	0.020	1.497	0.144	0.222	0.139	1.600	0.119
F+I 2/3 vs I	0.414	0.203	2.042	**0.049**	0.019	0.020	0.987	0.331	0.262	0.139	1.889	0.068
F+I 3/3 vs I	0.481	0.203	2.370	**0.024**	0.032	0.020	1.645	0.109	0.217	0.139	1.565	0.127
F+I 2/3 vs F+I 1/3	-0.229	0.144	-1.595	0.125	-0.010	0.020	-0.496	0.625	0.040	0.111	0.362	0.721
F+I 3/3 vs F+I 1/3	-0.162	0.144	-1.131	0.270	0.003	0.020	0.144	0.887	-0.005	0.111	-0.044	0.966
F+I 3/3 vs F+I 2/3	0.067	0.133	0.500	0.627	0.013	0.015	0.873	0.401	-0.045	0.102	-0.440	0.664

Table 4: Summary of the effects of management treatments on abundance, biomass and species richness of Auchenorrhyncha. Mean and Standard deviation for each treatments are given. For treatment abbreviations, see legend of Table 2.

	Abundance of Auchenorrhyncha		Biomass of Auchenorrhyncha		Species richness of Auchenorrhyncha	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
C-plots	59.17	53.70	0.07	0.07	9.42	3.20
F-plots	64.50	45.84	0.09	0.07	9.58	3.55
I-plots	61.50	47.72	0.10	0.09	10.08	3.94
F+I 1/3-plots	110.00	77.36	0.13	0.09	12.25	4.16
F+I 2/3-plots	85.50	64.37	0.12	0.08	12.00	1.54
F+I 3/3-plots	83.50	45.91	0.13	0.09	11.92	3.58

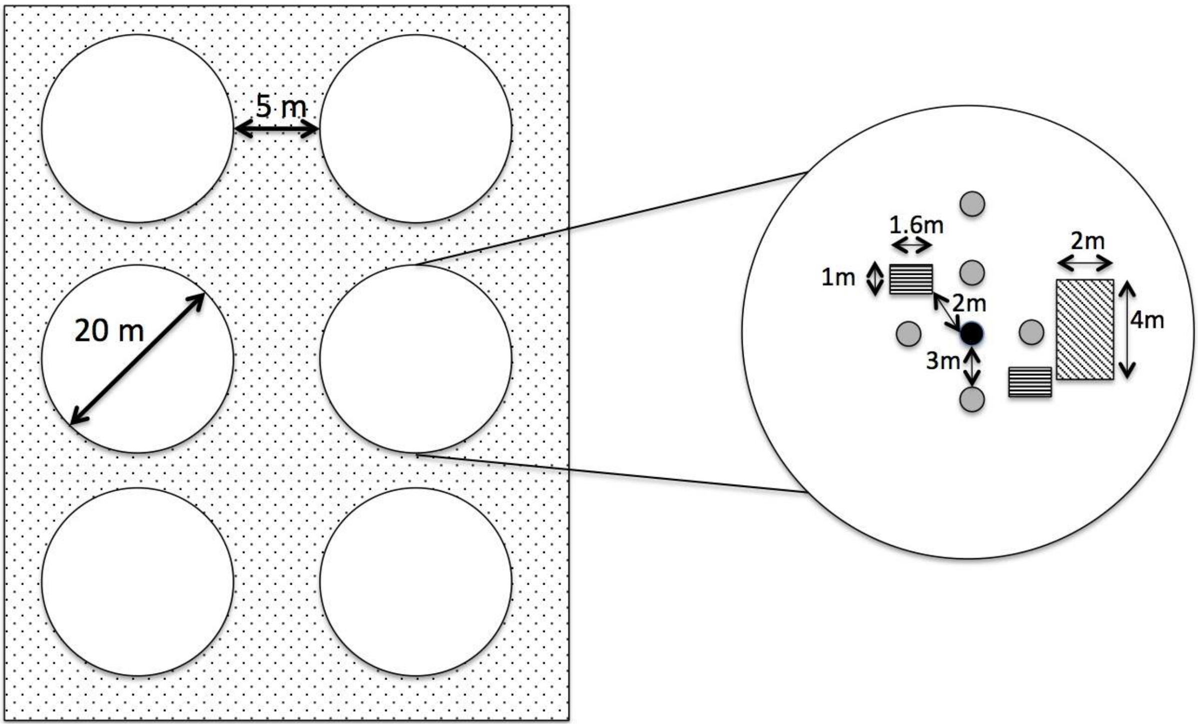


Figure 1. Experimental design. The six management treatments were applied randomly to six 20 m-diameter plots delineated within each meadow, with a 5 m buffer zone between the plots. In each plot, we sampled plant species richness and coverage of each functional group, i.e. Fabaceae, Poaceae and other plants (within a subplot of 4 x 2 m), hay yield (grass cut along two horizontal strips of 1 x 1.6 m) and Auchenorrhyncha (five grey circular sub-samples of 0.2 m^2).

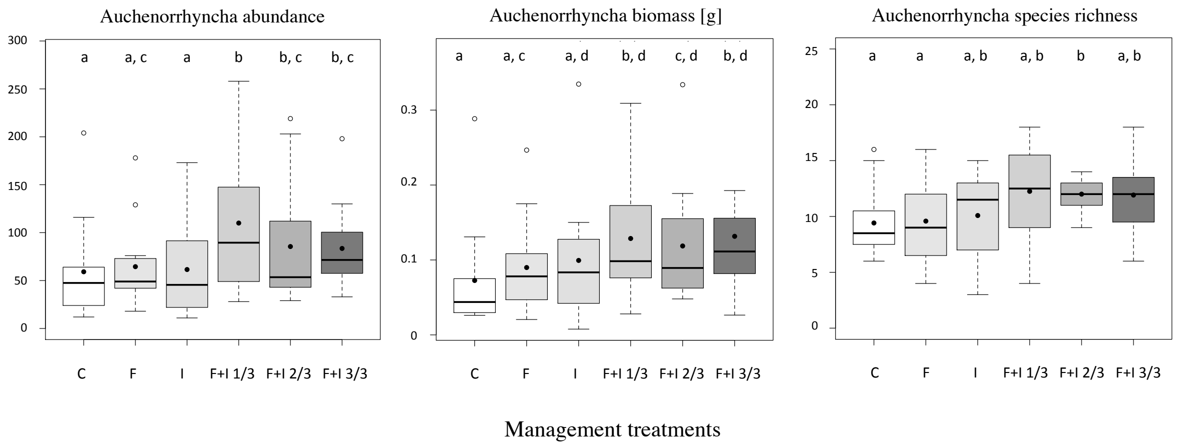


Figure 2. Auchenorrhyncha abundance, biomass and species richness in response to the different experimental treatments. Bold lines represent medians, solid points means, boxes the first and third quantiles, open circles outliers. Different letters indicate significant differences among treatments at an alpha rejection level of 0.05. For treatment abbreviations, see legend of Table 2. For statistical analyses see Table 3.

Supporting Information

Appendix S1. Quantity of liquid manure applied to F+I 3/3-plots to achieve the maximum theoretical local hay yield potential (corresponding to a medium intensity management of hay meadows). The pre-experimental hay yield of extensively managed meadows was measured in 2010 when meadows were still extensively managed. According to the management intensity applied to selected F+I 3/3-plots (mid-intensive) and the altitude of each meadow, the maximum potential increase of the hay yield was defined (according to Sinaj et al., 2009) and served to calculated the pre-experimental hay yield under a mid-intensive management. Maximum theoretical local hay production potential and standards for corrected liquid manure quantities were defined according to Sinaj et al. (2009) so as to achieve a maximal quantity of liquid manure application to F+I 3/3-plots.

	Sites											
	1	2	3	4	5	6	7	8	9	10	11	12
Pre-experimental hay yield of extensively managed meadows [dt MS/ha]	24	32	32	20	12	27	22	30	21	19	23	18
Site elevation [m]	798	1022	1028	880	980	1373	1270	1200	1153	1768	1589	1738
Management intensity considered (for F+I 3/3-plots)*	mid	mid	mid	mid	mid	mid	mid	mid	mid	mid	mid	mid
Maximum potential increase of the hay yield in function of management intensity and site elevation*	x 2-3	x 2-3	x 2-3	x 2-3	x 2	x 2	x 2	X 2	x 2	x 1-2	x 1-2	x 1-2
Pre-experimental hay yield calculated (mid-intensity management) [dt MS/ha]	48-72	64-96	64-96	40-60	24	54	44	60	42	38	46	36
Maximum theoretical local hay production potential [dt MS/ha]*	75	75	75	75	50	50	50	50	50	35	35	35
Standards of corrected liquid manure [kg Nitrogen/ha]*	60-80	60-80	60-80	60-80	40-60	40-60	40-60	40-60	40-60	30-40	30-40	30-40
Maximal quantity of liquid manure applied under mid-intensity management [kg nitrogen/ha] (for F+I 3/3-plots)	80	80	80	80	60	60	60	60	60	40	40	40

* according to Sinaj et al. (2009)

Appendix S2. Alphabetical list of all Auchenorrhyncha and determined according to their respective species, genus, sub-family or family levels. Feeding guilds refer to non-graminoid-feeders (non-graminoid), graminoid-feeders (graminoid), graminoid- and non-graminoid-feeders (graminoid and non-graminoid), and shrub- and tree-feeders (tree). Trophic specialisation for Auchenorrhyncha was defined as: 1) monophagous, feeding on a single plant species or plant genus; 2) oligophagous, feeding on one or two plant families or eat less than five species of lesser plant families; and 3) polyphagous, feeding on a broad spectrum of plants species and genus (based on Nickel 2003). Habitat specialisation information was drawn from diverse authors (Maczey, 2004; Nickel, 2003; Rombach, 2000) and consists of two types of species: stenotopic, who are able to tolerate only a small range of habitat conditions; and eurytopic, who are adapted to a variety of habitats. When a species could not be categorized due to lack of knowledge of its ecological requirements, “Na” (for “not applicable”) is indicated. The number of Auchenorrhyncha found per management treatment is also given. Treatment abbreviations: C = control; F = fertilised; I = irrigated; F+I 1/3, F+I 2/3 and F+I 3/3 = fertilised and irrigated at, respectively, 1/3, 2/3 or 3/3 of the dose that would be necessary to achieve the maximum theoretical local hay yield.

Determined at species level	Feeding guilds	Trophic specialisation	Habitat specialisation	Family	C	F	I	F+I 1/3	F+I 2/3	F+I 3/3
Acanthodelphax spinosa (Fieb.)	graminoid	monophagous	stenotopic	Delphacidae	0	1	0	1	2	2
Adarrus multinotatus (Boh.)	graminoid	monophagous	stenotopic	Cicadellidae	0	5	0	7	1	2
Agallia brachyptera (Boh.)	non-graminoid	oligophagous	stenotopic	Cicadellidae	18	46	36	53	25	30
Allygus mixtus (F.)	graminoid	polyphagous	eurytopic	Cicadellidae	0	0	1	0	0	1
Anaceratagallia ribauti (Oss.)	non-graminoid	oligophagous	stenotopic	Cicadellidae	17	11	5	52	22	6
Anakelisia perspicillata (Boh.)	graminoid	monophagous	stenotopic	Delphacidae	6	0	0	2	0	0
Anoscopus albifrons (L.)	graminoid	oligophagous	stenotopic	Cicadellidae	0	4	0	1	3	31
Anoscopus_serratulae (Fall.)	graminoid	oligophagous	eurytopic	Cicadellidae	1	5	1	2	5	4
Aphrodes bicinctus (Schrk.)	non-graminoid	oligophagous	stenotopic	Cicadellidae	3	7	21	22	8	18
Aphrophora alni (Fall.)	tree	polyphagous	eurytopic	Aphrophoridae	2	2	5	2	2	5
Arboridia simillima (Wagner)	non-graminoid	monophagous	stenotopic	Cicadellidae	0	0	0	1	0	0
Arocephalus languidus (Flor.)	graminoid	oligophagous	Na	Cicadellidae	2	4	1	4	4	3
Arthaldeus pascuellus (Fall.)	graminoid	oligophagous	eurytopic	Cicadellidae	0	0	0	0	1	0
Athysanus argentarius (Metc.)	graminoid	polyphagous	eurytopic	Cicadellidae	1	0	0	0	0	0
Athysanus quadrum (Boh.)	non-graminoid	monophagous	stenotopic	Cicadellidae	3	0	0	5	0	2
Batracomorphus irroratus (Lew.)	non-graminoid	monophagous	stenotopic	Cicadellidae	15	25	3	0	0	0
Bobacella corvina (Horv.)	Na	Na	eurytopic	Cicadellidae	0	0	1	0	0	0

Cicadula persimilis (Edw.)	graminoid	monophagous	stenotopic	Cicadellidae	1	3	0	8	4	5
Criomorphus albomarginatus (Curt.)	graminoid	oligophagous	stenotopic	Delphacidae	1	2	0	2	0	2
Deltocephalus pulicaris (Fall.)	graminoid	oligophagous	eurytopic	Cicadellidae	0	0	0	0	0	3
Dicranotropis divergens (Kirsch.)	graminoid	monophagous	stenotopic	Delphacidae	2	11	4	4	0	1
Dicranotropis hamata (Boh.)	graminoid	oligophagous	eurytopic	Delphacidae	0	4	6	12	6	10
Ditropsis flavipes (Sign.)	graminoid	monophagous	stenotopic	Delphacidae	36	20	48	184	170	86
Doratura stylata (Boh.)	graminoid	oligophagous	eurytopic	Cicadellidae	2	2	3	2	6	2
Elymana sulphurella (Zett.)	graminoid	oligophagous	eurytopic	Cicadellidae	9	1	2	0	3	1
Emeljanovianus medius (Mul.)	Na	Na	Na	Cicadellidae	0	0	0	0	1	0
Emelyanoviana mollicula (Boh.)	non-graminoid	polyphagous	eurytopic	Cicadellidae	26	29	9	12	14	21
Eupelix cuspidata (Fall.)	graminoid	monophagous	stenotopic	Cicadellidae	1	0	0	0	0	0
Eupteryx heydenii (Kirsch.)	non-graminoid	monophagous	stenotopic	Cicadellidae	49	20	19	88	119	48
Eupteryx collina (Flor)	non-graminoid	monophagous	stenotopic	Cicadellidae	2	0	0	0	0	0
Eupteryx notata (Curt.)	non-graminoid	oligophagous	stenotopic	Cicadellidae	29	24	4	17	4	5
Eupteryx origani (Zachv.)	non-graminoid	monophagous	stenotopic	Cicadellidae	0	1	2	3	2	3
Eupteryx atrapunctata (Goez.)	non-graminoid	polyphagous	eurytopic	Cicadellidae	2	4	1	0	1	3
Eurysa lineata (Perr.)	graminoid	oligophagous	Na	Delphacidae	0	0	0	0	1	0
Euscelis incisus (Kirsch.)	graminoid and non-graminoid	oligophagous	eurytopic	Cicadellidae	4	11	3	8	12	5
Euscelis venosus (Kbm.)	non-graminoid	monophagous	stenotopic	Cicadellidae	0	1	0	0	2	0
Evacanthus interruptus (L.)	non-graminoid	polyphagous	eurytopic	Cicadellidae	63	32	65	68	52	70
Forcipata citrinella (Zett.)	graminoid	monophagous	stenotopic	Cicadellidae	3	0	0	12	5	2
Graphocraerus ventralis (Fall.)	graminoid	oligophagous	stenotopic	Cicadellidae	6	10	11	12	14	6
Hardya Tenuis (Ger.)	graminoid	oligophagous	stenotopic	Cicadellidae	9	8	1	8	3	7
Hesium domino (Reu.)	tree	oligophagous	eurytopic	Cicadellidae	0	2	0	0	0	0
Idiodonus cruentatus (Pan.)	tree	polyphagous	eurytopic	Cicadellidae	4	3	1	1	2	0
Javesella pellucida (F.)	graminoid	polyphagous	eurytopic	Delphacidae	0	1	0	14	5	9
Kelisia haupti (Wag.)	graminoid	monophagous	stenotopic	Delphacidae	0	0	1	0	0	0
Kelisia monoceros (Rib.)	graminoid	monophagous	stenotopic	Delphacidae	0	0	0	1	0	0
Leypyronia coleoptrata (L.)	graminoid and non-graminoid	polyphagous	eurytopic	Aphrophoridae	6	5	8	13	8	7
Macrosteles cristatus (Rib.)	graminoid	polyphagous	eurytopic	Cicadellidae	0	0	0	0	0	1
Macrosteles laevis (Rib.)	graminoid and non-graminoid	polyphagous	eurytopic	Cicadellidae	0	1	1	0	1	2
Macustus grisescens (Zett.)	graminoid	polyphagous	eurytopic	Cicadellidae	0	3	2	5	1	0
Megadelphax sordidula (Stål)	graminoid	monophagous	stenotopic	Delphacidae	4	39	29	98	47	55
Megophthalmus scanicus (Fall.)	non-graminoid	oligophagous	eurytopic	Cicadellidae	1	9	10	18	15	16
Mocydia crocea (H.-S.)	graminoid	oligophagous	eurytopic	Cicadellidae	2	0	1	0	0	4
Neophilaenus albipennis (F.)	graminoid	monophagous	stenotopic	Aphrophoridae	1	0	1	0	0	0
Neophilaenus campestris (Fall.)	graminoid	oligophagous	eurytopic	Aphrophoridae	0	0	0	0	0	1
Oncopsis flavicollis (L.)	tree	monophagous	stenotopic	Cicadellidae	0	0	0	0	0	1
Philaenus spumarius (L.)	graminoid and non-graminoid	polyphagous	eurytopic	Aphrophoridae	94	96	122	127	121	128
Planaphrodes bifaciata (L.)	graminoid	polyphagous	eurytopic	Cicadellidae	40	65	49	45	51	28
Psammotettix helvolus (Kbm.)	graminoid	oligophagous	stenotopic	Cicadellidae	8	6	7	9	13	11
Recilia coronifera (Mar.)	graminoid	oligophagous	eurytopic	Cicadellidae	0	0	1	0	0	1
Rhopalopyx adumbrata (C. Shlb.)	graminoid	monophagous	stenotopic	Cicadellidae	2	0	0	0	0	0
Ribautodelphax albostriata (Fieb.)	graminoid	monophagous	stenotopic	Delphacidae	0	0	3	10	7	1
Ribautodephax angulosus (Rib.)	graminoid	monophagous	stenotopic	Delphacidae	1	1	0	0	0	0
Sonronius dahlboni (Zett.)	non-graminoid	monophagous	stenotopic	Cicadellidae	0	0	1	0	2	2

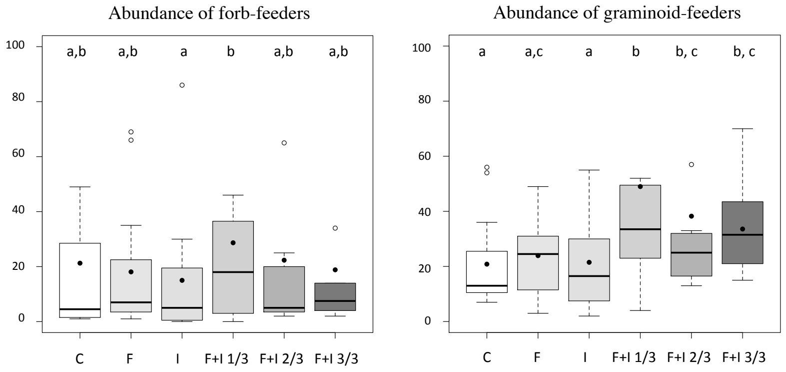
Stenocranus minutus (F.)	graminoid	monophagous	stenotopic	Delphacidae	1	0	1	2	4	2
Stictocoris picturatus (Sah.)	non-graminoid	oligophagous	stenotopic	Cicadellidae	0	1	0	1	0	2
Stiroma affinis (F.)	graminoid	oligophagous	stenotopic	Delphacidae	4	23	8	26	37	19
Streptanus aemulans (Kirsch.)	graminoid	oligophagous	eurytopic	Cicadellidae	3	6	1	0	3	3
Turrutus socialis (Fl.)	graminoid	oligophagous	stenotopic	Cicadellidae	43	21	22	34	33	57
Utecha trivia (Ger.)	non-graminoid	monophagous	stenotopic	Cicadellidae	27	7	4	4	2	0
Verdanus abdominalis (F.)	graminoid	oligophagous	eurytopic	Cicadellidae	61	42	53	80	29	43
Xantodelphax straminea (Stål)	graminoid	monophagous	stenotopic	Delphacidae	0	0	0	2	0	0
Zyginidia mocsaryi H.	graminoid	oligophagous	stenotopic	Delphacidae	0	0	0	0	1	0
Zyginidia scutellaris (H.-S.)	graminoid	oligophagous	eurytopic	Cicadellidae	0	0	1	1	0	0
Determined at genus level										
Anoscopus sp.	Na	Na	Na	Cicadellidae	15	39	45	88	54	74
Aphrodes sp.	Na	Na	Na	Cicadellidae	28	48	23	66	53	61
Diplocolenus sp.	Na	Na	Na	Cicadellidae	2	1	5	0	1	1
Eupteryx sp.	Na	Na	Na	Cicadellidae	0	0	1	0	3	0
Jasarrgus sp.	Na	Na	Na	Cicadellidae	0	1	0	0	0	0
Javesella sp.	Na	Na	Na	Delphacidae	0	0	0	1	1	6
Macrosteles sp.	Na	Na	Na	Cicadellidae	0	0	1	0	1	0
Muellerianella sp.	Na	Na	Na	Delphacidae	1	4	2	5	2	7
Planaphrodes sp.	Na	Na	Na	Cicadellidae	5	3	1	3	4	6
Psammotettix sp.	Na	Na	Na	Cicadellidae	6	5	10	5	9	9
Ribautodelphax sp.	Na	Na	Na	Delphacidae	1	0	0	3	1	1
Streptanus sp.	Na	Na	Na	Cicadellidae	0	0	3	0	1	1
Determined at sub-family level										
Typhlocybinae sp.	Na	Na	Na	Cicadellidae	0	1	0	0	0	0
Aphrodinae sp.	Na	Na	Na	Cicadellidae	3	6	17	6	6	2
Deltocephalinae sp.	Na	Na	Na	Cicadellidae	28	38	41	44	11	41
Determined at family level										
Delphacidae sp.	Na	Na	Na	Delphacidae	6	4	10	16	5	16

Appendix S3.

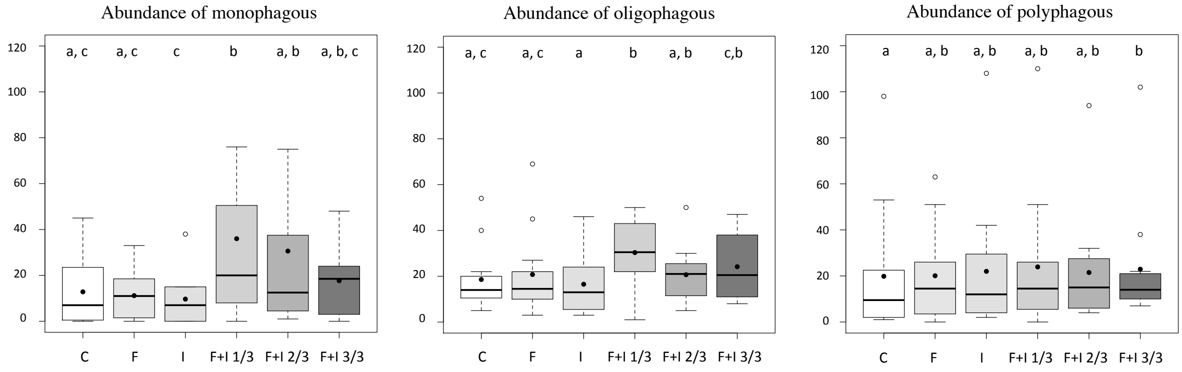
Table S3. Outputs of the linear mixed-effects models carried out on the effects of management treatment on feeding guilds (abundance of non-graminoid-feeders and graminoid-feeders), trophic specialisation (abundance of monophagous, oligophagous and polyphagous species) and habitat specialisation (species richness for stenotopic and eurytopic Auchenorrhyncha). Treatment abbreviations are as follows: C = control; F = fertilised; I = irrigated; F+I 1/3, F+I 2/3 and F+I 3/3 = fertilised and irrigated at, respectively, 1/3, 2/3 or 3/3 of the dose that would be necessary to achieve the maximum theoretical local hay yield. Estimate, standard error (SE), t-value and *P*-value (*P*) are given. The fixed factor was the management treatment. The random factor was study site. Significant *P*-values are highlighted in bold.

Auchenorrhynch	**non-graminoid-feeders abundance (log + 1 scale)**				**graminoid-feeders abundance (log + 1 scale)**			
	Estimate	SE	t-value	*P*	Estimate	SE	t-value	*P*
F vs C	0.171	0.296	0.577	0.566	0.162	0.227	0.712	0.479
I vs C	-0.290	0.296	-0.978	0.332	-0.057	0.227	-0.252	0.802
F+I 1/3 vs C	0.378	0.296	1.275	0.208	0.692	0.227	3.047	**0.004**
F+I 2/3 vs C	0.237	0.296	0.800	0.427	0.506	0.227	2.230	**0.03**
F+I 3/3 vs C	0.187	0.296	0.631	0.531	0.598	0.227	2.636	**0.011**
I vs F	-0.461	0.297	-1.553	0.128	-0.219	0.232	-0.945	0.35
F+I 1/3 vs F	0.207	0.297	0.696	0.49	0.530	0.232	2.288	**0.027**
F+I 2/3 vs F	0.066	0.297	0.222	0.825	0.345	0.232	1.488	0.144
F+I 3/3 vs F	0.016	0.297	0.054	0.958	0.437	0.232	1.885	0.066
F+I 1/3 vs I	0.667	0.297	2.246	**0.032**	0.749	0.231	3.243	**0.003**
F+I 2/3 vs I	0.527	0.297	1.773	0.085	0.564	0.231	2.440	**0.02**
F+I 3/3 vs I	0.477	0.297	1.605	0.118	0.656	0.231	2.839	**0.008**
F+I 2/3 vs F+I 1/3	-0.141	0.318	-0.442	0.662	-0.185	0.165	-1.122	0.274
F+I 3/3 vs F+I 1/3	-0.191	0.318	-0.600	0.555	-0.093	0.165	-0.566	0.577
F+I 3/3 vs F+I 2/3	-0.050	0.247	-0.203	0.843	0.092	0.151	0.609	0.555

(a) Feeding guilds



(b) Tropic specialisation



(c) Habitat specialisation

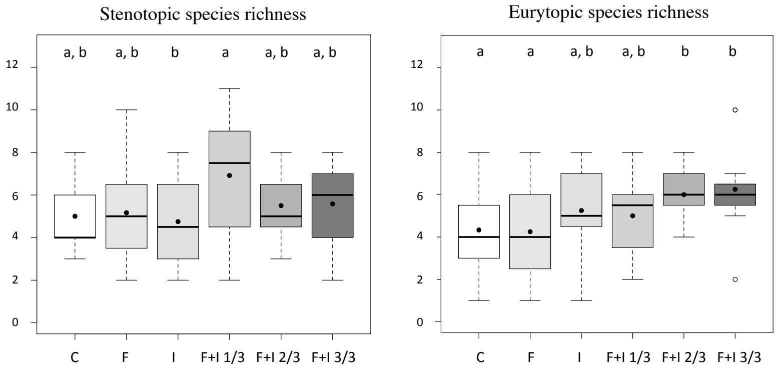


Figure S3. Graphical responses of Auchenorrhyncha to different intensification treatments with regard to: (a) feeding guilds, and (b) trophic or (c) habitat specialisation. Bold lines represent medians, solid points the means, boxes the first and third quantiles, open circles the outliers. Different letters indicate significant differences among treatments at an alpha rejection level of 0.05. Treatment abbreviations: C = control; F = fertilised; I = irrigated; F+I 1/3, F+I 2/3 and F+I 3/3 = fertilised and irrigated at, respectively, 1/3, 2/3 or 3/3 of the dose that would be necessary to achieve the maximum theoretical local hay yield.

Appendix S4. Dominant species of Auchenorrhyncha (occurring in ≥ 6 meadows) listed in alphabetical order, with indications on total abundance and number of meadows in which they were found (occurrence).

Auchenorrhyncha species	Total abundance	Occurrence (in *x* meadows)
Adarrus multinotatus (Boh.)	15	7
Agallia brachyptera (Boh.)	208	6
Anaceratagallia ribauti (Oss.)	113	11
Aphrodes bicinctus (Schrk.)	79	8
Aphrophora alni (Fall.)	18	9
Arocephalus languidus (Flor.)	18	7
Cicadula persimilis (Edw.)	21	7
Ditropsis flavipes (Sign.)	544	10
Doratura stylata (Boh.)	17	6
Emeljanovianus medius (Mul.)	111	10
Eupteryx notata (Curt.)	83	7
Euscelis incisus (Kirsch.)	43	9
Graphocraerus ventralis (Fall.)	59	10
Leypyronia coleoptrata (L.)	47	11
Megadelphax sordidula (Stål)	272	7
Philaenus spumarius (L.)	688	12
Planaphrodes bifaciata (L.)	278	12
Psammotettix helvolus (Kbm.)	54	11
Turrutus socialis (Fl.)	210	10
Verdanus abdominalis (F.)	308	9

Appendix S5. Outputs of the linear mixed-effects models carried out on three Auchenorrhyncha species found on at least six meadows (*Adarrus multinotatus*, *Agallia brachyptera* and *Megadelphax sordidula*), and showing the effects of management treatment on their abundance (log + 1 scale). Estimate, standard error (SE), t-value and *P*-value (*P*) are given. The fixed factor was the management treatment. Treatment abbreviations: C = control; F = fertilised; I = irrigated; F+I 1/3, F+I 2/3 and F+I 3/3 = fertilised and irrigated at, respectively, 1/3, 2/3 or 3/3 of the dose that would be necessary to achieve the maximum theoretical local hay yield. The random factor was the experimental study site. Significant *P*-values are highlighted in bold.

Auchenor-rhyncha	***Adarrus multinotatus (Boh.)*** **abundance (log + 1 scale)**				***Agallia brachyptera (Boh.)*** **abundance (log + 1 scale)**				***Megadelphax sordidula (Stål)*** **abundance (log + 1 scale)**			
	Estimate	SE	t-value	*P*	Estimate	SE	t-value	*P*	Estimate	SE	t-value	*P*
F vs C	0.231	0.131	1.762	0.083	0.144	0.238	0.603	0.549	0.530	0.267	1.986	0.052
I vs C	0.000	0.131	0.000	1.000	0.279	0.238	1.169	0.248	0.394	0.267	1.476	0.146
F+I 1/3 vs C	0.289	0.131	2.203	**0.031**	0.517	0.238	2.168	**0.035**	1.126	0.267	4.223	**0.000**
F+I 2/3 vs C	0.058	0.131	0.441	0.661	0.047	0.238	0.199	0.843	0.814	0.267	3.053	**0.003**
F+I 3/3 vs C	0.116	0.131	0.881	0.381	0.292	0.238	1.225	0.226	0.864	0.267	3.238	**0.002**
I vs F	-0.231	0.144	-1.609	0.113	0.135	0.237	0.571	0.571	-0.136	0.244	-0.558	0.580
F+I 1/3 vs F	0.058	0.144	0.402	0.689	0.373	0.237	1.578	0.122	0.597	0.244	2.445	**0.019**
F+I 2/3 vs F	-0.173	0.144	-1.207	0.233	-0.096	0.237	-0.407	0.686	0.285	0.244	1.167	0.250
F+I 3/3 vs F	-0.116	0.144	-0.804	0.425	0.148	0.237	0.627	0.534	0.334	0.244	1.369	0.178
F+I 1/3 vs I	0.289	0.132	2.196	**0.033**	0.238	0.190	1.257	0.218	0.733	0.260	2.820	**0.008**
F+I 2/3 vs I	0.058	0.132	0.439	0.663	-0.231	0.190	-1.220	0.231	0.421	0.260	1.620	0.115
F+I 3/3 vs I	0.116	0.132	0.879	0.384	0.013	0.190	0.070	0.944	0.470	0.260	1.810	0.079
F+I 2/3 vs F+I 1/3	-0.231	0.152	-1.522	0.138	-0.470	0.188	-2.503	**0.020**	-0.312	0.249	-1.254	0.223
F+I 3/3 vs F+I 1/3	-0.173	0.152	-1.141	0.262	-0.225	0.188	-1.199	0.243	-0.263	0.249	-1.056	0.303
F+I 3/3 vs F+I 2/3	0.058	0.097	0.596	0.557	0.245	0.114	2.146	0.055	0.049	0.193	0.256	0.802

GENERAL DISCUSSION

This thesis is part of a long lasting ecological experiment that inquires the rapid changes of farming practices in mountain regions. The main aim is to investigate the effect of modern agricultural practices (irrigation with sprinklers and fertiliser with slurry) on hay productivity and biodiversity that maintains economically attractive and environmentally sustainable hay meadow management in mountain regions to ensure their multifunctionality. The first results obtained through the three first years of the experiment (2010-2012) are presented in this thesis. This general discussion seeks to incorporate the findings from the different studies and to draw more general conclusions on the effects of mountain hay meadow management on plants and arthropods.

A meta-analysis, presented in first chapter and focused mainly on the effect of nitrogen applications on the biodiversity and productivity of meadows in temperate mountain regions, was firstly conducted to review the current state of scientific knowledge. This allowed us to identify several gaps in research: firstly, numerous studies addressed the effects of fertilising on mountain grassland vegetation (chapter 1), however, through this meta-analysis, it was evidenced the lack of studies related to the arthropod responses to intensification. Moreover, the effects of irrigation and the combination of both fertilising and irrigation have, to our knowledge, not been examined yet.

A comprehensive and fully-controlled experimental study analysing various treatments combining fertilisation and irrigation on the response of vegetation and arthropods in mountain hay meadows was conducted to answer, partially, to the questions raised in the meta-analysis. Six different treatments imitating different grassland management regimes, both applied and applicable by farmers, were set up using a randomised block design and replicated on 12 mountain meadows in the South-West of the Swiss Alps. They consisted of varying amounts of fertiliser (slurry) and irrigation water (distributed by sprinklers) applied separately or as a combination of both, constituting a site-adapted intensification gradient ranging from extensive to mid-intensive management. The main aim of this thesis was to quantitatively define whether it exists an optimal management intensity increasing hay productivity but sustaining biodiversity.

Previous studies showed that intensification of mountain meadows, which enhances phytomass production, usually alters biodiversity by inducing a marked reduction of plant species richness (Dietschi et al. 2007; Homburger & Hofer 2012; Humbert et al. 2015) as well as arthropod species richness and abundance (Marini et al. 2008; Niu et al. 2014). However, regarding the experimental set up we applied on species rich-meadows within the inner Alps, we expected based on the hump-shaped curve model (Grime 1973), that the addition of fertilisers and irrigation at an intermediate level of management intensity may support a higher plant species richness, diversity and grass and hay production than in very intensively or extensively managed hay meadows. This increased plant-growth would benefit in turn the abundance, biomass and diversity of arthropods because several ecological niches are co-occuring simultaneousely at very small scale.

Treatment effects

By documenting immediate changes occurring just one and two years after the experiment was set up, the studies described in chapters 2 and 3 highlighted the effects of intensification via fertilisers and irrigation or a combination of both on hay productivity, structure of herbaceous vegetation, plant species richness and arthropods abundance, biomass and species richness, including especially Auchenorrhyncha.

Although nitrogen plays a central role in mountain meadows (chapter 1), fertiliser applications alone showed limited positive effects on vegetation in the meadows studied. The plant species richness, hay productivity and arthropod biomass increased slightly after just one year of treatments (chapter 2). However, no positive or negative effects were observed on either the vegetation structure or arthropod abundance or on Auchenorryncha.

Adding irrigation water showed a different trend than fertiliser treatment, as water is clearly a limiting factor for the hay meadows studied (chapter 2). In the short-term, treatments only irrigated have the same benefits as treatments only fertilised but they increase also the vegetation structure and abundance of arthropods. The study described in chapter 2 showed that vegetation structure is not a key factor, as often described (e.g. Brown, Gibson & Kathirithamby 1992; Morris 2000; Woodcock et al. 2009; Dittrich & Helden 2011), but rather acts as a limiting factor on arthropod abundance. This means, that although high vegetation structure is essential for high arthropod abundance, it does not guarantee it. However, when irrigation is applied alone, no positive effect was observed on Auchenorrhyncha after two years of treatments (chapter 3).

By contrast, the application of water combined with fertiliser shows a significant advantage compared to fertiliser- or irrigation-only treatments. All three treatments immediately increase plant species richness, vegetation structure, hay productivity and arthropod abundance. They also boost Auchenorryncha abundance, species richness and biomass after two years of treatments. These interesting results confirm that it is the interaction between fertilisation and irrigation which induces major changes on hay meadows biodiversity in mountain regions.

However, as these three treatments have different effects from each other, it confirms the fact that the ecological response depends on the degree of management intensity (Fig. 1). As expected, data collected in 2012 shows that hay productivity increases along the intensity gradient. Moreover, even if some results are not statistically detectable (probably because the first phase of the study could not be undertaken over the long-term), Auchenorrhyncha abundance and plant species richness reached an optimum level with treatment receiving low-inputs of fertilisers and water combined as did specialist species of Auchenorrhyncha (monophagous, oligophagous and stenotopic). By contrast, treatments receiving medium and high inputs of fertilisers and water favoured generalist species (polyphagous and eurytopic), which could cause functional homogenisation at the community level in the long-term (Clavel, Julliard & Devictor 2010).

Recommendations

The alteration of the vulnerable mountain meadows is an ongoing process. Meadows on advantageous sites that are easily accessible or on flat areas are increasingly intensively managed, while others are being abandoned and reforested. To avoid a massive loss of biodiversity, it is crucial to maintain hay meadow management in mountain regions that prevents abandonment but also that avoids high intensification. Different agricultural practices and their effects were analysed in this thesis to achieve this aim. The results are unequivocal: the shift to modern agriculture practices through the use of sprinklers or slurry has a very rapid effect on mountain hay meadows which are not as stable and resistant as expected (Tilman & Downing 1994).

These studies were conducted using a fully-controlled experimental design with the ability to isolate the effects of treatments from those of confounding factors such as abiotic factors or local conditions. Consequently, the results presented in this thesis allow us to provide concrete guidance for the management of mountain meadows that require fertilisation and irrigation to ensure decent hay production. Finally, as the treatments imitate actual agricultural practices and manipulate the amounts of fertiliser and water (two easily controllable processes), farmers can easily implement these recommendations.

Thus, in the present state of knowledge, the following recommendations can be given:

If hay productivity of meadows is profitable enough and meadows can be managed without adding water or fertiliser, maintaining traditional mowing at least once a year is the preferred option to avoid compromising plant species diversity (Tasser & Tappeiner 2002; Maurer *et al.* 2006; Vassilev *et al.* 2011).

If meadows are threatened by abandonment or highly intensive agricultural practices due to economic pressures, a slight intensification increase of the management is still preferable to guarantee hay production and safeguard species diversity. An application of a dose of fertiliser and water equivalent of a maximum of one third of the amount required to achieve maximum theoretical local hay production (according to Sinaj *et al.* 2009) and one cut per year after flowering can be recommended as these measures will ensure the maintenance of the specialised species of Auchenorrhyncha, a key indicator taxon. This low-level of intensification therefore provides a good management option to avoid abandonment or high intensification and emphasises the fact that the best management practice should incorporate the meadow's characteristics as altitude, botanical composition as well as the potential productivity of the meadow.

If meadows have not been fertilised but previously irrigated using a gravitational terrestrial system and are currently threatened by abandonment, the use of sprinkler irrigation system and an application of maximum 20 mm water per week can be recommended. This measure ensures productivity while requiring far less labour input and sustains biodiversity (see also Riedener, Rusterholz & Baur 2013; Melliger et al. 2014).

Adding fertiliser has, in itself, shown no adverse effects on all organisms tested. However, this treatment is not recommended for management use as the intended effects of this measure, i.e. to benefit biodiversity, were not achieved. As such, the effects detected after two years of experiment on both the hay production volumes of hay and biodiversity outputs are generally in favour of a treatment equivalent to one-third of the dose required for maximum theoretical hay production.

Study limitations and conclusions

The analysis of different management treatments on plant and arthropods increases our understanding on the short-term effects of intensification in mountain hay meadows. This thesis clearly shows the rapid impact fertilisation and irrigation, separately or combined, has on productivity and biodiversity.

However, a specific disadvantage in the design of this experiment is the time needed by plants and arthropods to respond to experimental treatments and that the system displays transient dynamics (Tilman 1989; Woodcock et al. 2010). It is therefore not possible to evaluate the long-term effects of different treatments over two field-seasons. In particular, although intensification shows some positive effects on plants and arthropods at first, the equilibrium has probably not yet been reached. Although we have already observed trends for each treatment, we can view the effects of long-term intensification with some trepidation. Chapter 1 highlighted the effect that adding nitrogen depends on various biotic and abiotic factors, including the amount of nitrogen and how long it is applied. These results subsequently confirm the hump-shaped model (Grime 1973) and suggest that a low-dose nitrogen application over the long-term could have a similar effect on plant species richness as a high dose over the short-term. Thus, even a low intensification treatment could have daunting effects on biodiversity over the long-term.

To draw definitive conclusions about each treatment, plant and arthropod monitoring will have to be conducted over a longer timescale. Analysis of other taxa may also provide a comprehensive overview on the advantages and disadvantages of each treatment. In particular, analysis of arthropods with other ecological niches or belowground organisms, such as bacteria or springtails could improve our understanding of this complex ecosystem. Finally, there is the issue of arthropod accessibility, as they constitute an important food resource for higher trophic levels, like birds (Benton et al. 2002). On one hand, their abundance increases with some treatments while on the other hand, the increase in vegetation biomass can potentially protect them and make them less accessible to their predators.

To be sustainable, the farming practices must evolve on the basis of economic, environmental and social dimensions for the entire meadow system. Identifying mountain meadow management treatments that ensure decent hay productivion but which are resource-conscious and preserve the environment is a challenge that must involve each farmer by assessing the needs and potential of each of their meadows.

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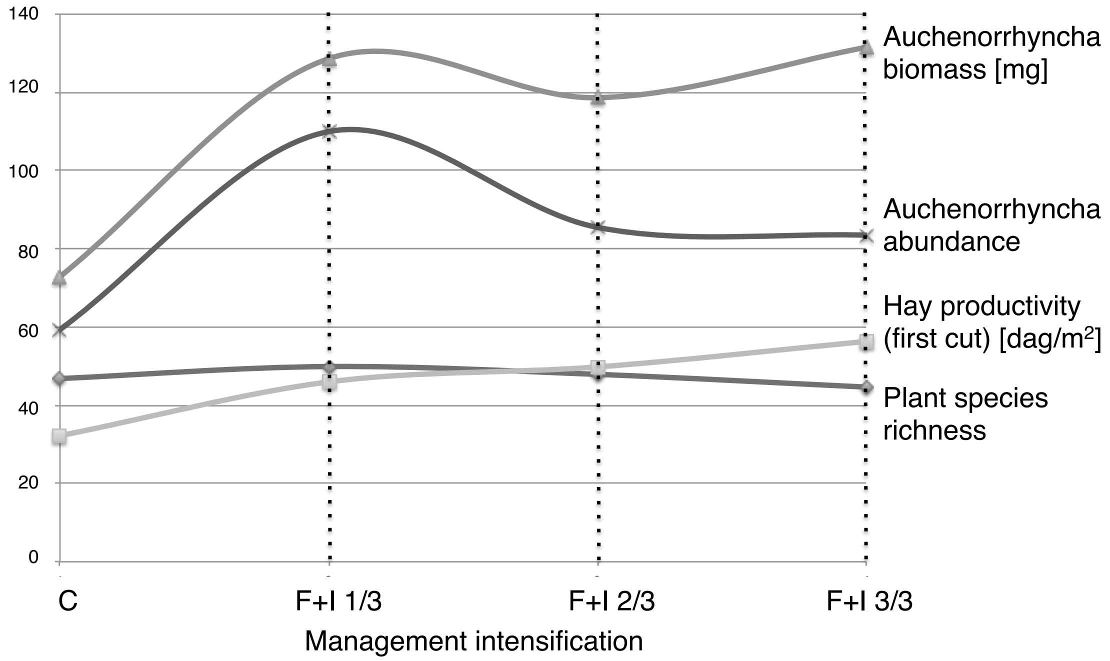

Figure 1: Graphical representation of the mean of the hay productivity [dag/m2], plant species richness, Auchenorryncha biomass [mg] and abundance measured in 2012 on the twelve hay meadows. Treatment abbreviations are as follows: C = control; F+I 1/3, F+I 2/3 and F+I 3/3 = fertilised and irrigated at, respectively, 1/3, 2/3 or 3/3 of the dose that would be necessary to achieve the maximum theoretical local hay yield.

APPENDIX 1

Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review

Global Change Biology

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RESEARCH REVIEW

Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review

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Abstract

Although the influence of nitrogen (N) addition on grassland plant communities has been widely studied, it is still unclear whether observed patterns and underlying mechanisms are constant across biomes. In this systematic review, we use meta-analysis and metaregression to investigate the influence of N addition (here referring mostly to fertilization) upon the biodiversity of temperate mountain grasslands (including montane, subalpine and alpine zones). Forty-two studies met our criteria of inclusion, resulting in 134 measures of effect size. The main general responses of mountain grasslands to N addition were increases in phytomass and reductions in plant species richness, as observed in lowland grasslands. More specifically, the analysis reveals that negative effects on species richness were exacerbated by dose (ha^{-1} $year^{-1}$) and duration of N application (years) in an additive manner. Thus, sustained application of low to moderate levels of N over time had effects similar to short-term application of high N doses. The climatic context also played an important role: the overall effects of N addition on plant species richness and diversity (Shannon index) were less pronounced in mountain grasslands experiencing cool rather than warm summers. Furthermore, the relative negative effect of N addition on species richness was more pronounced in managed communities and was strongly negatively related to N-induced increases in phytomass, that is the greater the phytomass response to N addition, the greater the decline in richness. Altogether, this review not only establishes that plant biodiversity of mountain grasslands is negatively affected by N addition, but also demonstrates that several local management and abiotic factors interact with N addition to drive plant community changes. This synthesis yields essential information for a more sustainable management of mountain grasslands, emphasizing the importance of preserving and restoring grasslands with both low agricultural N application and limited exposure to N atmospheric deposition.

Keywords: conservation, cumulative effects, fertilization, fertilisation, global change, nitrification, nutrient, vegetation

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Introduction

Reactive nitrogen (N) addition to terrestrial ecosystems through agricultural fertilization or atmospheric deposition has increased substantially in recent decades and is today considered to be one of the most widespread drivers of global change (Galloway *et al.*, 2008). This dramatic increase concerns both the rate of N application or deposition, and its spatial extent (Galloway *et al.*, 2004; Erisman *et al.*, 2008). Although N addition to terrestrial ecosystems has recently levelled off in some areas of the globe, it is predicted to increase further on a global scale (Dentener *et al.*, 2006; Erisman *et al.*, 2008). Among terrestrial ecosystems, grasslands, especially in the lowlands, have received considerable research attention. Several empirical studies and reviews have demonstrated the general response of grassland plant communities to N addition, notably in terms of decreases in species richness and resulting increases in phytomass productivity (e.g. Bobbink *et al.*, 2010; Maskell *et al.*, 2010; De Schrijver *et al.*, 2011; Borer *et al.*, 2014). These results have raised several conservation concerns and policy responses, such as the critical load policy concept (a policy tool for the control of air pollution, see Payne *et al.*, 2013 and Roth *et al.*, 2013). Some studies, however, have shown that responses can

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differ among plant communities as well as along large environmental gradients, with considerable variation in the magnitude of the responses (Clark *et al*., 2007).

The composition of the original plant community, including the relative proportion of functional groups (i.e. grasses, forbs, legumes and sedges), can influence the direction and magnitude of the changes to N addition (e.g. Tilman *et al*., 2001; Bassin *et al*., 2007; Marquard *et al*., 2009; Bai *et al*., 2010; Onipchenko *et al*., 2012). Grasses are generally favoured by N addition, while legumes are not, and forb responses tend to be species specific (Theodose & Bowman, 1997; Leto *et al*., 2008; Niu *et al*., 2008; Duprè *et al*., 2010).

Regional environmental conditions such as climate and local soil characteristics also influence the response of the original plant community to N addition. Climate may influence responses to N addition by controlling important aspects of energy supply that contribute to plant productivity and diversity maintenance (Hawkins *et al*., 2003; Cross *et al*., 2015), or by influencing secondary stress impacts such as frost damage in cold climates (e.g. Clark *et al*., 2007), and heat and water constrains in hot climates (Rustad *et al*., 2001; Ren *et al*., 2010). It has been shown that relative productivity responses to N addition increase with latitude (Lebauer & Treseder, 2008). At very local scales, responses can further differ due to different original soil nutrient limitation and/or soil moisture (Theodose & Bowman, 1997; but see Seastedt & Vaccaro, 2001). Relevant to these findings, resource ratio theory (Tilman, 1982) predicts that responses to N addition may be contingent on the supply of other limiting resources such as phosphorus or potassium (Ren *et al*., 2010; Harpole *et al*., 2011). Soil pH may also alter responses to N supply by influencing soil microbial activity and rates of N and carbon cycling (Kemmitt *et al*., 2006; Duprè *et al*., 2010).

In addition to environmental conditions, plant community responses may vary according to the form (oxidized vs. reduced) and type (ammonium nitrate, urea, etc.) of N addition (Gaudnik *et al*., 2011). Nitrogen fertilizer origin (mineral or organic) also appears to play a role, but we lack quantitative evidence about its effects (but see Kirkham *et al*., 2014; Pacurar *et al*., 2012). Finally, biomass removal via grazing and mowing, especially within seminatural agricultural grasslands, also influences grassland community diversity and composition (e.g. Marriott *et al*., 2009; Humbert *et al*., 2012) and responses to N addition (e.g. Kampmann *et al*., 2008; Lanta *et al*., 2009; Pavlů *et al*., 2011; Borer *et al*., 2014).

Overall, research to date tends to show that local biotic and abiotic conditions play an important role in moderating plant response to N addition. As the vast majority of studies were carried out in lowland grasslands, conclusions drawn from experiments at low altitude are not readily transferable to other types of ecosystems, notably to mountain grasslands (Sebastia, 2007). Biome-specific systematic syntheses are therefore required before we can generalize (Pullin, 2012). This systematic review thus focuses on temperate mountain ecosystems including montane, subalpine and alpine grasslands. It aims at assessing the available evidence regarding the effects of N addition upon biodiversity and productivity of temperate zone mountain grasslands. In particular, it investigates how abiotic factors that potentially interact with N addition drive the variable plant community responses that are commonly observed in nature. The term N addition refers here to N from anthropogenic origin, either in the form of agricultural fertilization (sometimes in combination with other nutrients) or atmospheric deposition. The present review not only provides a basis for sound predictions about community changes but also informs land managers and policymakers about the conservation threats potentially affecting mountain grasslands and remedies for biodiversity more friendly management (Maurer *et al*., 2006; Maskell *et al*., 2010; Bobbink & Hettelingh, 2011).

Materials and methods

We followed the review methodology of the Collaboration for Environmental Evidence partnership (Pullin & Stewart, 2006) and published an *a priori* protocol that was peer-reviewed (Dwyer *et al*., 2010 provided in Appendix S1).

Search strategy

The following Web databases were searched for documents: ISI Web of Science, Science Direct, JSTOR, Google (100 first hits) and Google Scholar (100 first hits). A high-sensitivity and low-specificity approach was used to ensure that all important relevant information was found (Dwyer *et al*., 2010 in Appendix S1; Pullin & Stewart, 2006). The databases searches were carried out between September 2012 and January 2013 (see Appendix S2 for exact term lists and dates). Any apparently relevant citations or links were followed one step away from the original hit. In addition, national and international experts on the subject were asked for any related literature and unpublished data.

Study inclusion criteria

All references retrieved from the Web search (2285) were scanned at the title, abstract and full-text filter levels by a first reviewer. From the 2285 initial references, 20% were randomly selected and rescanned by a second reviewer to check for inclusion consistency. The following inclusion criteria were used:

- Relevant subjects: natural or seminatural grasslands in temperate mountain zones. Grasslands were defined as generally treeless, dominated by graminoid and forb species (>50% graminoid and herbaceous cover prior to interventions), which excluded heath or other shrub-dominated lands. Mountain grasslands were defined as those occurring on mountain ranges within temperate regions that experience winter snow cover. Temperate regions were defined as those: (i) within temperate latitudes and (ii) classified in the Köppen–Geiger climate classification system as: Cfb, Cfc, Cwb, Cwc, Dfb, Dfc, Dfd, Dwb, Dwc, Dwd and Et (Kottek *et al.*, 2006). The use of these eleven categories excluded Mediterranean, subtropical and arid climates that occur within the temperate latitudes.
- Types of intervention: addition of nitrogen (alone or in combination with other nutrients).
- Types of comparator: nonfertilized control plots (experimental studies) or suitable reference areas that have not been fertilized (observational studies). Control plots had to be managed in the same way as treatment plots with the exception of fertilizer addition.
- Types of outcome: species richness or Shannon index of diversity (H') of at least one taxonomic group. Changes in biomass production or absolute abundance of functional groups were also recorded if provided.

Manipulative micro- and mesocosm experiments were not included as they cannot be considered 'seminatural' grasslands. While the definition of what is or what is not a mountain grassland is difficult to state, the majority of the authors defined their study sites as mountain, montane, subalpine or alpine grasslands. Importantly, study sites had to be located on the slopes or on the top of a recognized mountain range. Elevation *per se* was not a criterion, as high plateaus, such as the Xilin River Basin, Inner Mongolia, were not considered mountain sites (e.g. Bai *et al.*, 2010; study site at 1250 m). Included taxonomic groups were restricted to aboveground communities, which excluded soil microbial, faunal and fungi communities as well as seed banks. Inclusion consistency was checked with kappa statistics, and agreement between the reviewers was satisfactory ($k = 0.81$) (Pullin & Stewart, 2006).

Data extraction

Many studies reported more than one treatment (different amounts or types of fertilizer added), and some studies reported the results of the same experiment replicated in different habitats [e.g. Theodose & Bowman (1997) duplicated their experiment in dry and wet meadows]. In these cases, all comparisons were recorded as independent data points, and this is why there are more data points (units of analysis) than studies (Pullin & Knight, 2003; Humbert *et al.*, 2012). The majority of studies (40 of 42) that respected inclusion criteria were on vascular plants, with only two studies on either bryophytes (Bergamini & Pauli, 2001) or Coleoptera (Majzlan & Gajdoš, 2007). We therefore decided to limit this review to vascular plants.

The following information was extracted for each relevant treatment from the selected studies: (i) species richness and/or H'; (ii) total vascular plant biomass and biomass per functional group (i.e. grass, sedge, legume or forb); (iii) number of replicates per treatment; (iv) study duration in years; (v) Köppen–Geiger climate (hereafter 'K-G climate'); (vi) country where the study was carried out; (vii) precipitation per year in mm; (viii) mean monthly temperature; (ix) altitude; (x) latitude; (xi) soil pH before the experiment started; (xii) nitrogen fertilizer origin, classified as mineral or organic; (xiii) dose of N [$\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$] applied; (xiv) dose [same units] of phosphorus (P) and potassium (K) if applied with N; (xv) fertilization frequency per year; and finally (xvi) management type of the plots during the experiment, classified as unmanaged, grazed, mown, or grazed and mown.

For studies with multiple replicates per treatment, standard deviations (SD) were retrieved from published standard errors (SE) or variances. If no estimate of variance was provided, we requested it from the original authors. If original authors could not provide estimate of variance, or sample size was equal to one (i.e. no variance), the corresponding study was included only in the unweighted analyses (see Statistical analysis section below). K-G climates were rarely provided in the publications, but could be inferred from study site averaged monthly precipitations and temperatures. These values were either found in the original publication, provided by the authors, or found on web pages linked to the corresponding publication (e.g. from the Niwot Ridge Long-Term Ecological Research Site at http://niwot.colorado.edu/index.html). In some cases, we relied on the data from the nearest available weather station (e.g. for Swiss studies at: http://www.meteosuisse.admin.ch/) or highest resolution (30 arc-seconds latitude, i.e. ca 1 km) WorldClim global climate data (www.worldclim.org). Values for soil pH could not always be extracted from the publication or obtained by the authors and therefore some values were missing.

Additional potential sources of heterogeneity were also extracted such as exact fertilizer form (e.g. ammonium nitrate or urea), type of geological substrate, habitat type (e.g. dry, wet or mesophilous), plot size of vegetation relevés, original plant community, and former management. However, these factors could not be sourced for all studies and were later disregarded from analyses due to insufficient data.

Statistical analysis

Meta-analyses (MAs) were conducted on three response variables: (i) species richness; (ii) H'; and (iii) biomass. Studies lasted up to 65 years, and if multiple time-points were available along the time series, only the data for the last year (longest time period) were considered.

The response ratio statistic was used to estimate effect sizes. The response ratio (lr) for a given comparison is the difference between the mean ln-transformed treatment value and the mean ln-transformed control value (Hedges *et al.*, 1999):

$$\mathrm{lr} = \ln(\bar{X}_F) - \ln(\bar{X}_C),$$

where $\bar{X}_F$ and $\bar{X}_C$ are the means of the fertilized and control (unfertilized) groups. The lr is symmetric around 0, and negative values indicate a negative effect of N addition on the response variable. The lr and its variance (see Hedges *et al.*, 1999; eq. 1) were calculated using the function *escalc* of the R package *metafor* (Viechtbauer, 2010).

Random- and mixed-effects meta-analytical models were used (Gurevitch *et al.*, 2001). Under random- and mixed-effects models, the true effect size, that is the effect size as if there were no sampling error, can vary from study to study, but is assumed to do so under a normal distribution (Perera, 2009; Viechtbauer, 2010). Here, the Q test and I^2 statistic were used to assess heterogeneity among studies. The Q test is the test of significance, and the I^2 statistic estimates how much of the total variability in the mean effect size (composed of heterogeneity and sampling error) can be attributed to heterogeneity among the true effect sizes (Borenstein *et al.*, 2009; Perera, 2009).

Following Johnson & Omland (2004), a set of candidate models was generated including moderator(s) related to potential biotic and abiotic processes than can be biologically or agronomically interpreted. Candidate models comprised all models including one of the following moderators: study duration in years; K-G climate; mean summer temperature (i.e. mean monthly averages of May–August); number of month(s) with mean temperature ≥ +10°C; fertilizer origin (organic vs. mineral); fertilizer type (i.e. N, NP, NK or NPK); fertilization doses of N, P, and K; management type (nominal variable with four classes: unmanaged, grazed, mown, or grazed and mown); and management occurrence (binary variable with two classes: managed or unmanaged, with managed including grazed, mown, and grazed and mown). Candidate models also consisted of all models that included N dose applied plus one of the above-mentioned moderators, and the following more complex model: N dose + P dose + K dose. Influences of mean yearly precipitation, altitude and latitude were not tested independently as they are all encompassed in the K-G climate variable. The set of candidate models were ranked based on AIC values (Akaike information criterion) and on the level of significance of the estimates (Johnson & Omland, 2004; Borenstein *et al.*, 2009). Influences of soil pH and productivity ratio (phytomass production in fertilized plots/phytomass production in control plots) were also investigated, but could not be included in the model selection process because of missing values for several studies. Publication bias was assessed using funnel plots, by applying a regression test for funnel plot asymmetry (Borenstein *et al.*, 2009; Viechtbauer, 2010). Normal quantile–quantile (Q-Q) plots were inspected to assess normality of the residuals. Only the results where corresponding Q-Q and funnel plots were satisfactory are presented here.

In addition to the weighted MAs that utilized variances among replicates, unweighted meta-analyses were applied to a larger data set that included effect sizes without associated variances. Unweighted MAs were limited only to null models (i.e. without moderators) to assess overall effects of N addition. Bootstrapping was used to calculate 95% confidence interval (CI) of the estimated effect size; if CIs overlapped zero, the effect size was considered to be nonsignificant. All statistics were performed using R version 3.1.2 (R Core Team, 2014).

Results

A total of 2285 references were retrieved from the Web. Only 43 articles matched inclusion criteria, that is dealt with the influence of N addition on grassland biodiversity (Appendix S3). Among them, eight

Table 1 List of studies included in the meta-analysis, with study area and number of extracted data point(s). Appendix S4 provides a more comprehensive overview of variables considered for each data point

Source (study reference)	Country	Number of data points
Bassin *et al.* (2007)	Switzerland	4
Bassin *et al.* (2012)	Switzerland	4
Baumberger *et al.* (1996)	Switzerland	2
Bergamini & Pauli (2001)	Switzerland	2
Bonanomi *et al.* (2009)	Italy	3
Bowman *et al.* (2006)	USA, Colorado	3
Bowman *et al.* (2012)	USA, Colorado	3
Brinkmann & Reif (2006)	Romania	12
Britanak *et al.* (2007)	Slovakia	2
Chytrý *et al.* (2009)	Germany	4
Delpech (1984)	France	1
Elisseou *et al.* (1995)	Greece	9
Fahnestock & Detling (1999)	USA, Wyoming	2
Gross *et al.* (2009)	France	2
Jeangros & Troxler (2008)	Switzerland	2
Kassioumi (2003)	Greece	4
Kohler *et al.* (2004)	Switzerland	4
Kohler *et al.* (2005)	Switzerland	4
Krajčovič *et al.* (1990)	Slovakia	3
Kralovec *et al.* (2009)	Czech Republic	4
Lanta *et al.* (2009)	Czech Republic	3
Leto *et al.* (2008)	Croatia	2
Li *et al.* (2010)	China, Tibet	1
Majzlan & Gajdoš (2007)	Slovakia	3
Mamolos *et al.* (2005)	Greece	4
Mudrak & Leps (2010)	Czech Republic	1
Niu *et al.* (2012)	China, Tibet	2
Olofsson & Shams (2007)	Sweden	2
Onipchenko *et al.* (2012)	Russia	4
Pauli *et al.* (2002)	Switzerland	2
Pavlů *et al.* (2011)	Germany	4
Pavlů *et al.* (2012)	Czech Republic	2
Ren *et al.* (2010)	China, Tibet	4
Rixen *et al.* (2008)	Switzerland	1
Seastedt & Vaccaro (2001)	USA, Colorado	6
Sebastia (2007)	Spain, Pyrenees	2
Song *et al.* (2012)	China, Tibet	3
Suding *et al.* (2008)	USA, Colorado	1
Tenz *et al.* (2010)	Switzerland	1
Theodose & Bowman (1997)	USA, Colorado	4
Veresoglou *et al.* (2011)	Greece	4
Wang *et al.* (2010)	China, Tibet	4

articles were excluded due to paper content duplication. This was apparent when two distinct references stemming from the same experiment presented the same data to address different questions or presented results over different time periods. Seven additional articles were found in the bibliography sections of the retained papers or obtained after contacting experts, which resulted in a total of 42 suitable studies. In some studies, more than one treatment or more than one habitat type were investigated, resulting in a total of 134 treatment–control comparisons (i.e. data points, Table 1).

From these 134 data points, 98 reported results on plant species richness, 60 on Shannon index (H') for plants (in some cases, the original reference did not report results on H', but the corresponding author provided the raw data for its calculation), and 103 on changes in phytomass production. In 43 cases (12 for plant species richness, 20 for H' and 11 for phytomass), the study did not report SD, or replication per treatment was one. Consequently, these data could only be included in the unweighted MA. All studies were experimental, except one which was observational (Jeangros & Troxler, 2008) but of sufficient quality to be included (quality category II-2 of Pullin & Knight, 2003). Most observational studies were excluded because they did not report the quantity of nitrogen applied (e.g. Spiegelberger *et al.*, 2006). A list of all studies considered and of all treatment–control comparisons is provided in Appendix S4. Appendix S5 lists the articles excluded after full-text filtering, mentioning reasons for exclusion.

We present the results of the different MAs as follows: (i) all null models (models without moderators) for plant species richness, H' and biomass production; (ii) the best-supported models for species richness and H'; and (iii) several single-moderator models that did not emerge as 'best' models, but included moderators of high agronomical or biological relevance.

Null models

Species richness was reduced by N addition in weighted and unweighted MAs (Fig. 1). Regarding H' (Shannon index) weighted MA indicated no effect of N addition, while the unweighted MA indicated a significant decrease of H' in response to N addition. Overall, vascular plant and grass biomass were higher in fertilized compared to unfertilized plots. Sedge and legume biomass did not change following N addition, while forb biomass exhibited a significant increase with weighted MA and no change with unweighted MA (Fig. 1; see Appendix S6 for detailed model outputs).

Best-supported models

The model with best-support explaining changes in species richness was the bivariate model including N dose and study duration as moderators (Fig. 2 and Appendix S7). Both moderators had a significant negative effect on plant species richness effect size (N dose: lr = −0.0007, z = 0.0003, P = 0.026; study duration: lr = −0.0041, z = 0.0014, P = 0.003). Heterogeneity among studies was significant (Q = 605.28, df = 83, $P < 0.001$), indicating that other moderators likely influence responses to N addition.

The best model explaining H' was the single-moderator model including the number of months with mean temperature ≥ +10°C (Fig. 3b and Appendix S7), which indicated reduced H' in warmer regions. However, the resulting funnel plot showed significant asymmetry due to an outlying data point ('Site.T ii', from Kassioumi, 2003 unpublished PhD Thesis). Excluding this data point improved model diagnostics, but did not alter conclusions (number of months with mean temperature ≥ +10°C: lr = −0.044, z = 0.008, $P < 0.001$) or selection of the 'best' model. As for the species richness models, heterogeneity among studies was significant, indicating that other moderators likely influence effect sizes.

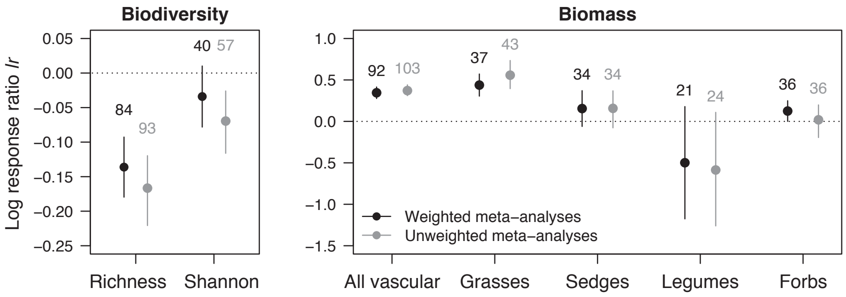

Fig. 1 Influence of N addition on plant species richness, diversity (Shannon index) and biomass production. Effect sizes are response ratios (lr), with negative values meaning a negative effect of N addition. Points represent means and error bars ± 95% CI. Sample size (numbers of data points) is given above each bar.

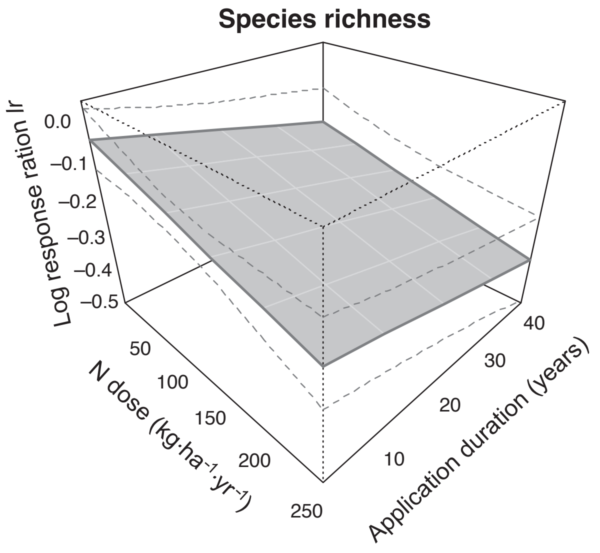

Fig. 2 3D perspective plot of species richness response ratio (lr) vs. the amount of nitrogen applied per year and application (study) duration. The grey plane represents the mean response and dashed lines are corresponding 95% CI.

Other single-moderator models

The negative effect of N addition on species richness was more pronounced in warmer than in cooler regions (Fig. 3a) and where phytomass responses to N addition were largest (higher productivity ratios; Fig. 4a). In contrast, there was no significant relationship between H' effect size and the productivity ratio (Fig. 4b). The negative effect of N addition on species richness was significantly more pronounced where experimental plots were managed ($\text{lr} = -0.1070$, $z = -2.5717$, $P = 0.0101$; Fig. 5). Species richness effect sizes were also moderated by fertilizer type (i.e. N, NP, NK or NPK): there was a stronger negative effect on species richness when NPK was applied compared to N alone (Appendix S8). Initial soil pH did not influence the effect of N addition on species richness (Appendix S8).

Unsatisfactory funnel plots prevented further metaregression on H'.

Discussion

Overall, this systematic review indicates that N addition generally reduces plant species richness and diversity and increases biomass in temperate mountain grassland systems, but these responses are also strongly influenced by N dose, application duration and management practice. Our results also suggest that plant community responses to N addition are modulated by mean summer monthly temperatures.

Both weighted and unweighted MAs showed that N addition typically induced a decrease in plant species richness. This is in agreement with the general negative pattern found in broad-scale studies of lowland grassland fertilization (e.g. Bobbink & Hettelingh, 2011; De Schrijver *et al.*, 2011; Van Den Berg *et al.*, 2011). Shannon index (H') also decreased with N addition but to a lesser extent, and the decrease was significant only when using unweighted MA. Vascular plant biomass increased with N addition, and this pattern was mostly driven by an increase in grass biomass. Biomass of sedges and legumes did not change with N addition while forb biomass exhibited a small significant increase, but only when weighted MA was applied. Mean effect sizes for legume biomass were clearly negative, but the variances of both weighted and unweighted MAs were large with the 95% CI overlapping 0 (i.e. no effect). It suggests that adding N has the tendency to decrease legume biomass, although other factors, such as the addition of P with N, can have interactive effects (e.g. Willems *et al.*, 1993; Ren *et al.*, 2010).

Best-supported models

The model with best support for explaining changes in species richness following N addition was the bivariate

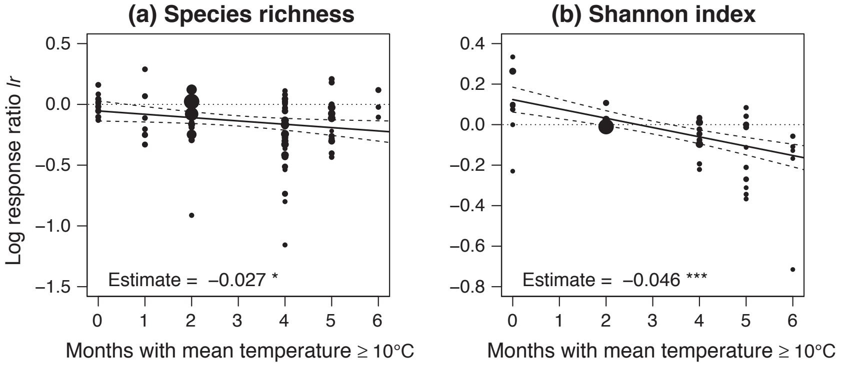

Fig. 3 Species richness and Shannon index response ratios (lr) vs. the number of months with mean temperature ≥ +10°C. Significance levels for slope estimates are given for each regression line ($^*P < 0.05$; $^{***}P < 0.001$). Symbol size depicts study weight.

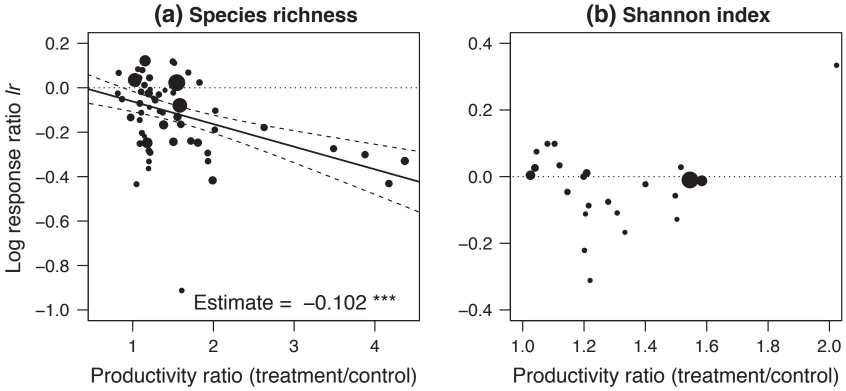

Fig. 4 Species richness and Shannon index (H') response ratios (lr) vs. phytomass productivity ratio (treatment/control). Significance level for slope estimate is given (***$P < 0.001$). Symbol size depicts study weight.

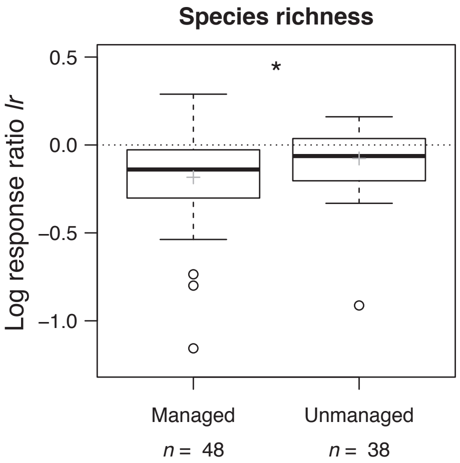

Fig. 5 Boxplot presentation of species richness response ratio (lr) with respect to management occurrence. Managed control and fertilized plots were grazed, mown, or grazed and mown. Bold transversal bars represent medians; grey crosses means; box boundaries the first and last quartiles; whiskers the interquartile distance multiplied by 1.5; and open circles the outliers. The asterisk indicates a significant difference between managed and unmanaged plots at $P < 0.05$.

model including N dose and study duration (years of N application, which indicates cumulative N; see Fig. 2). Both moderators had pronounced additive negative effects on species richness, revealing that low N doses applied for long periods lead to similar richness declines as high N doses applied for short periods. This additive effect has been recurrently presumed in the literature about grasslands. To the best of our knowledge, it is here demonstrated for the first time (but see Clark & Tilman, 2008). Because the effect sizes are on a log scale, significant negative linear relationships with N dose and study duration indicate that the steepest richness declines occur at low N doses and in the first few years of N application, respectively. These results are consistent with common grassland models that predict the steepest species declines occurring as N supply initially increases (Kleijn *et al.*, 2009), and with findings from longer-term fertilization and N atmospheric deposition studies (Clark *et al.*, 2007; Clark & Tilman, 2008; Duprè *et al.*, 2010; De Schrijver *et al.*, 2011).

For both species richness and H', the negative effect of N addition was weaker in cooler regions (regions with fewer months with ≥ +10°C mean monthly ambient temperature) than in warmer regions. This is consistent with some findings from alpine and Arctic tundra regions (Bowman *et al.*, 2006; Ditommaso & Aarssen, 1989; but see Seastedt & Vaccaro, 2001), but contrasts with findings from multiple sites across North America where the greatest species losses were observed in colder regions (Clark *et al.*, 2007). It is likely that colder growing season temperatures limit the extent to which plant species can respond to increased N supply (Cross *et al.*, 2015). Slower growth rates in cold adapted species have been widely reported and attributed to a trade-off between freezing tolerance and growth rate (Savage & Cavender-Bares, 2013). It has also been shown in cold ecosystems that warming alone can enhance plant productivity (Rustad *et al.*, 2001), mostly because warming increases nitrogen and phosphorus uptake capacity by plants (Jonasson *et al.*, 1999).

Influences of management, productivity ratio and initial soil pH

Further analyses of data showed that responses varied according to management occurrence (presence or absence of management). The relative negative effect of N addition on species richness was significantly more pronounced in managed communities (grazed, mown, or grazed and mown). This result reflects our choice of control–treatment comparisons – we always compared the effects of N addition between experimental communities that experienced the same management. Control plots in managed communities generally had higher richness than control plots in unmanaged communities, and this richness was maintained through time in longitudinal studies (e.g. Krajčovič *et al.*, 1990; Kohler *et al.*, 2004; Bonanomi *et al.*, 2009). As such, these managed communities had more species to 'lose' after N addition. In unmanaged communities, it is likely that successional processes had already reduced diversity in control plots (Pavlů *et al.*, 2012; Gaisler *et al.*, 2013), and so further losses due to N addition were not as pronounced in relative terms. In the subset of studies that applied combinations of fertilization and management treatments to the same community (Kohler *et al.*, 2004, 2005; Bonanomi *et al.*, 2009; Lanta *et al.*, 2009), fertilized-managed plots displayed higher species richness than fertilized-unmanaged plots in all cases, indicating that mowing and grazing actually maintains a higher level of species richness following fertilization compared with no phytomass removal. This corroborates the findings of Borer *et al.* (2014) that grazing can rescue richness losses in fertilized plots by allowing more light to reach ground level and by preventing competitive exclusion via intense light competition.

Species richness, but not H', was strongly negatively related to N-induced increases in phytomass, that is the greater the phytomass response to N addition, the greater the decline in species richness (Fig. 4). Clark *et al.* (2007) found a similar pattern, suggesting that changes in productivity play a key ecological role regarding species richness responses to N addition.

It is known that fertilization often decreases soil pH and that subsequent soil acidification has negative effects on plant communities (e.g. Duprè *et al.*, 2010; Bobbink & Hettelingh, 2011; Van Den Berg *et al.*, 2011; Liu *et al.*, 2012); however, the influence of initial soil pH on plant community responses to N addition is still unclear (De Schrijver *et al.*, 2011; Veresoglou *et al.*, 2011). Although Clark *et al.* (2007) found greatest species losses following N addition in plant communities with lower soil cation exchange capacity, that is in soils most prone to acidification, we did not find evidence of such a link with soil pH.

Mechanistic link to species loss

There are several ecological mechanisms that can drive grassland plant community changes following N addition. First, it has been demonstrated that fertilization can negatively impact species richness by reducing the number of available limiting resources, which diminishes trade-off opportunities that allow species coexistence (Levine & Hillerislambers, 2009; Harpole *et al.*, 2011). This increases the biomass of exploitative species which deter smaller species with low growth rates through intensified light competition (Hautier *et al.*, 2009). Accordingly, species losses would be expected to be smaller in sites with smaller relative biomass responses to N, because small changes in foliage quantity would induce only small changes in overall light availability for the whole plant community (Ren *et al.*, 2010; Borer *et al.*, 2014). This mechanism is supported by the data at hand. In line with the concept of limiting resources reduction, stronger negative effects on plant species richness were found when P and K were jointly added to N, compared to N alone, suggesting nutrient colimitation [Appendix S8, see also Ren *et al.* (2010) and Harpole *et al.* (2011)]. Fertilization can also increase belowground root competition, causing additional competitive exclusion among species (Dickson & Foster, 2011; Rajaniemi, 2002; but see Hautier *et al.*, 2009).

Limitations and research gaps

Other factors that have been shown to influence plant community responses to N addition include the following: the form of N input (i.e. ammonium, nitrate; see Song *et al.*, 2012), the scale (i.e. size of the experimental plot; see Gross *et al.*, 2009 and Spiegelberger *et al.*, 2006), habitat (e.g. dry or wet; see Theodose & Bowman, 1997) and grassland type (e.g. Wang *et al.*, 2010). Unfortunately, the effects of these factors could not be investigated in this MA due to incomplete data. We have also to recognize that changes in management from pre-experimental conditions may also generate confounding effects (Kralovec *et al.*, 2009); such changes could not be investigated as information about pre-experimental conditions was rarely provided.

The main research gap identified by this systematic review is certainly the lack of studies on invertebrate responses to grassland N addition. All studies that met inclusion criteria were on vascular plants, except two: one on bryophytes (Bergamini & Pauli, 2001) and one on Coleoptera (Majzlan & Gajdoš, 2007). While there are few observational studies on invertebrate responses to fertilization (e.g. Grandchamp *et al.*, 2005; Boschi & Baur, 2008), these did not meet our inclusion criteria. Given that insect herbivory has been demonstrated to influence both nutrient cycling and plant production (Blumer & Diemer, 1996; Belovsky & Slade, 2000), the dearth of information about the role of herbivory in grassland responses to abiotic change is a serious issue (Scherber *et al.*, 2010; Borer *et al.*, 2014). We need more comprehensive experimental research on the influence of nutrient addition on both plant and invertebrate communities, and interactions in between, to better appraise the functional ecology of grasslands (Scherber *et al*., 2010; Littlewood *et al*., 2012; Andrey *et al*., 2014).

Conclusions

Plant species losses and biomass increases following N addition appear to be a universal pattern across grassland systems. Here, we further establish that effects on species richness are negatively and additively influenced by the dose of N applied and duration of application. This finding has important conservation implications; it implies that sustained addition of relatively small N doses will ultimately reduce plant diversity in the long term. Consequently, it is important to protect grasslands not only where N application is limited if not null, but also which are not affected by N atmospheric deposition (Payne *et al*., 2013). In addition, refined estimates of exposure thresholds below which no harmful effects can be detected (e.g. in the critical load concept, Bobbink & Hettelingh, 2011) have to be defined from the dual point of view of quantity and time, this given their additive effects (see also Clark & Tilman, 2008).

We also found that the effects of N addition on species richness and diversity are less pronounced in cool summer mountain areas than in warm summer mountain areas (see also Ditommaso & Aarssen, 1989). These two areas closely match the segregation between subalpine and alpine grasslands. We even observed a positive effect of N addition on H' in the coldest summer mountain areas, that is where <4 months have a mean ambient temperature $\geq +10°C$. In the face of global warming, this finding indicates that the magnitude of the effects of N addition upon mountain plant communities might increase as summer temperatures increase (Rustad *et al*., 2001). Given that climate warming is more pronounced in mountain ranges and in boreal regions (Nogues-Bravo *et al*., 2007; Engler *et al*., 2011), it is in subalpine and boreo-alpine grassland ecosystems that we might expect major changes in vegetation.

Of course, it remains to be seen how subtle changes in the species richness and diversity and productivity of these mountain systems may alter their functioning and resilience to further environmental change. Anthropogenic N enrichment is likely to become more widespread in the future, touching remote mountain regions that have so far remained unaffected. Research is urgently needed to predict its impacts on ecosystems and their services so as to take appropriate conservation action (Bobbink & Hettelingh, 2011; Manning, 2012).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Systematic review protocol published in 2010.
Appendix S2. Search terms used and dates when literature searches were conducted.
Appendix S3. Flow diagram reporting the number of references identified, excluded and added during the literature screening process.
Appendix S4. Lists of all data points included in the review with details on extracted variables.
Appendix S5. List of all studies excluded after abstract or full text filtering and reasons for exclusion.
Appendix S6. Influence of N addition on plant species richness, Shannon index and biomass production. The statistical outputs of all null models are presented (related to Fig. 2).
Appendix S7. Model selection processes and detailed statistical outputs of the most parsimonious models about the influence of N addition on plant species richness and Shannon index.
Appendix S8. Model outputs on the influence of fertiliser type or soil pH on species richness.

APPENDIX 2

Experimental evidence for the immediate impact of fertilization and irrigation upon the plant and invertebrate communities of mountain grasslands

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Experimental evidence for the immediate impact of fertilization and irrigation upon the plant and invertebrate communities of mountain grasslands

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Summary

The response of montane and subalpine hay meadow plant and arthropod communities to the application of liquid manure and aerial irrigation – two novel, rapidly spreading management practices – remains poorly understood, which hampers the formulation of best practice management recommendations for both hay production and biodiversity preservation. In these nutrient-poor mountain grasslands, a moderate management regime could enhance overall conditions for biodiversity. This study experimentally assessed, at the site scale, among low-input montane and subalpine meadows, the short-term effects (1 year) of a moderate intensification (slurry fertilization: 26.7–53.3 kg N·ha^{-1}·year^{-1}; irrigation with sprinklers: 20 mm·week^{-1}; singly or combined together) on plant species richness, vegetation structure, hay production, and arthropod abundance and biomass in the inner European Alps (Valais, SW Switzerland). Results show that (1) montane and subalpine hay meadow ecological communities respond very rapidly to an intensification of management practices; (2) on a short-term basis, a moderate intensification of very low-input hay meadows has positive effects on plant species richness, vegetation structure, hay production, and arthropod abundance and biomass; (3) vegetation structure is likely to be the key factor limiting arthropod abundance and biomass. Our ongoing experiments will in the longer term identify which level of management intensity achieves an optimal balance between biodiversity and hay production.

Introduction

Numerous studies have documented that grassland management intensification alters biodiversity, leading to decline of plant and arthropod species richness and modifying plant traits as well as community structure (e.g., Marini et al. 2008; Riedener et al. 2013; Niu et al. 2014). Similarly, but on the other extreme of the grassland management intensity gradient, abandonment occurring in steep and less accessible mountain regions leads to forest encroachment and the disappearance of many open-habitat species (MacDonald et al. 2000; Tasser et al. 2007). However, alternatives to this dichotomous trend (agriculture intensification versus abandonment) exist in the form of an intermediate intensity of management in terms of mowing regime (e.g., Tonn and Briemle 2010; Bernhardt-Romermann et al. 2011), irrigation (Jeangros and Bertola 2000), and fertilization (e.g., Pauli et al. 2002; Bowman et al. 2006). This moderate management is likely to have conjugated positive effects on plant and invertebrate diversity, hay production, and forage nutritional quality. Different theories and factors can explain why an intermediate or moderate management intensity is likely to benefit grassland flora and fauna communities. For example, based on the hump-shaped species diversity curve of Grime (1973; see also Mittelbach et al. 2001), a moderate addition of resources should enhance plant species growth and richness. This phenomenon is especially expected in nutrient-poor montane and subalpine grasslands (Peter et al. 2009). In turn, an increase in plant growth will provide more food, space, and shelters for arthropods, boosting their abundances (e.g., Haddad et al. 2000; Perner et al. 2005; Dittrich and Helden 2011; Buri et al. 2013). Higher plant species richness not only provides more potential host plants for herbivores, but also greater horizontal and vertical vegetation structure complexity, which seems to be crucial to support higher diversity and abundance of arthropods (e.g., Brown et al. 1992; Morris 2000; Woodcock et al. 2009; Dittrich and Helden 2011). A more abundant arthropod community will promote higher trophic levels up to vertebrates through a cascading process (Hunter and Price 1992; Britschgi et al. 2006). In seminatural mountain meadows, the exact management practices that would permit decent hay production without degrading the functional integrity of the system remain unknown, thus meriting further investigation.

We launched a two-way factorial experiment on the short-, mid-, and long-term effects of fertilization and irrigation on plant and arthropod communities of montane and subalpine hay meadows of the inner European Alps (Valais, SW Switzerland). The main objective of this study is to document the short-term changes that occurred just 1 year after the onset of differential experimental management treatments. While end-user management recommendations will be based on the longer-term outputs of the study, thoroughly assessing the short-term effects clarifies the ecological mechanisms at play during the temporal process of grassland intensification. More specifically, we addressed two questions: (1) What are the short-term effects of fertilization and irrigation, considered separately and in combination, on plant species richness, vegetation structure, hay production, and arthropod abundance and biomass? and (2) what is the relationship between vegetation and arthropod parameters?

Plants and arthropods were hypothesized to respond differently to the fertilization and irrigation treatments in the short-term, that is, after just 1 year of experimental manipulation, partly because plants typically have a slower reaction time than animals to changes in environmental conditions (Mortimer et al. 1998; Cole et al. 2010). More specifically, we expected slight positive effects of fertilization on plant species richness and hay production (Grime 1973), while an increase in plant growth and richness was expected to increase vegetation structure, which would in turn promote arthropod populations (Woodcock et al. 2009). On the other hand, we predicted that irrigation would have no effect on plant species richness (Riedener et al. 2013), but still positive effects on arthropod abundance through an increased phytomass productivity and protection against dessication (Nielsen 1955). Fertilization was also predicted to increase herbivorous arthropod abundances, owing to an increase in plant tissue nitrogen content (Haddad et al. 2000; Dittrich and Helden 2011). However, due to a highly diverse plant species pool among all our meadows (given that they have been extensively managed over the past decades), a high ecological stability and resistance against the experimental treatments were expected in the short term (Tilman and Downing 1994), therefore translating into few contrasted effects.

Materials and Methods

Study sites

In 2010, twelve extensively managed montane and subalpine hay meadows were selected according to their management history. The meadows had to be managed extensively for at least the last 10 years with no or very low levels of fertilization (with solid manure only) and irrigation (terrestrial only), and only a single cut per year. Their homogeneous topography and their size were also considered (>4000 m^2). The study sites were situated in the inner Alps (Valais, SW Switzerland) between 790 and 1740 m above sea level, encompassing a wide gradient of altitudes and ambient temperatures (Table 1). This region experiences a continental climate with cold and wet winters, and dry and hot summers.

Design

A two-way full factorial design was applied in our experiments. At each study site, that is, in each meadow, four circular plots of 20 m in diameter were established with at least 5 m between plot boundaries. The different management treatments were randomly assigned to the four plots within a given meadow. The first plot served as a control (C-plot: neither irrigation nor fertilization). The second plot was only irrigated (I-plot) at regular time intervals with sprinklers. The third plot was only fertilized (F-plot) with liquid manure, and the fourth plot was irrigated and fertilized (I + F-plot). C-plots were cut once a year, which corresponds to local standards for extensively managed meadows, while I, F, and I + F-plots were cut twice a year. Although this discrepancy deviated the design from a purely speaking two-way full factorial design, it made agronomical sense; local farmers would not irrigate or fertilize their field without doing a second cut. Treatments I and I + F were irrigated weekly from mid-May to the beginning of September, except when heavy rainfall occurred (>20 mm over the previous week).

Table 1. Description of the twelve study sites with altitude, exact coordinates, and quantity of fertilizer, that is, nitrogen (N), phosphorus (P), and potassium (K), applied per hectare per year. The fertilizer consisted of organic NPK pellets, and mineral K_2O dissolved in water to reach the equivalent of standard-farm liquid manure.

			Coordinates		Fertilizer applied [$kg \cdot ha^{-1} \cdot year^{-1}$]		
Site	Name	Altitude [m]	Latitude	Longitude	N	P	K
1	La Garde	980	46°3′45″N	7°8′35″E	40.0	33.3	133.3
2	Sembrancher	798	46°4′24″N	7°8′36″E	53.3	44.4	177.7
3	Orsières	1022	46°1′44″N	7°9′8″E	53.3	44.4	177.7
4	Vens	1373	46°5′7″N	7°7′24″E	40.0	33.3	133.3
5	Euseigne	1028	46°10′9″N	7°25′27″E	53.3	44.4	177.7
6	Eison	1768	46°9′18″N	7°28′10″E	26.7	22.3	89.0
7	St-Martin	1589	46°11′8″N	7°26′43″E	26.7	22.3	89.0
8	Grimentz	1738	46°11′22″N	7°34′35″E	26.7	22.3	89.0
9	Arbaz	1270	46°16′42″N	7°22′47″E	40.0	33.3	133.3
10	Icogne1	1200	46°17′56″N	7°26′31″E	40.0	33.3	133.3
11	Icogne2	880	46°17′6″N	7°26′10″E	53.3	44.4	177.7
12	Cordona	1153	46°19′45″N	7°33′8″E	40.0	33.3	133.3

Weekly sprinkler irrigation amounted to 20 mm of water column. The fertilizer consisted of organic dried manure NPK pellets (MEOC SA, 1906 Charrat, Switzerland), and mineral potassium oxide (K_2O) dissolved in water to reach the equivalent of standard-farm liquid manure (Sinaj et al. 2009), consisting namely of 2.4 kg of usable nitrogen, 2 kg of phosphate (P_2O_5), and 8 kg of potassium oxide (K_2O) per m^3 of solution. 174, 262, or 349 l of liquid manure per plot, corresponding to, respectively, 26.7, 40.0, or 53.3 kg $N \cdot ha^{-1}$ $year^{-1}$, were applied three times in August 2010, May 2011, and August 2011 (Table 1). The exact amount of manure applied at each site depended on the theoretical local hay production potential calculated using pre-experimental hay yield (when extensively managed) and site elevation, and it matched the local mid-intensive management norm recommended in Sinaj et al. (2009). In each plot, a 4 × 2 m permanent rectangle subplot was established at a distance of 4 m from plot center, randomly placed along the slope axis on the right or the left side of the plot. In each subplot, we measured plant species richness, vegetation structure, hay production, and abundance and biomass of arthropods (Fig. 1).

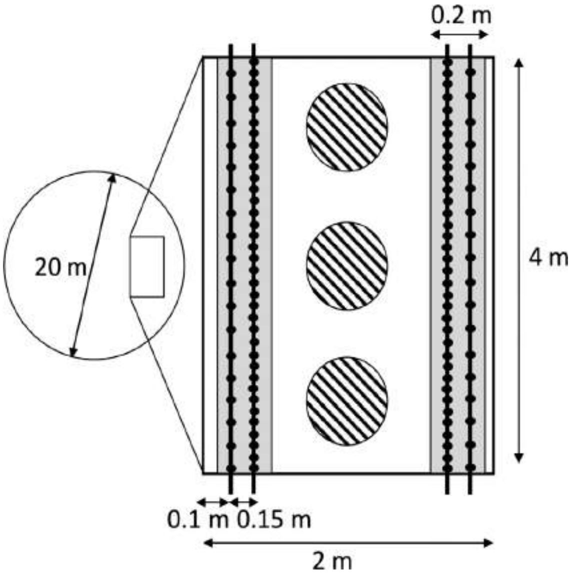

Figure 1. Experimental design. Four management treatments were applied at random onto 20-m-diameter circles delineated on each meadow. In each circle (excerpt), vegetation (*n* = 122 records per circle, black dots), hay production (gray strips), and arthropods (three dashed circles of 0.2 m^2) were sampled.

Vegetation sampling

In 2011, plant species richness, vegetation structure, and hay production were assessed twice: once just before the first cut (from mid-June to end of July, at a similar vegetation stage, depending on altitude; hereafter referred to as July samples) and once just before the second cut (from August to September; hereafter August samples). Vegetation surveys were performed using the point quadrat method in order to obtain information on the vertical distribution of each plant species (Stampfli 1991). For that purpose, we developed an ad hoc device that consisted of a 4.10-m-long steel bar (supported by two tripods) that contained 41 holes distant of 10 cm (Appendix 1). Graduated metal sticks of 5 mm in diameter were inserted vertically into the holes. Each plant species touching the stick was recorded, and the height at which the plant touched the stick was noted. If the same species touched more than once a single stick, the maximal height was retained. The sampling device was positioned along each long side of the permanent rectangular subplot, first 10 cm and then 25 cm from the long edge (Fig. 1). We recorded contacts between plants and sticks at 20 and 41 holes (points) when the device was positioned at 10 cm and 25 cm from the edge, respectively. Altogether, we thus recorded 122 points in each plot. A modified Shannon–Wiener diversity index (Woodcock et al. 2009) was used to define the structure of the vegetation:

$$Struct = \sum_{i=1}^{n} p_i \log_e p_i$$

where *Struct* is the index for vegetation structure and p_i the proportion of the number of contacts with the stick at each height *i*, in each subplot, at each sampling session. Thus, greater structural complexity of the vegetation results in a higher value.

Just before each grass cut, hay production was estimated by clipping two strips of grass with an area of 0.2 × 4 m along each long edge of the permanent subplot at 6 cm above the ground, exactly where the vegetation relevés had been performed (Fig. 1). The two samples from the same subplot were then pooled together. The collected plant material was dried at 105°C during 72 h and then weighed (±0.1 g) in order to quantify hay production.

Arthropod sampling

Arthropods were sampled using a suction sampler (Stihl SH 86 D; Stihl) equipped with a gauze sampling sack fixed inside the nozzle to collect arthropod items. This technique has been proved to be efficient for grassland vegetation-dwelling arthropods (Sanders and Entling 2011). All plots were sampled twice during the vegetation season, once before each grass cut. At each sampling session, three subsamples were collected at three regularly spaced locations in the middle of each permanent subplot (Fig. 1). Subsamples consisted of the vacuumed content of a metallic cylinder of 50 cm height and 50.5 cm diameter (0.2 m^2 area) that was placed directly on the ground. The content of the gauze sampling sack was transferred into a sealed plastic bag stored at low temperature in an ice-cooled box. Sampling was undertaken between 11:00 and 17:00, only under dry vegetation conditions and with low or moderate wind. Arthropod specimens were then stored in the laboratory at −20°C before being classified in six main taxonomic groups: spiders, Auchenorrhyncha (i.e., plant- and leafhoppers), weevils, leaf beetles, ants, and others (other arthropods not belonging to the previous groups). The number of specimens was counted prior to drying the arthropods at 60° during 72 h. Finally, all arthropod groups stemming from one subsample were weighed (±0.1 mg). For statistical analyses, the three subsamples per plot were summed. Ants had to be discarded because suction trapping proved to be inefficient for sampling this group due to their massive local colonial aggregations.

Statistical analysis

Treatment effects were analyzed with linear mixed-effects models (LMMs) using the *lmer* function from the *lme4* package for R (Bates et al. 2011). *P*-values and confidence intervals (CI) were computed with the *pvals.fnc* function from the *languageR* package using 100,000 Markov chain Monte Carlo iterations (Baayen 2011). Response variables were log-transformed plant species richness, vegetation structure, hay production, log-transformed arthropod abundance, and log-transformed arthropod biomass. As grass (Poaceae), legume (Fabaceae), and forb species may respond differently to the management treatments (e.g., Li et al. 2010), additional models on the relative cover of each functional group were run. Note that not all variables needed log-transformation prior to analysis to achieve normal distribution of residuals. The fixed effects were the treatments (C, I, F, or I + F) and the sampling sessions (July or August) which were added as a factor to take in account the fact that two measures were made per plot. For hay production, analyses were performed on the sum of the July and August (pooled samples). Thus, for this variable, fixed effects were limited to the treatments. The study sites (geographic replicates) were designated as a random effect. To better appraise differences between treatments, post hoc tests were performed using the function *relevel* of R to change the first reference level of the factor "treatment."

In order to further understand the relationship between the vegetation and arthropod parameters, simple linear regressions were performed using the *lm* function (Crawley 2007). The log-transformed abundance and biomass of arthropods were fitted against plant species richness, vegetation structure (index *Struct*), and hay production. Finally, to test whether the variance in arthropod abundance and biomass (variance of the nontransformed raw data) changes with respect to vegetation structure, a homoscedasticity test (Bartlett's test) was conducted between the values obtained from the first and the third quantiles of *Struct* (Crawley 2007). Thus, a significant *P*-value would indicate that with low vegetation structure, there are only few arthropods, while with a higher vegetation structure, it is possible to have either few or many arthropods (see Fig. 4). In other words, this value indicates whether vegetation structure limits arthropod abundance and/or biomass. All statistical tests were performed using R version 2.15.3 (R Core Team 2013).

Results

Effects of irrigation and fertilization on the vegetation

In total, 194 plant species belonging to 34 families were identified during the two sampling sessions across all meadows (see Appendix 2 for a complete list of the plant species recorded). F-plots, I-plots, and I + F-plots harbored significantly more plant species than C-plots (Fig. 2; and Table A3.1 in Appendix 3 for related model outputs). Moreover, irrigated plots (I and I + F) had significantly higher species richness than F-plots, but treatment I + F was not different from I. Irrigated plots exhibited a higher vegetation structure (index *Struct*) than C-plots and F-plots, while treatment F did not differ from C. The greatest vegetation structure was measured in July and the lowest in August; this pattern was consistent across all treatments. Annual hay production (sum of both sampling sessions) ranged from 96.5 to 1111 g·m^{-2} across all plots. It was approximately three times higher in the irrigated plots compared with C-plots, but I + F treatment did not differ from treatment I. Fertilization (F) had a lower effect compared with irrigation but still gave a significantly higher hay production than C.

Relative cover of grasses decreased in I, F, and I + F-plots compared with the control plots, while legumes increased their cover (Fig. 3). Relative changes were all significant at a $P < 0.01$ level (see Table A3.2 in Appendix 3 for exact values of models outputs). Forb species cover did not differ among treatments except I + F that had significantly less cover than C ($P = 0.011$).

Effects of irrigation and fertilization on the arthropods

In total, 7198 arthropods (ants excluded) were collected across all replicates (3923 in July and 3275 in August). The samples included *n* individuals of the following taxa: 629 spiders (Araneae), 1869 plant- and leafhoppers (Hemiptera: Auchenorrhyncha), 562 weevils (Coleoptera: Curculionidae), 587 leaf beetles (Coleoptera: Chrysomelidae), and 3551 others. Abundance of arthropods in I-plots and I + F-plots were significantly higher than in C-plots and F-plots (Fig. 2; and Table A3.1 in Appendix 3 for related model outputs). Treatment F did not deliver a higher abundance of arthropods compared with treatment C. The only significant differences within a single arthropod group were for plant- and leafhoppers where in I + F-plots, there were more individuals compared with C-plots (MCMC mean = 0.890, 95% CI = 0.281–1.511, *P* MCMC = 0.005) and to F-plots (MCMC mean = 0.766, 95% CI = 0.161–1.385, *P*

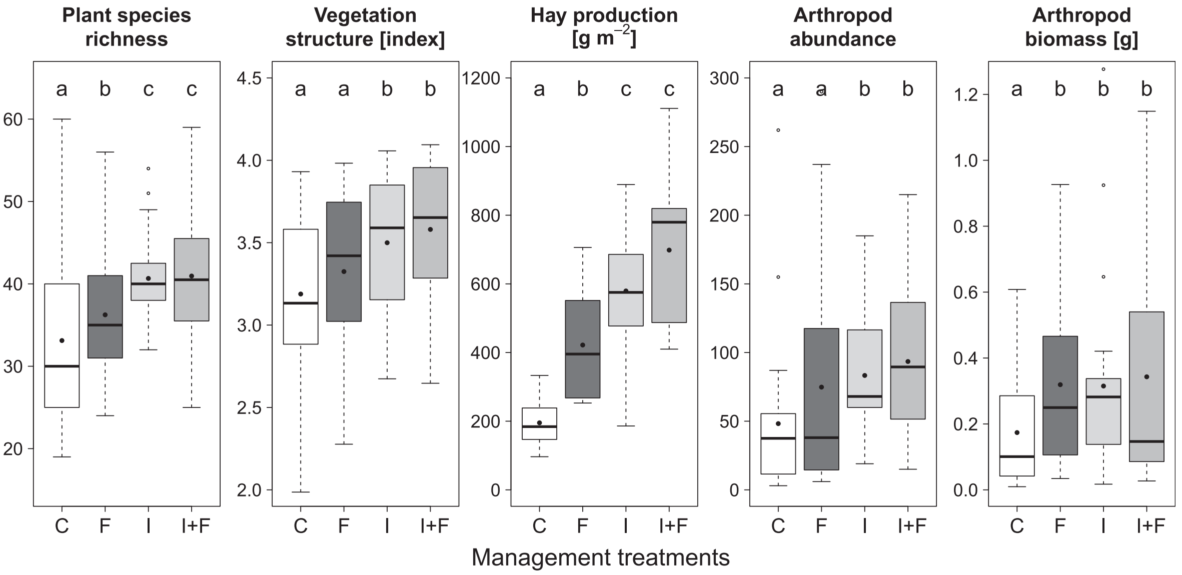

Figure 2. Responses of the vegetation (plant species richness, vegetation structure and hay production) and arthropod (abundance and dry biomass) variables to the different management treatments. Bold lines represent medians, solid points the means, boxes the first and third quantiles. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Treatments abbreviations are as follows: (C) control; (I) irrigated, (F) fertilized, and (I + F) irrigated and fertilized.

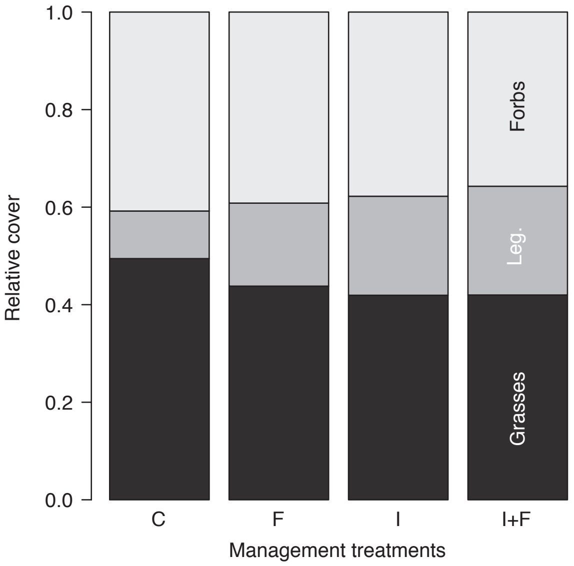

Figure 3. Responses of relative cover of grass (dark-gray), legume (mid-gray), and forb (light-gray) species to the different management treatments. Model outputs (including estimates, CIs, and *P*-values) are provided in Table A3.2 in Appendix 3. For treatment abbreviations, see legend of Fig. 2.

MCMC = 0.015). For spiders, abundance in I + F-plots was marginally significantly higher than in C-plots (MCMC mean = 0.375, 95% CI = −0.021–0.759, *P* MCMC = 0.060), while no differences were detected between I-plots and F-plots, on one side, and C-plots, on the other side.

In total, 26.92 g dry weight of arthropods was collected across all replicates (17.13 g in July and 9.79 g in August). The samples (excluding ants) included the following taxa: 1.856 g of spiders, 2.705 g of plant- and leafhoppers, 0.766 g of weevils, 0.458 g of leaf beetles, and 21.130 g for others. All treatments affected positively the biomass of arthropods (Fig. 2; and Table A3.1 in Appendix 3). The biomass of plant- and leafhoppers was significantly higher in I + F-plots than in the C-plots (MCMC mean = 0.019, 95% CI = 0.001–0.037, *P* MCMC = 0.038), while there were no significant biomass differences between treatments and controls in another arthropod taxonomic group.

Relationships between arthropods and vegetation

The total abundance of arthropods was positively linked to hay production (estimate = $2.60 \cdot 10^{-3}$ $t = 4.767$, $P < 0.001$; adjusted $R^2 = 0.186$, i.e. 18.6% explained variance), plant species richness (estimate = $6.79 \cdot 10^{-2}$ $t = 6.696$; $P < 0.001$, $R^2 = 0.316$), and vegetation structure (estimate = 0.572, $t = 2.752$, $P = 0.007$, $R^2 = 0.065$). The variance in arthropod biomass was explained in about the same order of magnitude by hay production (estimate = $2.905 \cdot 10^{-3}$ $t = 5.085$, $P < 0.001$, $R^2 = 0.207$), plant species richness (estimate = $5.580 \cdot 10^{-2}$ $t = 4.747$, $P < 0.001$, $R^2 = 0.185$), and vegetation structure (estimate = 1.049, $t = 5.182$, $P < 0.001$, adjusted $R^2 = 0.214$). Note that estimates are on the log scale. Regarding the analyses about whether vegetation structure limits arthropods, for both arthropod abundance (Bartlett's $K^2 = 6.933$, df = 1, $P = 0.008$) and biomass (Bartlett's $K^2 = 23.145$, df = 1, $P < 0.001$), Bartlett's test showed a greater variance at the third than at the first quantile of vegetation structure (Fig. 4).

Discussion

This study shows that among low-input montane and subalpine hay meadows, plant species richness, vegetation structure, hay production as well as arthropod abundance and biomass all immediately and positively react to moderate experimental fertilization and irrigation. It should be noted, however, that the starting conditions in our study meadows were typical of the traditional, extremely extensive management practices that have been prevailing for centuries in the inner Alps, with very low fertilizer application and limited terrestrial irrigation. It is thus not totally surprising that our experimental treatments improved both biodiversity and hay yield in the very short term. These traditional grasslands typically are poor in nitrophilous species with specialized taxa present due to a very constraining edaphic context and watering regime (Peter et al. 2009). The speed at which these changes operated in response to intensification was, however, unexpectedly rapid. A powerful advantage of our full block design approach is certainly that it allows a direct comparison of the effects of both irrigation and fertilization, which were either separated or conjugated, upon meadowland ecological communities regardless of other potentially confounding abiotic factors such as altitude, exposition, or soil properties.

Effects of fertilization and irrigation on the vegetation

Fertilizing with liquid manure and watering with sprinklers are two modern, currently spreading management practices, even in remote areas of the Alps (Riedener et al. 2013). Our treatments thus mimic the trends of modern agriculture in these areas. Although we had predicted slower effects on plant species richness, basing our predictions on the dynamics observed in most long-term studies in alpine and arctic regions (e.g., Carlen et al.

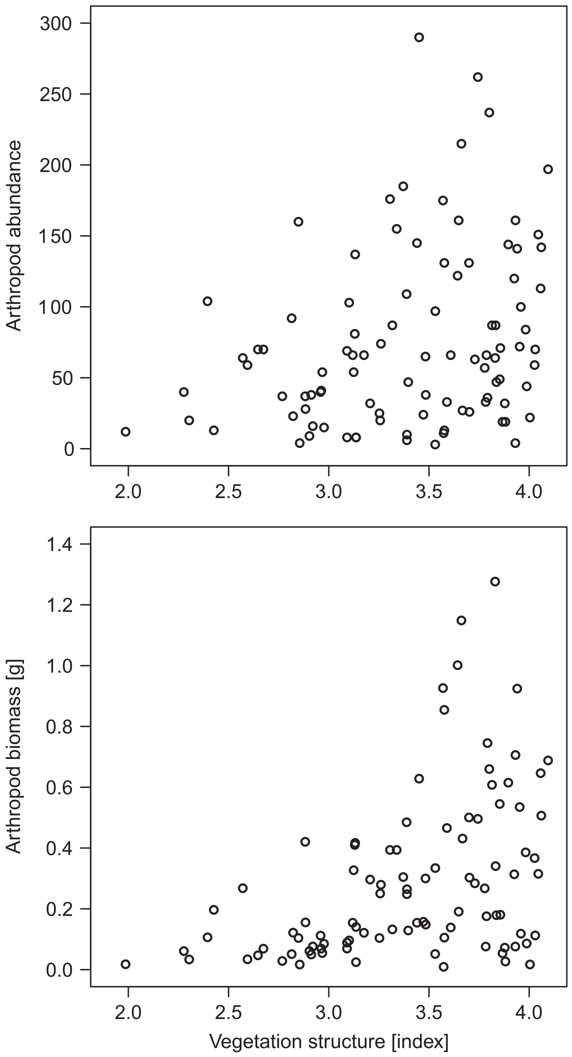

Figure 4. Relationships between arthropod abundance and biomass versus vegetation structure (index *Struct*). Greater the structure of the vegetation, higher the *Struct* index.

1998; Yang et al. 2011), our findings are in accordance with the predictions of the hump-shaped model of plant diversity (Grime 1973; Mittelbach et al. 2001). This model stipulates that an intermediate level of intensification must support a higher plant species richness than low- or high-input systems. Yet, we cannot exclude, given that we measured effects just 1 year after the onset of the experimental treatments, that abiotic factors, such as interannual weather variation, might have interacted with the treatment effects, amplifying the signal (Walker et al. 1994). What is certain, however, is that no plant community would ever reach an equilibrium after just 1 year of this management (Yang et al. 2011). Hence, a short-term, moderate intensification as applied here may indeed promote high plant species richness because it rapidly offers favorable conditions to nitrophilous and mesophilous species that are normally absent on nutrient-poor and dry soils. Some of the original plant species pool consisting of heliophilous species, tolerant to reduced nutrients and water supply but particularly intolerant to intensification and shade, may actually have persisted in the community merely because they were already extant. This suggests the possibility of a short-term coexistence of plants with different life-history traits and varied ecological requirements (Bowman et al. 2006). In the mid- and long run, however, one would expect that interspecific competition for resources such as light will especially increase among some species. Species exhibiting characteristics such as low growth rate could become progressively disadvantaged and possibly decline to local extinction (Rajaniemi 2002; Hautier et al. 2009).

Irrigating and fertilizing increased the relative cover of legumes, which appears to be mostly at the expense of the cover of grasses. While this seems in contradiction with most grassland fertilization studies that found the reverse pattern regarding their biomasses (e.g. DiTommaso and Aarssen 1989; Carlen et al. 1998; Li et al. 2010), it must be stressed that relative cover does not necessarily correlate with biomass, especially when comparing grasses that grow tall and thin with legumes that tend to grow wider. In addition, fertilization studies that found positive effects of intensification on grasses and negative effects on legumes usually applied mineral fertilizers, while the application of organic fertilizers is known to have slightly different influences, typically favoring legume species (e.g., Vintu et al. 2011).

In contradiction to our prediction that fertilization would have a positive short-term effect on all vegetation parameters, addition of liquid manure alone did not increase vegetation structure, while the combination of fertilization and irrigation did not elicit a greater response from vegetation parameters than did irrigation alone. This indicates that in the short term, application of fertilizer (only) might enhance the sensitivity of the vegetation to water stress (Huston 1997) or that our meadows were more likely to be limited by water supply than nitrogen supply. Indeed, the climatic context in the inner Alps is characterized by its dryness (Central Valais, around Sion-Visp, is the pole of xericity in the whole Alpine massif, with ca 500 mm annual precipitation), with even April-June 2011 slightly drier than interannual average (94 mm vs. 136 mm mean rainfall during 2006–2010 in Sion; MeteoSwiss). Plant nutrient uptake may also have been improved by water addition thus enhancing plant growth (Davis et al. 2000). Future vegetation surveys in the same study meadows will enable disentangling climatic from agronomic effects, while characterizing mid- and longer-term changes in plant communities.

Effects of fertilization and irrigation on arthropod communities

Irrigation in turn had a positive effect on arthropod species richness, as predicted. This indicates that water might be a limiting factor for arthropods (e.g., intolerance to desiccation; Nielsen 1955), or that there is an indirect effect mediated via plants onto arthropods. In contrast, fertilization per se led to no discernible effect on arthropods, corroborating previous findings in comparable montane ecosystems (Grandchamp et al. 2005). The less complex vegetation structure achieved via fertilization alone compared with irrigation means that the offer of microhabitats and the resulting ecological niche opportunities are less favorable when only fertilization is augmented (Reid and Hochuli 2007). Irrigation and fertilization were also expected to increase the rate of herbivory, that is, the abundance of plant- and leafhoppers, and as a result increase the abundance of their predators such as spiders (Kirchner 1977). However, only plant- and leafhoppers showed a numeric response to irrigation and fertilization suggesting that a steady state had not been achieved with no discernible effects being propagated to the upper trophic levels along the food chain at this stage. It is also important to note that a much smaller sample size for predator taxa than for prey taxa could have blurred the pattern due to lower statistical power.

Relationships between arthropods and vegetation

Vegetation parameters such as plant species richness, plant biomass, and vegetation structure all influence arthropod community to some extent (Knops et al. 1999; Haddad et al. 2000). There is still an ongoing debate about which factor has the greatest impact on arthropods (Perner et al. 2005), but recent studies have pointed out that vegetation structure might be the crux (Woodcock et al. 2009; Dittrich and Helden 2011). Our analyses show that all vegetation parameters influence arthropods to a certain degree. However, neither plant species richness (31.6% of explained variance for abundance/18.5% for biomass) nor hay production (18.6%/20.7%) or vegetation structure (6.5%/21.4%) individually accurately predicted arthropod abundance and biomass. This seems to contradict the view that vegetation structure is a key factor. However, there is evidence that vegetation structure did profoundly influence the number of arthropods in our meadows (Fig. 4), yet vegetation structure is more likely to act as a limiting than a predictive factor. Indeed, at low vegetation structure, low arthropod abundance and biomass always prevail, whereas at high vegetation structural diversity, arthropod abundance and biomass can either be low or high. This pattern is in line with the predictions of the habitat heterogeneity hypothesis (Brown et al. 1992). A higher entanglement of plant aboveground parts can increase the mobility of grass-dwelling arthropods (Randlkofer et al. 2009) through better vertical and horizontal connectivity while it offers a broader palette of ecological niches (Duffey 1962). Thus, if complex vegetation structure is a *sine qua non* condition for high arthropod abundance and biomass, it does not guarantee it. It is likely that source populations must exist in the surrounding matrix to colonize any newly emerging, highly structured vegetation patches. Moreover, new detrimental factors generated by high vegetation structure might also obliterate the ability of arthropod populations to develop, such as microclimatic conditions that adversely affect some taxa (increase moisture or shade) or altered diffusion of plant volatiles that hampers resource location (e.g. Van Wingerden et al. 1992; Finch and Collier 2000; Després et al. 2007).

Conclusion

Although plant community stability was likely not achieved after just 1 year of experimental fertilization and irrigation, our findings demonstrate that on a short-term basis, a moderate level of intensification positively affects biodiversity and hay production of low-input, extensively managed montane and subalpine meadows. Tremendous land-use changes steadily affect mountainous regions, leading either to abandonment of marginal grasslands or to intensification of fields accessible to machinery (Tasser et al. 2007). This rather dichotomous trend should be reversed, which calls for more intermediate management practices if one wants to concomitantly promote grassland biodiversity and acceptable agricultural revenue. Although this short-term study only provides insights into the mechanism of intensification within upland grasslands, the continuation of our experiments will deliver detailed prescriptions in the mid term for optimizing slurry fertilization and aerial irrigation so as to achieve the best possible compromise between hay production, biodiversity preservation, and ecosystem functioning among montane and subalpine hay meadows.

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Conflict of Interest

None declared.

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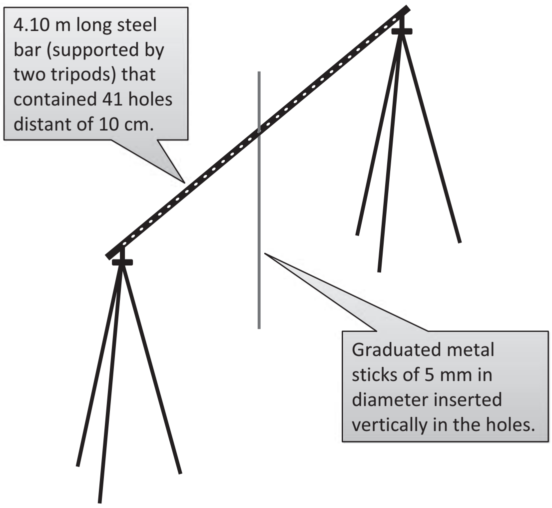
Appendix 1: Drawing of the ad hoc device used to sample the vegetation (point quadrat method).



4.10 m long steel bar (supported by two tripods) that contained 41 holes distant of 10 cm.

Graduated metal sticks of 5 mm in diameter inserted vertically in the holes.

Appendix 2: A complete list of the plant species identified during the two sampling sessions across all treatments in all meadows.

Table A2.1. In total, 194 plant species belonging to 34 families were identified during the two sampling sessions across all meadows.

Plant species name	Family	Plant species name	Family
Achillea millefolium l.	Asteraceae	Crepis conyzifolia (Gouan)	Asteraceae
Acinos alpinus (l.) Moench	Lamiaceae	Crepis pyrenaica (l.) Greuter	Asteraceae
Agrimonia eupatoria l.	Rosaceae	Crocus albiflorus Kit.	Iridaceae
Agrostis capillaris l.	Poaceae	Cynosurus cristatus l.	Poaceae
Agrostis stolonifera l.	Poaceae	Dactylis glomerata l.	Poaceae
Ajuga pyramidalis l.	Lamiaceae	Dactylorhiza fuchsii (Druce) sod	Orchidaceae
Ajuga reptans l.	Lamiaceae	Descampsia sp	Poaceae
Alchemilla vulgaris aggr.	Rosaceae	Elymus repens (l.) Gould.	Poaceae
Allium oleraceum l.	Liliaceae	Erucastrum nastrurtiifolium	Brassicaceae
Anthericum ramosum l.	Liliaceae	Euphorbia cyparissias l.	Euphorbiaceae
Anthoxanthum odoratum l.	Poaceae	Euphorbia verrucosa l.	Euphorbiaceae
Anthriscus sylvestris (l.) Hoffm.	Apiaceae	Euphrasia rostkoviana aggr.	Scrophulaceae
Anthyllis vulneraria l.	Fabaceae	festuca arundinacea schreb.	Poaceae
Arabis ciliata Clairv.	Brassicaceae	festuca ovina l.	Poaceae
Arabis hirsuta (l.) scop.	Brassicaceae	festuca pratensis Huds.	Poaceae
Arenaria serpyllifolia l.	Caryophyllaceae	festuca rubra l.	Poaceae
Arrhenatherum elatius (l.)	Poaceae	festuca valesiaca Gaudin	Poaceae
Asperula cynanchica l.	Rubiaceae	filipendula vulgaris Moench	Rosaceae
Avenella flexuosa (l.) Drejer	Poaceae	Galium anisophyllum Vill.	Rubiaceae
Botrychium lunaria (l.) sw.	Ophiolglossaceae	Galium boreale l.	Rubiaceae
Brachypodium pinnatum (l.)	Poaceae	Galium mollugo aggr.	Rubiaceae
Briza media l.	Poaceae	Galium pumilum Murray	Rubiaceae
Bromus erectus Huds.	Poaceae	Galium verum l.	Rubiaceae
Bunium bulbocastanum l.	Apiaceae	Gentiana acaulis l.	Gentianacees

Table A2.1. Continued.

Plant species name	Family	Plant species name	Family
Campanula glomerata l.	Campanulaceae	Gentiana campestris l.	Gentianacees
Campanula rhomboidalis l.	Campanulaceae	Gentiana verna l.	Gentianacees
Campanula rotundifolia l.	Campanulaceae	Geranium sanguineum l.	Geraniaceae
Campanula scheuchzeri Vill.	Campanulaceae	Geranium sylvaticum l.	Geraniaceae
Cardamina hirsuta	Brassicaceae	Geum montanum l.	Rosaceae
Carex caryophyllea latourr.	Cyperaceae	Gymnadenia conopsea (l.) r. Br.	Orchidaceae
Carex flacca schreb.	Cyperaceae	Helianthemum nummularium (l.) Mill.	Cistaceae
Carex montana l.	Cyperaceae	Helictotrichon pubescens (Huds.) Pilg.	Poaceae
Carex ornithopoda Willd.	Cyperaceae	Hepatica nobilis schreb.	Renonculaceae
Carex pallescens l.	Cyperaceae	Heracleum sphondylium l.	Apiaceae
Carex sempervirens Vill.	Cyperaceae	Hieracium murorum aggr.	Asteraceae
Carlina acaulis l.	Asteraceae	Hieracium piloselloides Vill.	Asteraceae
Carum carvi l.	Apiaceae	Hippocrepis comosa l.	Fabaceae
Centaurea jacea l.	Asteraceae	Hypericum perforatum l.	HypEricaceae
Centaurea scabiosa l.	Asteraceae	Hypochoeris maculata l.	Asteraceae
Cerastium arvense l.	Caryophyllaceae	Inula salicina l.	Asteraceae
Cerastium fontanum	Caryophyllaceae	Knautia arvensis (l.) Coult.	Dipsacaceae
Chaerophyllum hirsutum l.	Apiaceae	Knautia dipsacifolia Kreutzer	Dipsacaceae
Cirsium acaule scop.	Asteraceae	Koeleria pyramidata (lam.) P. Beauv.	Poaceae
Cirsium arvense (l.) scop.	Asteraceae	laserpitium latifolium l.	Apiaceae
Clinopodium vulgare l.	Lamiaceae	laserpitium siler l.	Apiaceae
Colchicum alpinum DC.	Liliaceae	lathyrus pratensis l.	Fabaceae
Colchicum autumnale l.	Liliaceae	leontodon hispidus l.	Asteraceae
Crepis aurea (l.) Cass.	Asteraceae	leucanthemum vulgare aggr.r	Asteraceae
Crepis biennis l.	Asteraceae	linaria vulgaris Mill.	Scrophulaceae
linum catharticum l.	Linaceae	Prunella vulgaris l.	Lamiaceae
listera ovata (l.) r. Br.	Orchidaceae	Pulmonaria australis (Murr)	Lamiaceae
lolium perenne l.	Poaceae	Pulsatilla alpina (l.) Delarbre	Renonculaceae
lotus corniculatus l.	Fabaceae	ranunculus acris l.	Renonculaceae
luzula campestris (l.) DC.	Joncaceae	ranunculus bulbosus l.	Renonculaceae
luzula nivea (l.) DC.	Joncaceae	ranunculus montanus aggr.	Renonculaceae
luzula sylvatica aggr.	Joncaceae	ranunculus tuberosus lapeyr.	Renonculaceae
Medicago lupulina l.	Fabaceae	rhinanthus alectorolophus (scop.)	Scrophulaceae
Molinia arundinacea schrank	Poaceae	rosa pendulina l.	Rosaceae
Molinia caerulea (l.) Moench	Poaceae	rubus caesius l.	Rosaceae
Myosotis arvensis Hill.	Boraginaceae	rumex acetosa l.	Polygonaceae
Myosotis sylvatica Hoffm.	Boraginaceae	salvia pratensis l.	Lamiaceae
Nardus stricta l.	Poaceae	sanguisorba minor scop.	Rosaceae
Onobrychis viciifolia scop.	Fabaceae	sanguisorba officinalis l.	Rosaceae
Ononis repens l.	Fabaceae	scabiosa columbaria l.	Dipsacaceae
Ononis spinosa l.	Fabaceae	securigera varia (l.) lassen	Fabaceae
Paradisea liliastrum (l.) Bertol.	Liliaceae	selaginella selaginoides (l.)	Selaginellaceae
Pastinaca sativa l.	Apiaceae	sesleria caerulea (l.) Ard.	Poaceae
Peucedanum oreoselinum (l.)	Apiaceae	silene nutans l.	Caryophyllaceae
Phleum alpinum l.	Poaceae	silene vulgaris (Moench) Garcke	Caryophyllaceae
Phleum pratense l.	Poaceae	soldanella alpina l.	Primulaceae
Phyteuma betonicifolium Vill.	Campanulaceae	stachys recta l.	Lamiaceae
Phyteuma orbiculare l.	Campanulaceae	Taraxacum officinale aggr.	Asteraceae
Phyteuma spicatum l.	Campanulaceae	Thalictrum minus aggr.	Renonculaceae
Picris hieracioides l.	Asteraceae	Thesium alpinum l.	Santalaceae
Pimpinella saxifraga l.	Apiaceae	Thesium pyrenaicum Pourr.	Santalaceae
Plantago atrata Hoppe	Plantaginaceae	Thymus serpyllum aggr.	Lamiaceae
Plantago lanceolata l.	Plantaginaceae	Tragopogon pratensis l.	Asteraceae
Plantago media l.	Plantaginaceae	Trifolium alpestre l.	Fabaceae
Poa alpina l.	Poaceae	Trifolium badium schreb.	Fabaceae
Poa bulbosa l.	Poaceae	Trifolium dubium sibth.	Fabaceae

Table A2.1. Continued.

Plant species name	Family	Plant species name	Family
Poa pratensis l.	Poaceae	Trifolium medium l.	Fabaceae
Poa trivialis l.	Poaceae	Trifolium montanum l.	Fabaceae
Polygala alpestris rchb.	Polygalceae	Trifolium pratense l.	Fabaceae
Polygala chamaebuxus l.	Polygalceae	Trifolium repens l.	Fabaceae
Polygala comosa schkuhr	Polygalceae	Trisetum flavescens (l.) P. Beauv.	Poaceae
Polygala sp.	Polygalceae	Trollius europaeus l.	Renonculaceae
Polygala vulgaris l.	Polygalceae	Vaccinium myrtillus l.	Ericaceae
Polygonatum odoratum	Liliaceae	Verbascum nigrum l.	Scrophulaceae
Polygonum viviparum l.	Polygonaceae	Veronica arvensis l.	Scrophulaceae
Potentilla aurea l.	Rosaceae	Veronica chamaedrys l.	Scrophulaceae
Potentilla crantzii fritsch	Rosaceae	Veronica teucrium l.	Scrophulaceae
Potentilla erecta (l.) raeusch.	Rosaceae	Vicia cracca l.	Fabaceae
Potentilla pusilla Hostr	Rosaceae	Vicia sativa l.	Fabaceae
Potentilla rupestris l.	Rosaceae	Vicia sepium l.	Fabaceae
Potentilla thuringiaca link	Rosaceae	Viola hirta l.	Violaceae
Primula veris l.	Primulaceae	Viola rupestris f. W. schmidt	Violaceae
Prunella grandiflora (l.) scholler	Lamiaceae	Viola tricolor l.	Violaceae

Appendix 3: Outputs of the linear mixed-effects models (LMMs) carried out on: (1) the effects of fertilization and irrigation on plant species richness, vegetation structure, hay production, arthropod abundance and biomass; and (2) the effects of fertilization and irrigation on the relative cover of grass, legume, and forb species. Table A3.1 refers to figure 2, and Table A3.2 refers to figure 3.

Table A3.1. Results of the linear mixed-effects models (LMMs) carried out on the effects of fertilization and irrigation on plant species richness, vegetation structure, hay production, arthropod abundance and biomass. Table refers to figure 2 in the article. The fixed factors were the experimental treatments (with four levels: C = control plots; F = fertilized; I = irrigated; I + F = irrigation and fertilization combined) and the sampling sessions (two levels: July and August). Random factor were the experimental study sites. *P*-values and 95% confidence intervals (CI) were computed with 100,000 Markov chain Monte Carlo (MCMC) iterations. MCMC mean parameter estimates (differences between expected mean densities) are given for the paired treatments comparisons, and significant contrasts are highlighted in bold.

Response variable and comparison	MCMC mean	Lower 95% CI	Upper 95% CI	MCMC *P*-value
Plant species richness (log scale)				
F vs. C	0.109	0.016	0.205	**0.023**
I vs. C	0.240	0.145	0.333	**<0.001**
I + F vs. C	0.236	0.144	0.331	**<0.001**
I vs. F	0.130	0.035	0.223	**0.007**
I + F vs. F	0.127	0.033	0.221	**0.009**
I + F vs. I	−0.003	−0.097	0.092	0.947
Structure of vegetation (index)				
F vs. C	0.136	−0.001	0.272	0.051
I vs. C	0.311	0.176	0.450	**<0.001**
I + F vs. C	0.392	0.255	0.529	**<0.001**
I vs. F	0.175	0.039	0.311	**0.012**
I + F vs. F	0.256	0.121	0.395	0.001
I + F vs. I	0.081	−0.054	0.219	0.247
Hay production [$g \cdot m^{-2}$]				
F vs. C	226.8	101.1	352.5	**0.001**
I vs. C	384.4	262.6	514.1	**<0.001**

Table A3.1. Continued.

Response variable and comparison	MCMC mean	Lower 95% CI	Upper 95% CI	MCMC *P*-value
I + F vs. C	503.2	379.7	630.8	**<0.001**
I vs. F	157.6	29.0	280.2	**0.015**
I + F vs. F	276.7	150.2	400.7	**<0.001**
I + F vs. I	118.8	−7.2	245.6	0.065
Arthropod abundance (log scale)				
F vs. C	0.403	−0.039	0.845	0.072
I vs. C	0.935	0.497	1.378	**<0.001**
I + F vs. C	1.014	0.579	1.452	**<0.001**
I vs. F	0.534	0.087	0.966	**0.018**
I + F vs. F	0.612	0.164	1.044	**0.006**
I + F vs. I	0.077	−0.365	0.514	0.730
Arthropod biomass [g] (log scale)				
F vs. C	0.829	0.327	1.303	**0.001**
I vs. C	0.824	0.325	1.306	**0.001**
I + F vs. C	0.734	0.237	1.219	**0.004**
I vs. F	−0.005	−0.501	0.477	0.983
I + F vs. F	−0.094	−0.579	0.397	0.706
I + F vs. I	−0.091	−0.587	0.389	0.716

Table A3.2. Results of the linear mixed effects models (LMMs) carried out on the effects of fertilization and irrigation on the relative cover of grass, legume and forb species. Table refers to figure 3 in the article. The fixed factors were the experimental treatments (with four levels: C = control plots; F = fertilized; I = irrigated; I+F = irrigation and fertilization combined) and the sampling sessions (two levels: July and August). Random factors were the experimental study sites. *P*-values and 95% confidence intervals (CI) were computed with 100,000 Markov chain Monte Carlo (MCMC) iterations. MCMC mean parameter estimates (differences between expected mean densities) are given for the paired treatments comparisons and significant contrasts are highlighted in bold.

Response variable and comparison	MCMC mean	Lower 95% CI	Upper 95% CI	MCMC *P*-value
Grasses (Poaceae)				
F vs. C	−0.056	−0.099	−0.014	**0.009**
I vs. C	−0.075	−0.117	−0.033	**0.001**
I + F vs. C	−0.075	−0.116	−0.032	**0.001**
I vs. F	−0.019	−0.061	0.023	0.380
I + F vs. F	−0.018	−0.059	0.024	0.390
I + F vs. I	0.001	−0.042	0.043	0.974
Legumes (Fabaceae)				
F vs. C	0.073	0.037	0.108	**<0.001**
I vs. C	0.105	0.070	0.140	**<0.001**
I + F vs. C	0.125	0.091	0.162	**<0.001**
I vs. F	0.033	−0.003	0.068	0.070
I + F vs. F	0.053	0.018	0.088	**0.004**
I + F vs. I	0.020	−0.015	0.055	0.261
Forbs				
F vs. C	−0.016	−0.055	0.024	0.415
I vs. C	−0.030	−0.070	0.009	0.131
I + F vs. C	−0.051	−0.090	−0.012	**0.011**
I vs. F	−0.014	−0.054	0.025	0.479
I + F vs. F	−0.035	−0.074	0.005	0.083
I + F vs. I	−0.021	−0.059	0.020	0.302

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ERKLÄRUNG

Erklärung

gemäss Art. 28 Abs. 2 RSL 05

Name/Vorname:	Hayoz-Andrey Aline
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LeiterIn der Arbeit:	Prof. Dr. R. Arlettaz Dr. Jean-Yves Humbert

Bachelor ☐ Master ☐ Dissertation X

Ich erkläre hiermit, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen benutzt habe. Alle Stellen, die wörtlich oder sinngemäss aus Quellen entnommen wurden, habe ich als solche gekennzeichnet. Mir ist bekannt, dass andernfalls der Senat gemäss Artikel 36 Absatz 1 Buchstabe r des Gesetzes vom 5. September 1996 über die Universität zum Entzug des auf Grund dieser Arbeit verliehenen Titels berechtigt ist. Ich gewähre hiermit Einsicht in diese Arbeit.

Ort/Datum : Bern,

Unterschrift

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EDUCATION

2010-present	**Institute for Ecology and Evolution, Conservation Biology, University of Bern,** Switzerland PhD thesis: *Optimal balance between yield and biodiversity in montane and subalpine meadows*
2009	**Institute of Biology, University of Iceland, Reykjavík, Iceland** Master thesis: *Physiological responses and PAHs uptake in relation to tidal impact under winter condition on blue mussel (Mytilus Edulis L.).* Supervisor: Prof. Jörundur Svavarsson (University of Iceland), Dr. Nathalie Chèvre (EPFL)
2004-2009	**School of Architecture, civil and environmental engineering, environmental engineering section, Swiss Federal Institute of Technology,** Lausanne (EPFL)
2006-2009	Master degree Specialized study: Natural Water, Soil and Ecosystems Engineering, including a minor in glaciology (ETHZ).
2004-2006	Bachelor degree
2002-2003	**Department of Environmental Systems Science, Agricultural Science Swiss Federal Institute of Technology,** Zurich (ETHZ) Propedeutic degree
1997 - 2001	**Collège du Sud,** Bulle Federal maturity in sciences

POSITIONS HELD

2014	**Research project** *Infoflora,* Bern, Switzerland Surveys of threatened plant species in Switzerland for the National Red List.
2010	**Internship** *Swiss Federal Institute for Forest, Snow and Landscape Research,* Davos, Switzerland Research project : Snow distribution dynamics under forest canopy.
2008	**Research project** *Regional Nature Park Gruyères - Pays-*d'Enhaut, Switzerland Environmental management system and life cycle assessment of a wood product used as roof cover ("le Tavillon").
2008	**Research project** *Stucky SA,* Lausanne, Switzerland Integrated watershed management project for a swiss river (Haute-Sarine)
2004	**Internship** *Institut agricole de Grangeneuve, (IAG)*, Posieux, Switzerland Assistant for research projects in plant production and Person in charge of detection of fire blight in the canton of Fribourg, Switzerland
2004	**Internship** *Mr. Schneider's Farm,* Misery, Switzerland Active participation in agricultural works (crops and animal care)
2003	**Internship** *«Los Cedros» Natural Reserve,* Quito, Ecuador Collect and classification of tropical weeds
2002	**Internship** *«Montana Verde Association»,* San Isidro del General, Costa Rica Young farmers group leader's assistant, production of organic fertilisers

LIST OF PUBLICATIONS

2015	Humbert, J. Y., Dwyer, J., **Andrey, A**., & Arlettaz, R. (2015). Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review. *Global change biology*.
2014	**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(12), 2610-2623.

PRICES AND AWARDS

2013	**ScienceComm'13 Conference**, (La Chaux-de Fond, Switzerland) from Swiss Interacademic Commission for Alpine Studies ICAS for the best presentation.
2012	**ProMontesPreis Price**, (Thun, Switzerland) from the Swiss Foundation for Alpine Research («Schweizerischen Stiftung für Alpine Forschung (SSAF) ») for the best presentation and innovative project.

ORAL PRESENTATIONS

2014	**La Murithienne, Société valaisanne des Sciences Naturelles**, (Sion, Switzerland) Andrey A., Humbert J.-Y., Arlettaz R. *Effets de la fertilisation et de l'irrigation sur les prairies de montagne.*
2013	**Swiss Alpine Club, Section Dent-de-Lys,** (Châtel-St-Denis, Switzerland) Andrey A., *Agriculture de montagne et biodiversité.*
2013	**ScienceComm'13 Conference,** (La Chaux-de-Fond, Switzerland) Science Slam : *Intensification of subalpine meadows.*

2012 **Interacademic commission for alpine studies,** (Thun, Switzerland)

Andrey A., Humbert J.-Y., Arlettaz R. *Compte-rendu des effets à court-terme de l'intensification des prairies de montagne.*

3rd European Conference of Conservation Biology (Glasgow, UK)

Andrey A., Humbert J.-Y., Arlettaz R. *Short-term effect of land-use management on montane meadows.*

2008 **Regional Nature Park Gruyères - Pays-d'Enhau**t, (Charmey, Switzerland)

Andrey A., *Analyse du cycle de vie du Tavillon comme couverture des chalets d'alpage.*

POSTERS

2013 **Mountains under watch, Observing climate change effects in the Alps**, (Aosta Valley, Italy)

Andrey A., Humbert J.-Y., Arlettaz R. « Shifting land-use systems: short-term effects on biodiversity in subalpine meadows »

2012 **13th Student conference in Conservation Sciences** (University of Cambridge, UK)

Andrey A., Humbert J.-Y., Arlettaz R. « Direct effects of fertilisation and irrigation on extensive meadows at subalpine levels. »

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Andrey A., Humbert J.-Y., Arlettaz R. « Changes in irrigation and fertilisation regime of extensively managed montane meadows. »