Natural and anthropogenic determinants of biodiversity of grasslands in the Swiss Alps

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Chapter 1

General introduction

The aim of this thesis

This thesis comprehensively studies biodiversity at all levels of biological integration (communities within a landscape, species diversity within communities, biological interactions, diversity within a species) in grasslands in the Swiss Alps. It evaluates the relative importance of potential drivers of biodiversity in the alpine landscape, such as land use, altitude or, specifically in Switzerland, cultural traditions. Identifying determinants of biodiversity at all levels creates the basis for political measures for its protection. Moreover, the thesis analyzes whether drivers of biodiversity act opposingly upon different levels of biodiversity and wants to detect potential conflicts between these levels as well as between biodiversity and agriculture.

Biodiversity

Since the United Nations Conference on Environment and Development (UNCED), held in Rio de Janeiro 1992, the importance and the value of biodiversity are recorded in the Convention on Biological Diversity (United Nations 1992) and are widely accepted. The 170 contracting countries are responsible for conserving their biological diversity and for using their biological resources in a sustainable way. Biodiversity comprises several levels, such as the diversity of landscapes and communities, the diversity of biological interactions, and genetic diversity within species (Primack 2002). These levels are all of equal importance. Moreover, these levels of biodiversity need to be considered at different spatial scales, within (alpha-diversity) and between (beta-diversity) study sites (Primack 2002). To be able to comprehensively conserve biodiversity, a good knowledge of all levels and spatial scales is necessary (Till-Bottraud & Gaudeul 2002). However, comprehensive studies providing such knowledge are very scarce.

A high biodiversity is important because it increases the stability of ecosystems (Hector et al. 1999) and enhances the sustainability of resource exploitation (Klaus et al. 2001). Moreover, while a high diversity of species and genotypes increases stress tolerance, redundancy of functional relationships protects ecosystems against disturbances (Hooper et al. 2005). These ecological reasons to protect biodiversity are completed by further motivations. For example, ethical reasons confirm every individual's right of existence (Aus der Au 2003). Furthermore, the existing biodiversity is a cultural heritage that should be conserved for future generations. And finally, from the esthetic point of view, communities of high plant species diversity are more appreciated by the public than those of low diversity (Junge 2004).

Limitations of biodiversity conservation

Although, from the scientific point of view, the importance of biodiversity conservation is widely accepted, its conservation is limited by its costs. Biodiversity usually benefits society as a whole but the costs of its conservation fall upon the individual, leading to a close connection of ecology and economy (Edwards & Abivardi 1998). Furthermore, different perception of biodiversity by different stakeholders complicates its conservation (Baur 2004). As an example, tourists and hikers enjoy colorful meadows rich in flowers, while for farmers their yield is more important. Parcels of land laborious to manage and returning only a small yield are most likely to get abandoned if farmers are not rewarded for their service for society. Therefore, in the Swiss Alps socio-economically motivated changes in land use have been taking place and are still going on, or are even enhanced by international treaties.

Alpine biodiversity

The Alpine landscape is characterized by strong natural gradients such as topography and climate creating different habitats and a high biodiversity. Furthermore, human land use has shaped the biodiversity of mountain areas (Nagy et al. 2003). A recent inventory of alpine biodiversity in Europe focused on taxonomic richness of plant and animal communities including their pattern and diversity in space and time and the underlying ecosystem processes above timberline studied in different European mountain systems (Nagy et al. 2003). In the Swiss Alps plant species diversity above timberline was found to decrease with altitude (Theurillat et al. 2003) as it has been observed in a variety of alpine habitats (Grabherr et al. 1995). Comparing invertebrates, a decreased diversity with increasing altitude can also be observed in many invertebrate groups (Brandmayr et al. 2003) with the exception of mobile taxa, such as butterflies, where habitat type and grazing impact were found to be more important than altitude (Tontini et al. 2003). However, all these studies concentrated on the level of species diversity and did not consider other levels of biodiversity. Therefore, comprehensive studies including all levels of biodiversity are necessary.

Agricultural land use in the Swiss Alps

Human land use is one of the most important determinants of grassland biodiversity. In Switzerland alpine grasslands have been influenced by humans for more than 5000 years (Bätzing 2003). Most grasslands below tree line are man-made, substituting cleared forests, and their plant species diversity is up to three times higher than in the forests they are replacing (Zoller & Bischof 1980). In the cultural landscape of the Alps, these grasslands contribute to a diverse mosaic of pastures, meadows and forests resulting in one of the most plant species rich areas in Europe (Väre et al. 2003). Plant species diversity within parcels depends on the type and intensity of land use regimes which include stocking rates or cutting frequency and levels of fertilization. Some parcels are fertilized, mostly with liquid manure or dung, while others are not fertilized. Pastures are grazed by cattle, dairy cows, mother cows, sheep or goats, either only for a few days or weeks, or during the whole summer. Depending on climatic conditions, stocking rate and duration of grazing can vary considerably among years (Pykälä 2005). Unfertilized meadows are usually cut once a year while fertilized meadows are cut between two and four times per season.

Changes in land use

Since the Second World War severe socio-economically motivated land use changes have been taking place which are still going on. A development towards abandonment of parcels used at low intensity far away from farms, which led to a dramatic decline in grassland area, has been observed in Switzerland and other European countries (Haefner & Günter 1984; Olsson et al. 2000; Lindborg & Eriksson 2004). Simultaneously, easily accessible parcels are used more intensively as fertilizer application is increased, meadows are cut more often, and pastures are grazed with more and larger cattle (Bätzing 2003). Although these changes are likely to affect biodiversity negatively, larger-scale and comprehensive studies investigating these effects are missing in the Alps.

Cultural traditions

In the Swiss Alps we distinguish three main old cultural traditions. The Romanic culture developed with the increase of the human population in Europe after 1800 BC, when settlements and land use were extended into Alpine valleys. It is characterized by a self-sufficient alpine economy practicing mixed farming with permanent compact villages at the valley bottoms, transient summer settlements at higher altitudes, and (sub)alpine pastures (Im Hof et al. 1986). Hereditary partitioning of parcels was frequent in the Romanic culture. The small-grained and frequently terraced parcel structure is still visible in many Romanic villages (Bätzing 2003).

The Germanic culture developed after 600 AD, when Alemannic people from the North immigrated into the wetter northern and northeastern parts of the Alps. Germanic villages typically consisted of scattered single farm houses surrounded by fields and meadows, and the farmers practiced mainly dairy farming. Permanent fields for grain cultivation were rare, the farm was inherited as a whole by a son of the farmer, hereditary partitioning of parcels was uncommon and farms were usually larger than in Romanic villages (Bätzing 2003).

After 1200 AD, the Walser, Alemannic people from the canton Valais, migrated eastwards and settled at relatively high altitudes, as lower parts of the valleys were already occupied. As a consequence the Walser had to abandon cultivation of grain. Apart from farming they lived on trade and maintenance of alpine passes (Bätzing 2003).

In spite of important changes during the last century, remarkable socio-economic differences can still be detected among these cultural traditions (Pfister 2004). Hence, we hypothesize that the observed socio-economic differences and the ongoing socio-economic changes act differently upon land use in villages of the three cultural traditions. Therefore, we expect to find differences in biodiversity among cultural traditions to be detectable via different land use regimes.

The Swiss agricultural payment system

Swiss agriculture is supported by a two-stage payment system (Bundesamt für Landwirtschaft 2004). In contrast to former times, these payments are less and less based on production but rather on achievements and ecological services rendered by the farmers. General direct payments are intended to ensure area-wide utilization and maintenance of agricultural land. Additionally, farmers' extra efforts due to aggravating topography are rewarded. To obtain general direct payments, farmers must comply with certain minimal ecological requirements concerning e.g., livestock husbandry or soil protection, and every farm has to manage 7 % of its land as ecological compensation area. This comprises structures of high biological value like hedges or extensively used meadows. Additionally, farmers can get ecological direct payments to reward further efforts for a sustainable agriculture. Ecological direct payments comprise payments for diverse kinds of ecological compensation areas as mentioned above, payments for a certain ecological quality and for interconnectedness of ecological compensation areas, for biological agriculture, or for special conditions for livestock husbandry. In the mountainous regions where this study takes place and where the yield per area is considerably lower than in the lowlands, direct payments made up 36.8 % of farmers' gross profit in 2003. Although achievements have been made to reward high ecological quality of agriculturally used land (measured by the occurrence of certain plant species), due to the system's voluntariness only 4 % of ecological direct payments are really based on ecological quality. Therefore, although ecology and biodiversity are getting more and more considered, the Swiss agricultural payment system is still rather based on the area of agriculturally used land and the number of livestock than on the ecological value of the managed land. Biodiversity is only a minimal criteria for financial incentives.

The National Research Programme 48 „Landscapes and Habitats of the Alps“

Within the framework of the National Research Programme 48 „Landscapes and Habitat of the Alps“ (NRP 48) of the Swiss National Science Foundation, scientists in 35 inter- and transdisciplinary projects study economic, cultural and ecological processes concerning landscape and habitat dynamics in the Swiss Alps. These research projects' aim is to acquire knowledge on goals and actions needed for a landscape development which is socially desired, economically acceptable and politically feasible. Obtained results will contribute to implement norms for the sustainable use and shaping of landscapes and habitats. With the NRP 48, public awareness of the collective goods of landscape and habitat with their extensive social significance should be increased. The NRP 48 concentrates on five main topics a couple of research projects. Land use and biodiversity are among the major issues and are investigated by several projects. Our project considers all levels of biodiversity from the landscape to the gene to address the complexity of biodiversity and considers the effects of different cultural traditions on the landscape of the Swiss Alps which makes it particular within the NRP 48.

This doctoral thesis

Design

We comprehensively studied the effects of potential natural and anthropogenic drivers of biodiversity at all levels of biodiversity using a hierarchical parcel-based design. In 12 villages in the Swiss Alps (see Chapter 2, Fig. 1) we selected grassland parcels at three altitudinal levels, at the valley bottom (about 1000 m asl), at intermediate altitudes (in some regions called “Maiensäss”, about 1500 m asl), and at the alp level (about 2000 m asl). Each of the three cultural traditions Romanic, Germanic, and Walser was represented by four villages. We searched for grassland parcels defined by the combination of three altitudinal levels (as described above), traditional land use (mown or grazed), current land use (mown, grazed, or abandoned), and fertilization status (fertilized or unfertilized). Altogether we selected 216 grassland parcels, between 12 and 24 per village. All or a subset of these grassland parcels were the study objects of the seven chapters of this thesis. All chapters were written for publication in scientific journals.

Outline

Chapter 2 addresses the level of plant species diversity. We explored whether cultural traditions, altitude, or type of land use affect the likelihood of occurrence of a certain type of grassland parcel, plant species richness, Shannon index, and evenness, and productivity. Furthermore, we tested whether the diversity of land use types was linked with plant species diversity within a village. To assess plant species richness we took two vegetation records per parcel in randomly selected 5 m x 5 m plots by estimating ground cover for each species and counting the number of species per record. For each parcel we calculated mean cover per species and by using these values we obtained Shannon’s index of diversity and Evenness. To estimate productivity we harvested standing crop 4 cm above ground in a randomly selected sub-area of 0.5 m x 0.5 m in each plot and calculated mean standing crop per parcel.

Chapter 3 complements the level of plant species diversity. Using the same vegetations records as in Chapter 1, we tested whether cultural traditions, altitude, or type of land use affect plant species composition and ecological properties of the communities. We especially focused on the consequences of grazing or abandonment of formerly unfertilized meadows for grassland species typical for unfertilized meadows.

Chapter 4 addresses the level of biological interactions between plants, herbivores, and fungal pathogens. We asked how the extent of leaf damage by herbivory and fungal pathogen infection, as measures of level and diversity of plant-herbivore and plant-fungal pathogen interactions, are affected by plant functional group, land use, and altitude. Moreover, we explored whether extent and diversity of leaf damage are related to each other, to plant species richness, and to standing crop. We recorded the percent leaf area damaged by ten types of herbivory and five types of fungal pathogen infection on 12’054 plant leaves of legumes, other forbs, and graminoids collected in 215 of the 216 grassland parcels of different land use and altitude.

The following four chapters are dedicated to the genetic diversity of *Poa alpina* L.

In **Chapter 5** we characterize five microsatellite DNA markers in *P. alpina*. Five out of 15 microsatellite inserts turned out to be polymorphic in a tested sample. This allowed us to study molecular genetic variation in *P. alpina*. This chapter presents a first application of these microsatellite markers and is the prerequisite for the subsequent Chapter 6.

In **Chapter 6** we used these five microsatellite markers to study the genetic diversity of 569 *P. alpina* plants originating from 54 agriculturally used grassland parcels and 20 natural sites. We asked whether populations from agriculturally used grasslands were differentiated from natural populations. Furthermore, we analyzed whether genetic differentiation among villages and among parcels was related to geographic distances and to differences in land use. Moreover, we explored whether genetic variation within grassland parcels, estimated as numbers of alleles per plant and per parcel, was related to altitude, land use and reproductive mode.

Chapter 7 firstly relates the occurrence of the species to land use types and altitude. Secondly, we present results from a quantitative common garden field experiment with two plants of each of 615 genotypes originating from 57 grasslands parcels and from 21 natural sites. We wanted to answer the question whether plant performance in the common garden suggested local adaptation of *P. alpina* to land use and altitude by measuring several vegetative and reproductive traits and calculating allocation to reproductive biomass.

Chapter 8 extends the study of determinants of genetic diversity of *P. alpina* to quantitative genetic diversity. We asked whether there was heritable genetic variation in vegetative and reproductive traits in P. alpina and, if yes, whether this variation was related to abiotic factors. land use, and cultural traditions. As measures for quantitative genetic diversity we used broad-sense heritability of several vegetative and reproductive traits for 825 P. alpina plants originating from 53 grassland parcels measured in the common garden experiment. Furthermore, by combining the results of all studies of this thesis, we analyzed how different levels of biodiversity were related to each other.

In the **General Discussion** I analyze the effects of cultural traditions, altitude, and land use on all investigated levels of biodiversity.

In the **Summary** I give an overview of the most important results of this thesis, draw conclusions including all levels of biodiversity, and make suggestions for further research and conservation.

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Chapter 2

Old cultural traditions, in addition to land use and topography, are shaping plant diversity of grasslands in the Alps

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Abstract

Socio-economically motivated land use changes are a major threat for species diversity of grasslands throughout the world. Here, we comprehensively explore how plant species diversity of grasslands in the species-rich cultural landscape of the Swiss Alps depends on recent land use changes, and, neglected in previous studies, on old cultural traditions. We studied diversity in 216 grassland parcels at three altitudinal levels in 12 villages of three cultural traditions (Romanic, Germanic, and Walser). In valleys of Romanic villages more different parcel types tended to occur than in those of Germanic and Walser villages, suggesting that socio-economic differences among cultural traditions still play a role in shaping landscape diversity. Moreover, at the village level, higher man-made landscape diversity was associated with higher plant species richness. All observed changes in land use reduced the farmers' workload. Plant species richness was lower in fertilized than in unfertilized parcels and in abandoned compared with used parcels. Grazing slightly reduced species richness compared with mowing among unfertilized parcels, while in fertilized parcels it had a positive influence. The highest species diversity was found in mown unfertilized subalpine grasslands. Nevertheless, moderate grazing of former meadows can be a valuable alternative to abandonment. We conclude that the ongoing changes in land use reduce plant species richness within parcels and at the landscape level. To preserve plant species diversity at the landscape level a high diversity of land use types has to be maintained.

Keywords: biodiversity, grazing, mowing, species richness, conservation

Introduction

Human land use is among the most important determinants of grassland biodiversity. In the cultural landscape of the Alps, one of the most plant species rich areas in Europe (Väre et al., 2003), human impacts are particularly pronounced. In Switzerland Alpine grasslands have been influenced by humans for about 5000 years (Bätzing, 2003). Below timberline most grasslands are man-made. They replace cleared forests and harbor up to three times more species than those (Zoller and Bischof, 1980). In the Alps grassland plant species diversity has to a large extent been shaped by different agricultural traditions (Stebler and Schröter, 1892). Low-intensity farming promotes biodiversity. However, for farmers low-intensity farming often means intensive human labor and small yield (Bignal and McCracken, 1996). Therefore, current socio-economic changes such as abandonment of farms and a reduced number of people working in agriculture are bringing about severe land use changes. In recent decades low-intensity land use of many grassland parcels far away from farms has been abandoned, in Switzerland and in other European countries, which led to a dramatic decline in grassland area (Haefner and Günter, 1984; Olsson et al., 2000; Tasser and Tappeiner, 2002; Lindborg and Eriksson, 2004). On the other hand, easily accessible parcels are used more intensively (Kahmen et al., 2002). More fertilizer is used, meadows are cut more often and pastures are grazed by more and larger cattle (Bätzing, 2003). These land use changes may reduce plant species richness and therefore receive most attention in the current debate on how agricultural policy can balance socio-economic and conservation considerations. While many of these issues have been addressed in local studies, larger-scale and comprehensive studies investigating the changes in land use and their consequences for biodiversity are missing in the Alps. Moreover, despite the long history of grassland management in the Alps, the effect of cultural traditions on current biodiversity has not been studied.

In the Swiss Alps three main cultural traditions are distinguished. The Romanic culture developed after 1800 BC, when settlements and land use were extended into Alpine valleys. Self-sufficient mixed farming was practiced from permanent compact villages at the valley bottoms, transient summer settlements at higher altitudes, and in (sub)alpine pastures (Im Hof et al., 1986). Hereditary partitioning of parcels was frequent and led to the still visible small-grained parcel structure of many Romanic villages (Bätzing 2003). The Germanic culture developed after 600 AD, when

Alemannic people from the North immigrated into the wetter northern and northeastern parts of the Alps. Typical Germanic villages of scattered single farm houses were surrounded by fields and meadows mainly used for dairy farming. Farms were inherited as a whole and therefore were usually larger than in Romanic villages (Bätzing, 2003). After 1200 AD, the Walser, Alemannic people from the Valais, migrated eastwards and settled at higher altitudes, as lower parts of the valleys were already occupied. Apart from farming they lived on trade and maintenance of alpine passes (Bätzing, 2003). Despite important changes since World War II, socio-economic differences are still remarkably pronounced among these cultural traditions (Pfister, 2004).

Effects of cultural traditions on plant diversity may be superimposed by effects of topography, climate, and soil conditions. Altitudinal gradients therefore play an important role (Rahbek, 1995; Theurillat et al., 2003). Moreover, abiotic conditions can vary considerably among regions in the Alps. Thus, Swiss landscapes may differ in the size of their regional species pools which may potentially occur in grasslands (Welten and Sutter, 1982; Wohlgemuth, 1998; Koellner et al., 2004). Moreover, at the landscape level, the number of different habitat types can affect species richness as suggested by the habitat diversity hypothesis (Williams, 1964), the habitat heterogeneity hypothesis (Levin, 1974), or the mosaic concept (Duelli, 1997).

Swiss agriculture is supported by a two-stage payment system (Bundesamt für Landwirtschaft, 2004). General direct payments are intended to ensure area-wide maintenance of agricultural land and therefore reward farmers' extra efforts due to aggravating topography. The ecological requirements to receive these payments are quite modest. They include that at least 7 % of the farm area have to be so-called ecological compensation areas. Additionally to these general payments farmers can receive ecological direct payments rewarding further efforts for sustainability. However, because none of these payments takes cultural differences or local and regional land use diversity into account, they contribute to homogenize landscapes and agriculture.

We studied the effect of cultural traditions, altitude, and agricultural land use on plant species diversity in 216 grassland parcels in 12 villages in the Swiss Alps. Each cultural tradition was represented by four villages, and the parcels were situated at three altitudinal levels. We studied parcels that traditionally had been mown or grazed, that currently were mown, grazed or abandoned and that either were fertilized or unfertilized. We asked the following questions: (1) Does the likelihood of occurrence of a certain type of grassland parcel depend on cultural traditions, altitudinal level, and land use? (2) Did land use changes in the study parcels consistently indicate reduced labor for farmers? (3) Does the diversity of land use types affect plant species diversity in a village? (4) Does species diversity per parcel depend on cultural traditions, altitude, and land use, and which types of land use are associated with highest plant species richness?

Methods

Study area

We studied grasslands in 12 villages in the Swiss Alps, four of each of the three cultural traditions Romanic, Germanic, and Walser (Fig. 1). Villages are political municipalities that may comprise several smaller hamlets. The 12 villages are situated along an east-west gradient spanning 170 km, and each village belongs to a separate Alpine valley. The villages were randomly selected, with the restriction that their agricultural character had only changed modestly during the last 50 years, that they were not very touristy and did not have more than about 1500 inhabitants. Due to settlement history, the Germanic villages rather are located in the northern parts of the Alps and the Romanic and Walser villages in central and southern parts. The studied grasslands are mainly on siliceous bedrock forming brown podzolic soils, podzols, and rankers, and in a few sites they are on calcareous bedrock forming gray-brown podzolic soils and rendzinas (Frei et al., 1964).

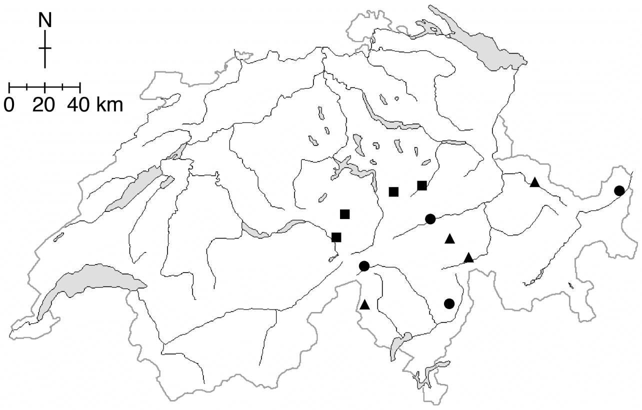

Fig. 1. Map of Switzerland with the 12 study villages and their cultural traditions.

● = Romanic
■ = Germanic
▲ = Walser

Regional species pools

As topography, climate, and soil conditions could vary considerably among villages, this could influence the number of species potentially inhabiting the grasslands of each village. The number of potential inhabitants is represented by the regional species pool (Wohlgemuth, 1998; Koellner et al., 2004). For each village we obtained the number of plant species of the regional species pool from "The Distribution Atlas of Pteridophytes and Phanerogams of Switzerland" (Welten and Sutter, 1982). For this atlas, species lists were compiled for almost 600 naturally defined sub-areas of about 100 km^2. Each area was classified as valley (from valley bottom to timberline) or mountain area (above timberline). For each of the 12 investigated villages we combined the species list of the concerned valley area with the surrounding mountain areas with the software VEGEDAZ (Küchler, 2004). To exclude species mainly occurring in wetlands or forests from this list we removed all species with ecological indicator values of 5 for moisture and 1 for light according to Landolt (1977). For each village we considered the number of remaining species on the corresponding list as size of the regional species pool. The sizes of the regional species pools were independent of the mapping area (between 154.8 and 324.6 km^2, Pearson's product-moment correlation, $r = -0.07$, $p = 0.8356$) and the altitudinal range of each pool (altitudinal differences between lowest and highest point of each area varied between 2 019 and 3 529 m, Pearson's product-moment correlation, $r = -0.27$, $p = 0.3909$).

Study design

In each village we selected grassland parcels at three altitudinal levels, at the valley bottom (about 1000 m asl), at intermediate altitudes (about 1500 m asl), and at the alp level (about 2000 m asl). We included parcels that had been used in the same way for as long as it was known and parcels whose use had been changed five years ago or earlier. We looked for parcels which had traditionally been mown or grazed, currently were either mown or grazed or had been abandoned some years ago, and that were either additionally fertilized or unfertilized. Thus, for each village and altitudinal level we looked for parcels of 12 different combinations of land use, leading to a theoretical maximum of 432 parcels.

We selected the parcels in close collaboration with local farmers in 2002. They knew the land use history of their villages and helped to select parcels representative for the agricultural practice in each village. When it was impossible to find parcels whose type of land use had changed, we selected two parcels of the concerned type of former land use to increase sample size. Altogether we selected 216 parcels, between 12 and 24 per village. The fertilized parcels were all fertilized with fertilizer produced on the farm, either with liquid manure or dung from the stables. According to the farmers the selected unfertilized parcels had never been fertilized. Unfertilized meadows were normally cut once a year while fertilized meadows were cut two to four times. Grazing intensity by sheep and cattle varied among years depending on climatic conditions.

Vegetation records

In each parcel we randomly selected two 5 m x 5 m plots at a distance of 5 m. We classified each plot according to three classes of structural heterogeneity (homogeneous: cover of bare ground, stones, and wood < 20% and relief ± even; little heterogeneous: cover of bare ground, stones, and wood between 20 and 30% or relief with a few small bumps; very heterogeneous: cover of bare ground, stones, and wood between 30 and 40% or relief with bumps). Additionally we recorded coordinates and altitude of the parcels with a GPS, aspect as deviation from south in degrees, slope in degrees, and soil pH with a Hellige set (AVM Analyseverfahren, Freiburg, Germany).

In each plot we took a vegetation record according to a refined Braun-Banquet (1951) method (for cover values above 15% we used categories of 10% difference: 16-25%, 26-35%, etc.). We visited each parcel once in 2002 or 2003, when the vegetation was best developed, usually shortly before the start of mowing or grazing. We estimated the ground cover percentage of all vascular plant species and counted the number of species S per record. For each record we calculated Shannon's index of diversity (Magurran, 1988) and Evenness as $E_{1/D} = \frac{1/D}{S}$. This evenness index based on a diversity index derived from Simpson's index of dominance D ($D = \sum_i p_i^2$, $p_i =$ relative abundance of species i) is independent of species richness (Smith and Wilson, 1996).

We also calculated mean ground cover of the two plots per parcel for each species and parcel and obtained Shannon's index of diversity and Evenness also for these mean cover values. To measure the similarity between species compositions of the two records per parcel we calculated the Jaccard index as described in Magurran (1988).

Because it was logistically not possible to repeatedly monitor productivity in the 216 parcels, we harvested standing crop once 4 cm above ground in a randomly selected sub-area of 0.5 m x 0.5 m when vegetation was best developed. In already slightly grazed parcels, we chose areas in the plot where the vegetation had been untouched, provided it could be considered representative for the vegetation of the whole parcel. In 16 cases we decided not to harvest at all. We first air-dried the samples in the field and later at 80°C for 24 hours before weighing them in the laboratory. We processed species lists with the software VEGEDAZ (Küchler, 2004).

Statistical analysis

Occurrence of land use types

To test which factors affect the occurrence of a specific land use type, we used logistic regression based on the binomial distribution including the factors culture, village, altitudinal level, fertilization, traditional land use, abandonment, and actual land use, and their two-way interactions. Villages were nested within cultures, and current land use, which here denotes differences between mown and grazed grassland parcels, was nested within abandonment.

Diversity of land use types and species richness per village

We used analysis of covariance (ANCOVA) with sequential sums of squares to test whether the total number of species found in all investigated grassland parcels in a village depended on the number of combinations of altitudinal level and land use types present. As covariables accounting for landscape heterogeneity and topography, we used the standard deviation of the aspects of all parcels in a village, mean slope, the standard deviation of the slopes, and the altitudinal range covered by the parcels per village.

Due to the study design the area investigated per village was correlated with the number of land use types. To test whether confounding between the number of land use types and investigated area per village could explain the number of species recorded per village, we compared it with the number of species expected based on the species-area curve calculated by Keel (1995) for vegetation records of species-rich Mesobromion grasslands. Because our records span a much larger altitudinal gradient than those of Keel's study, we only used the records of the valley parcels of our study to compare observed and expected species numbers with a paired t-test. The species numbers found at the valley bottom of the 12 villages were on average 62 % higher than expected from the species-area curve ($p < 0.001$), indicating that differences between villages were not simply due to different numbers of study parcels.

Species diversity at the parcel level

Basic unit of replication in our study was the parcel. To investigate effects of regional species pool, abiotic factors, cultural tradition, altitude, and land use combination on the measures of plant species diversity obtained per parcel and mean standing crop per parcel, we used hierarchical ANCOVA with sequential sums of squares. Regional species pools and abiotic factors were used to account for regional differences in soil and climate. To test for curvilinear relationships of pH and altitude we also included the terms pH^2 and $altitude^2$. Effects of species pool and culture were tested against remaining variation among villages and of all other factors against remaining variation among parcels (Table 2).

Analyses of mean and total species numbers per parcel and of the corresponding two values of Shannon index and Evenness yielded qualitatively identical results. Therefore, we present results for mean species numbers and Shannon index and Evenness calculated based on mean cover values of the two records per parcel. With a chi-square test and using mean heterogeneity of the two plots per parcel we tested whether pastures were more heterogeneous than meadows.

We performed all statistical analyses with the software R version 2.0.1 (R Development Core Team, 2004), except for the logistic regression for which we used GenStat (Version 6.1, GenStat Committee, VSN International, UK, 2002).

Results

Landscape diversity

Occurrence of land use types among cultural traditions

We found the highest diversity of land use types in valleys of Romanic villages (culture by altitude interaction, $p = 0.064$, Fig. 2). In the valley bottoms of the four Romanic villages, altogether eight abandoned parcels of three different types were present, while in the Germanic and Walser villages there were only one and two abandoned formerly unfertilized meadows present, respectively.

There were between eight and 21 combinations of land use types and altitudinal levels per village, with a mean of 12.5 (Table 1). We found significantly more unfertilized than fertilized parcel types ($p < 0.001$), more grazed parcel types than mown ones ($p < 0.001$), and more different parcel types in valleys than at higher altitudes

($p < 0.01$). Fertilized parcels were more likely to be found in valleys than at the alp level (altitude by fertilization interaction, $p < 0.05$).

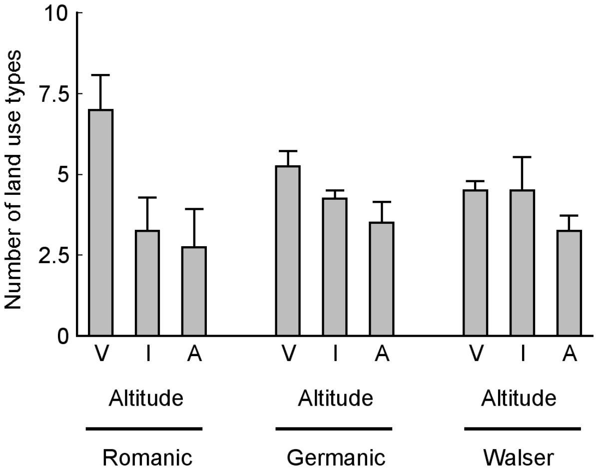

Fig. 2. Mean number of land use types per cultural tradition and altitudinal level ± SE.
V = Valley
I = Intermediate altitudes
A = Alp.

29 of the 126 formerly mown parcels now are grazed, while none of the 91 formerly grazed parcels now are mown. 23 formerly mown and ten formerly grazed parcels, mostly unfertilized, have been left abandoned.

Species richness per village

Regional species pools varied between 747 and 1186 species (Table 1). Altogether we recorded 561 plant species in the 216 studied grassland parcels. Per village we found between 176 and 284 plant species in all study parcels, with a mean of 229 species (Table 1). We found highest species numbers in Germanic villages (247 ± 16 species, mean ± SE), followed by Romanic (219 ± 22 species) and Walser villages (219 ± 15 species). However, these differences were not statistically significant. None of the topographic covariables explained significant variation in plant species diversity per village. The total number of species per village increased with the number of land use types per village ($n = 12$, $p < 0.05$, Fig. 3).

Table 1. Overview of study villages, their cultural traditions, numbers of land use by altitude combinations, total plant species numbers, and numbers of plant species in the regional species pool.

Village	Cultural tradition	Altitude of village	Altitudinal range of valley zone (m asl)*	Altitudinal range of intermediate altitudes (m asl)*	Altitudinal range of alp zone (m asl)*	No. of land use types	Total species number	No. of species in the regional species pool
Bedretto	Romanic	1400	1330-1540	1550-1740	2030-2050	12	213	939
Braggio	Romanic	1320	1330-1510	1720-1750	2060-2070	8	187	875
Ramosch	Romanic	1230	1100-1490	1580-1880	1950-2060	21	284	1186
Trun	Romanic	850	870-1040	1200-1650	1880-1990	10	193	1110
Gadmen	Germanic	1200	1170-1310	1490-1640	1830-1910	13	272	877
Guttannen	Germanic	1050	990-1270	1370-1460	1930-2110	12	199	747
Linthal	Germanic	670	770-1030	1230-1360	1800-1860	14	260	987
Unterschächen	Germanic	1000	1010-1130	1310-1680	1950-1990	12	258	1093
Bosco Gurin	Walser	1500	1460-1590	1670-1760	1860-2020	9	176	1021
Medels	Walser	1560	1500-1600	1650-1860	2030-2170	14	242	862
Pany / Luzein	Walser	950	800-1410	1540-1870	1880-1950	11	241	1129
Vals	Walser	1250	1260-1490	1680-1920	1990-2220	11	218	853

*in the ANCOVA model to test for species diversity at the parcel level (Table 2) exact values of altitude (m asl) were used for each parcel.

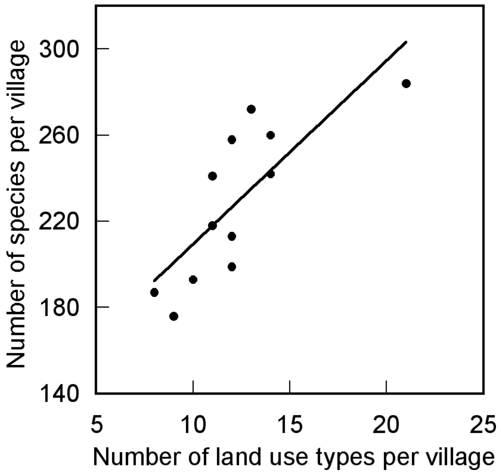

Fig. 3. Relationship between the number of plant species recorded per village and the number of combinations of altitudinal level and land use types investigated per village.

Species diversity per parcel

We recorded between 15 and 84 plant species per 5 m x 5 m plot and between 20 and 101 plant species per parcel (two plots of 5 m x 5 m). More species-rich parcels of land had a lower Jaccard index (r = -0.20, $p < 0.01$), indicating larger differences in species composition between the two plots in more species-rich parcels.

Neither cultural tradition nor the size of the regional species pool had a significant influence on species richness, Shannon index, or Evenness of the parcels. Species numbers increased with altitude ($p < 0.001$, Table 2), followed by a marginally significant decrease (squared altitude effect, Table 2). We found highest species richness at intermediate altitudes for unfertilized and fertilized meadows and pastures (Table 3). More southern aspect, steeper slope and intermediate pH-value were also associated with higher species richness (Table 2).

Grassland biodiversity was strongly influenced by the type of land use (Fig. 4a). Mean species richness was lower in fertilized (32.5 species) than in unfertilized parcels (46.0 species, $p < 0.001$, Table 2) and it was lower in abandoned compared with currently used parcels ($p < 0.001$, Table 2).

Among unfertilized, traditionally mown parcels, mean species richness was slightly higher in mown (49.1 species) than in recently grazed parcels (47.6 species, Fig. 4a), where it was still higher than in parcels that had always been grazed (47.0 species, Fig. 4a). In contrast to unfertilized parcels, mean species richness was significantly higher in grazed (37.9 species) than in mown (29.7 species, a priori contrast, $p < 0.05$, Fig. 4a) among the fertilized, traditionally mown parcels.

Table 2. Summary of sequential sums of squares ANCOVA testing effects of abiotic variables, cultural traditions, and human land use on mean plant species richness per parcel among 216 grassland parcels in the Swiss Alps. Effects of culture and species pool were tested against remaining variation among villages. n.s. denotes values of $p > 0.1$. The non-significant interactions between culture and land use factors, between village and land use factors, and between altitude and land use factors were omitted from the table.

Source of variation	df	SS	*F*	*p*
Regional species pool	1	30.5	0.06	n.s.
Aspect	1	826.5	12.26	$p < 0.001$
Slope	1	3482.8	51.68	$p < 0.001$
pH	1	41.5	0.62	n.s.
$(\mathrm{pH})^2$	1	716.1	10.63	$p < 0.01$
Culture	2	82.2	0.08	n.s.
Village[Culture]	8	3985.2	7.39	$p < 0.001$
Altitude	1	1691.6	25.10	$p < 0.001$
$(\mathrm{Altitude})^2$	1	191.0	2.83	$p < 0.1$
Fertilization	1	4805.9	71.32	$p < 0.001$
Traditional land use	1	44.5	0.66	n.s.
Abandonment	1	2139.2	31.74	$p < 0.001$
Current land use [abandonment]	1	164.1	2.44	n.s.
Fertilization*traditional land use	1	226.3	3.36	n.s.
Fertilization*abandonment	1	7.2	0.11	n.s.
Fertilization*current land use[abandonment]	1	131.8	1.96	n.s.
Traditional land use*abandonment	1	147.2	2.18	n.s.
Culture*altitude	2	72.7	0.54	n.s.
Village[Culture]*altitude	9	3310.2	5.46	$p < 0.001$
Residuals	133	8962.6		

Qualitatively, we obtained the same results for Shannon index and Evenness, with the only exception of higher Evenness in mown than in grazed among the fertilized parcels.

Analyses of the Jaccard index showed smaller differences in species composition between two records in fertilized than in unfertilized parcels ($p < 0.05$), in traditionally mown than in traditionally grazed parcels ($p < 0.01$), and in used compared with abandoned parcels ($p < 0.001$). Pastures were structurally significantly more heterogeneous than meadows ($p < 0.001$).

Standing crop increased with fertilization ($p < 0.001$) and abandonment ($p < 0.001$). Among used parcels, fertilized meadows were more productive than fertilized pastures (Fig. 4b). Higher standing crop values (mean of two plots) per parcel were associated with lower mean species richness ($r = -0.25$, $p < 0.001$).

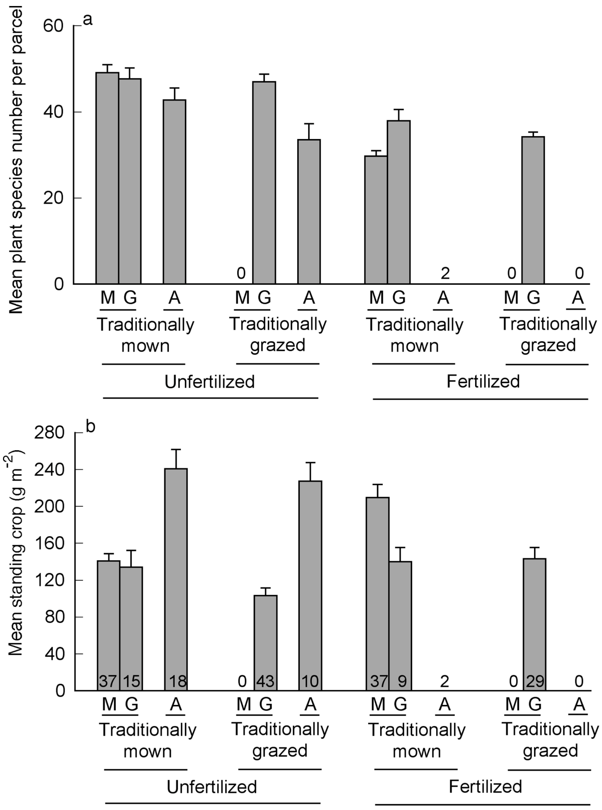

Fig. 4. Relationship between a) mean (± SE) plant species number per parcel or b) mean (g m^{-2},± SE) standing crop per parcel and combination of fertilization, traditional land use, and current land use.
M = Mown
G = Grazed
A = Abandoned
Figures in columns indicat numbers of replicate parcels.

Table 3. Plant species numbers per 5 m x 5 m (mean ± Standard error) of 216 grassland parcels of different land use at three altitudinal levels.

	Currently mown		Currently grazed	
	Unfertilized	Fertilized	Unfertilized	Fertilized
Valley	44.38 ± 2.06	28.57 ± 1.51	44.13 ± 1.93	34.21 ± 1.56
Intermediate	55.43 ± 3.26	31.23 ± 2.12	50.23 ± 2.72	36.62 ± 2.17
Alp	47.14 ± 4.15	37.0*	47.26 ± 2.75	34.7 ± 1.83

*only one parcel

Discussion

Cultural impact on landscape diversity

The trend towards a higher number of different land use types in valleys of Romanic villages suggests that cultural traditions in the Alps still influence landscape diversity. Because the land use decisions of Swiss farmers largely depend on a subsidy system which does not differentiate among cultural traditions, the observed differences in land use diversity must indeed result from persisting socio-economic differences between cultures. Because plant species richness per village is the higher the more different land use types there are per village (Fig. 3), this implies that cultural traditions indirectly affect plant species diversity in a village.

In Romanic villages land use structure at the valley bottom is likely to be more fine-grained, because of frequent hereditary partitioning of parcels and mixed farming in earlier decades (Bätzing, 2003). When parcels are partitioned over many generations, they can get too small for an efficient agricultural land use and are therefore likely to be abandoned, which could explain the higher frequency of abandoned parcels in the valleys of Romanic compared with Germanic villages. Hereditary partitioning was also common in Walser villages. However, Walser villages were situated at higher altitudes than Romanic villages and the harvest per area was smaller. Therefore, farmers might have been forced to use all grassland parcels that were available in order to get enough fodder for their cattle, which slowed down abandonment of agricultural land. Although in a single parcel species richness and diversity can decrease after abandonment, a structurally diverse landscape including abandoned parcels enhances overall species diversity of plants and other taxa, such as butterflies (Zoller and Bischof, 1980).

In a recent socio-economic study comparing 140 Romanic, Germanic and Walser villages, 29 of 72 investigated socio-economic variables significantly differed among these cultural traditions (Pfister, 2004). Socio-economic factors have an important influence on plant species richness via land use diversity. Differences in ecological compensation areas and organic farming among cultural traditions are potential mechanisms for such land use mediated diversity effects. We consider the observed cultural effects on land use diversity as especially noteworthy, as Swiss agricultural policy is rather promoting a homogenization of agricultural practices than taking cultural or regional peculiarities into account.

Diversity of plant species at the landscape level

Villages with more different land use types harbored more plant species, and observed species numbers by far exceeded numbers expected from the species-area curve. This indicates that land use diversity really played the decisive role and that each land use type contributes specific species. These results are in line with the mosaic concept (Duelli, 1997) and the habitat diversity hypothesis (Williams, 1964), which propose higher species numbers in landscapes with higher habitat diversity. Such patterns have been found for plants in Switzerland (Wagner et al., 2000) and other European countries (Crawley and Harral, 2001; Moser et al., 2002; Honnay et al., 2003; Waldhardt et al., 2004) and for certain animal taxa (Weibull et al., 2000; Dauber et al., 2003). The relationship between species diversity and landscape diversity suggests that maintaining high plant species diversity at the landscape level requires conserving high land use diversity. At the same time this will help conserve a high insect diversity (Oertli et al., 2005).

Direction of land use changes

While we found meadows that had been converted to pastures and formerly mown or grazed parcels that had been left abandoned, there were no pastures that had been converted to meadows. Pastures with a certain slope very often show remarkable horizontal cow tracks. They are therefore not suitable for mowing due to their structural heterogeneity, which may impede their conversion to meadows. Nevertheless, our findings clearly show the important socio-economic trend that all observed changes in land use reduced the farmers' workload (Bätzing, 2003).

Relationship between altitude and plant species diversity per parcel

Plant species richness was highest at intermediate altitudes. As reviewed by Rahbek (1995), different altitude-species richness patterns can be observed. Among plants, a hump-shaped pattern, as in our study, appears most common. Most likely explanation for the highest plant species diversity observed at intermediate altitudes appears to be the overlap of subalpine and alpine species pools (Grytnes, 2003).

Effects of fertilization and abandonment on plant species diversity per parcel

Plant species richness was significantly reduced in fertilized parcels confirming experimental studies in Switzerland (Tidow, 2002) and abroad (e.g., Foster and Gross, 1998; Jacquemyn et al., 2003; Baer et al., 2004).

Species richness was also clearly reduced in abandoned compared with used parcels as observed in more local studies (Zoller and Bischof, 1980; Zoller et al., 1984; Ryser et al., 1995). In abandoned parcels a thick litter layer of dead plant material covers the ground, and tall forbs and clonal and highly competitive grasses grow vigorously. As a consequence of increased competition and reduced light availability, mortality of sub-canopy plants increases (Jacquemyn et al., 2003). In the long run there is a shift in vegetation composition towards dwarf shrub or forest communities combined with a loss of species richness (Tasser and Tappeiner, 2002). Abandonment is not only a problem concerning biodiversity loss, but, depending on aspect, slope, and soil depth, it can also enhance the probability of landslides (Tasser et al., 2003), which may endanger roads and settlements.

Effects of mowing and grazing on plant species diversity at the parcel level

We found highest plant species richness in unfertilized meadows. However, the difference to formerly mown, unfertilized pastures was small. In contrast, species numbers in always grazed unfertilized pastures were lower than in the pastures that were previously mown. This indicates that in the long run species richness of meadows will decrease when they are converted to pastures (Fischer and Wipf, 2002).

In contrast to unfertilized grassland parcels, grazing enhanced species richness among the fertilized parcels. Such a reversed impact of grazing in nutrient-poor versus nutrient-rich ecosystems can be observed in mountain habitats (Austrheim and Eriksson, 2001) and many other habitat types (Proulx and Mazumder, 1998). Pastures are structurally more heterogeneous, probably because naturally homogeneous grassland parcels were rather selected to be mown than to be grazed, but also due to cow prints and more heterogeneously distributed nutrients and uneven seed input by feces. This heterogeneity offers a higher diversity of available ecological niches (Duelli and Obrist, 2003) and may promote species richness at high nutrient levels (MacArthur and MacArthur, 1961; Gigon and Leutert, 1996). In nutrient-poor habitats, however, intensive grazing reduces species richness because plants have a limited capacity for regrowth (Proulx and Mazumder, 1998). Although grazing reduces species richness compared with mowing, this loss of biodiversity is not as severe as after abandonment. Therefore, grazing can help to conserve at least part of the species richness while simultaneously reducing the workload for the farmer. Traditionally, however, many parcels that were too steep to be grazed by cattle had been mown, and nowadays their plant species richness is threatened by abandonment.

The analyses using Shannon index and Evenness largely confirmed our findings on species richness. The only exception was that Evenness was highest in mown fertilized grasslands, whereas species richness was highest in unfertilized ones. This is probably due to the few but rather abundant, highly competitive species in these grassland parcels and to the absence of cow prints, which could relieve competition pressure and serve as safe sites for recruitment of rarer species.

Relationship between species diversity and standing crop

Our data confirmed a conflict between the conservation goal of high plant species richness and the agricultural goal of high productivity. In fertilized meadows with the highest standing crop, only half as many species occurred than in unfertilized meadows. Standing crop of fertilized pastures was not much higher than that of unfertilized pastures, but their species richness was much lower. This implies little yield gain from fertilization of pastures but a severe loss of biodiversity. Liquid manure and dung used as fertilizers mainly result from milk production, which is supported by financial incentives. To reduce additional fertilization of pastures, agricultural policy could aim at rather promoting meat production where cattle are mostly outdoors.

Conclusions

Our comprehensive study across 12 villages along a 170 km east-west gradient suggests that cultural traditions still affect man-made landscape diversity. This cultural footprint on land use diversity can be explained by still persisting socio-economic differences among cultural traditions. High land use diversity in turn was related to high plant species richness in villages and thereby is important to conserve high plant species diversity.

The observed land use changes reduce farmers´ workload, but at the same time decrease plant species diversity. Unfortunately, unfertilized meadows with the highest species richness require much labor of the farmer and provide little yield. While the conversion of such unfertilized meadows to extensively grazed pastures could be a valuable alternative to abandonment, in the long run it will result in a reduction of species numbers.

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Chapter 3

The role of landuse and natural determinants for grassland vegetation composition in the Swiss Alps

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submitted

Abstract

The Alps constitute a highly diverse habitat for plant species, structured by altitude and abiotic site conditions, such as variation in slopes, aspects, and substrate conditions. Additionally, in man-made grasslands, vegetation composition is affected by differences in landuse. Comprehensive studies are scarce, however, which investigate effects of landuse and landuse change relative to effects of abiotic factors over large geographic and altitudinal ranges. We recorded vegetation composition in 216 parcels of grassland in 12 villages representing an area of 170 x 70 km^2 in the south-eastern part of the Swiss Alps. Each parcel was characterized by a combination of altitudinal level (valley, intermediate, alp), traditional landuse (mown, grazed), current management (mown, grazed, abandoned), and fertilization (unfertilized, fertilized). For each parcel we also assessed the abiotic factors aspect, slope, and pH-value and for each village mean yearly precipitation and geographic coordinates. We analyzed vegetation composition using partial CCA, cover of graminoids, non-legume forbs, and legumes, and dominance and frequency of species. Species composition was determined by, in decreasing order of explained variation, villages and their cultural traditions, landuse, abiotic factors, and altitude. Current socio-economically motivated landuse changes strongly affect vegetation composition, as in our study grazing of unfertilized former meadows or their abandonment both strongly reduced the frequency of characteristic meadow species. Changes from mowing to grazing suggested less severe consequences for vegetation composition than abandonment. Therefore, extensive grazing and mowing every few years should be considered valuable conservation alternatives to abandonment. Furthermore, because each landuse type was characterized by different species, a high variety of landuse types should be promoted to preserve plant species diversity in Alpine grasslands.

Keywords: mowing, grazing, abandonment, Switzerland, plant species composition, landuse changes

Introduction

The European Alps constitute a highly structured and diverse plant habitat. Environmental conditions and vegetation composition vary due to the altitudinal gradient and abiotic site factors, like variation in slopes, aspects, moisture, and soils (Ellenberg, 1996). Below timberline, many man-made grasslands replace cleared forests, and for hundreds of years these grasslands have been maintained by mowing or grazing (Bätzing, 2003). The intensity of these landuses and the economic return are largely determined by the fertilization level (van der Hoek, van Mierlo & van Groenendael, 2004). In recent decades agricultural landuse and its intensity have been changing for socio-economic reasons (Bätzing, 1993). These changes include intensified landuse of easily accessible parcels of land and reduced labor in parcels yielding only a small return (Tasser & Tappeiner, 2002). The latter mostly concerns unfertilized meadows, which frequently are very steep and laborious to mow. Some of these meadows have been abandoned while others nowadays are grazed by cattle or sheep instead of being mown. Previous more local studies suggest that these changes have severe impacts on the vegetation composition of Alpine grasslands (Fischer & Wipf, 2002; Stampfli & Zeiter, 2004; Tasser & Tappeiner, 2002; Zoller & Bischof, 1980). However, comprehensive studies covering larger geographic ranges, including abiotic site conditions, several combinations of landuse, and landuse changes at several altitudes as potential determinants of grassland vegetation, are still missing.

We studied vegetation composition of 216 grassland parcels of different landuse and geographic characteristics in the Swiss Alps. The parcels were situated at different altitudes in 12 villages representing an area of 170 by 70 km^2 ranging from Central to Eastern Switzerland. We studied grassland parcels that formerly had been mown or grazed, today are mown, grazed or abandoned, and are either additionally fertilized or unfertilized. To account for potential regional or cultural differences, each of the three main cultural traditions in the Swiss Alps (Romanic, Germanic, and Walser) was represented by four villages. The three cultural traditions with their characteristic agricultural practices have contributed to the landscape diversity of the Alps (Bätzing, 2003). Traditionally, self-sufficient mixed farming was practiced in the Romanic culture. Hereditary partitioning of parcels was frequent and led to a still visible small-grained parcel structure. In Germanic villages scattered single farm houses were surrounded by fields and meadows mainly used for dairy farming. The Walser, Alemannic people from the Valais in south-eastern Switzerland, migrated eastwards and settled at higher altitudes, as lower parts of the valleys were already occupied. Apart from farming, they lived on trade and maintenance of Alpine passes. Socio-economic differences are still remarkably pronounced among these cultural traditions (Pfister, 2004).

We characterized grassland vegetation composition based on two vegetation records in each of the studied parcels. To assess the relative importance of landuse and natural determinants of species composition we used multivariate analysis (Leps & Smilauer, 2003). Furthermore, we analyzed the frequency of dominant and characteristic species for the different types of parcels. Moreover, we analyzed the percentage cover of the life-forms graminoids, legumes, and non-legume forbs. In grasslands, the abundance of legumes is of particular importance due to their ability to directly use atmospheric N_2 as a nitrogen source. In graminoids, the leaf and apical meristems are situated below ground, which makes this group particularly resistant against grazing.

Our main hypothesis was that plant species composition and ecological properties of grasslands in the Swiss Alps are strongly affected by geographic factors (villages and their cultural traditions), altitude, and abiotic site conditions, but that differences in landuse play an equally important role. Moreover, we expected recent changes in landuse to largely affect species composition. More specifically, we asked: 1) How do cultural traditions and villages, landuse, altitude, and abiotic factors affect plant species composition of grassland parcels? 2) How do these factors affect the percentage cover of the life-forms graminoids, legumes, and non-legume forbs? 3) How do these factors affect dominance and frequency of grassland species? 4) Moreover, because the most common among the ongoing changes in landuse are grazing or abandonment of unfertilized former meadows, we especially addressed the changes of plant species composition after these specific landuse changes.

Methods

Study sites

We studied grasslands in 12 villages representing an area of approximately. 170 by 70 km^2 in south-eastern Switzerland. Each of the three cultural traditions Romanic, Germanic, and Walser was represented by four villages (Fig. 1, Maurer, Weyand, Fischer & Stöcklin, 2006). We randomly selected the villages with the restriction that their agricultural character had only changed modestly during the last 50 years, they were not very touristy and did not have more than about 1500 inhabitants. Due to settlement history, Germanic villages are located in the northern parts of the Alps, and Romanic and Walser villages in central and southern parts.

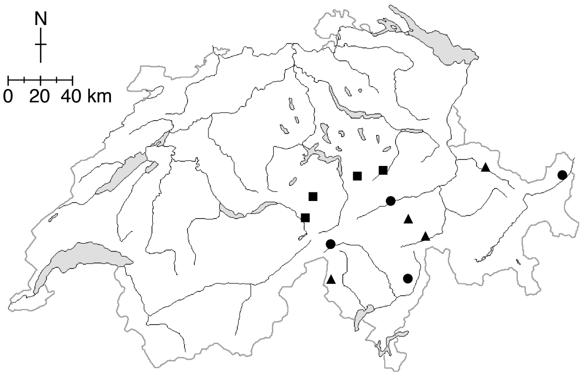

Fig. 1. Map of Switzerland with the 12 study villages and their cultural traditions.
● = Romanic
■ = Germanic
▲ = Walser

In collaboration with local farmers, we selected grassland parcels at the valley bottom (about 1000 m asl), at intermediate altitudes (about 1500 m asl), and at the alp (about 2000 m asl) in each village. We included grassland parcels without any known landuse changes and parcels whose use had been changed at least five years ago. Each grassland parcel was characterized by the combination of traditional landuse (mown or grazed), current management (mown, grazed, or abandoned), fertilization (fertilized or unfertilized), and altitude. The unfertilized meadows were usually cut once a year while the fertilized meadows were cut between two and four times. Grazing intensity varied among years depending on climatic conditions. Altogether, we selected 216 parcels, between 12 and 24 per village.

Vegetation records

We visited each grassland parcel once in 2002 or 2003 when the vegetation was best developed, shortly before the start of mowing or grazing. In each parcel, we took two vegetation records in plots of 5 m x 5 m at a distance of 5 m estimating the ground cover percentage of all vascular plant species. We calculated mean cover values of the two vegetation records for each species in a parcel and used these averaged species lists comprising a plot area of 50 m^2 for further calculations. For each parcel we calculated the percentage cover of the life-forms graminoids (Poaceae, Cyperaceae, Juncaceae), legumes, and non-legume forbs as a measure of species composition. We did not consider woody species or orchids because their frequency was very low. To measure the heterogeneity of the species composition of the two vegetation records per parcel we calculated the Jaccard index (Magurran, 2004). For the processing of species lists and the calculations of cover of life-forms and Jaccard indices we used the program VEGEDAZ (Küchler, 2004). Nomenclature follows Lauber & Wagner (2001) except for *Anthoxanthum,* where we aggregated *A. odoratum* and *A. alpinum* and for *Poa,* where we aggregated *P. annua* and *P. supina*. Furthermore, we recorded altitude of the parcel and the abiotic factors aspect as deviation from South, slope in degrees, and soil pH. Additionally, we assessed the mean yearly precipitation of the years 1998-2002 in mm using data of the nearest measuring station for each village (either in the study-villages or in the next village) and geographic coordinates of each village.

Statistical analysis

Gradients in species composition

To assess the relative impact of the variables aspect, slope, pH-value, cultural tradition, village, altitude, fertilization, traditional landuse (mowing or grazing), abandonment, and current landuse (mowing or grazing) on plant species composition, we used Canonical Correspondence Analysis (CCA, Leps & Smilauer, 2003). We partitioned total explained variation by introducing one variable after the other in the CCA-model in the order shown above according to the hierarchical structure of our data and assessed the percentage of variance explained by each factor. Significance of the variance partitioning was tested with 499 Monte Carlo permutations (Leps & Smilauer, 2003). Furthermore, because precipitation data had been measured only at the village level, additionally we calculated the percentage of the variance explained by the villages that could be assigned to the east-west- and north-south-gradients of the geographic location of the villages and the mean yearly precipitation.

Relations between abiotic and landuse variables

To investigate relations between abiotic and landuse variables we calculated one-way Analyses of variance (ANOVA). Of the significant ANOVAs between abiotic factors and current management (abandoned, mown, or grazed), we additionally calculated Tukey’s HSD of differences among the three levels.

Percentage cover of life-forms and Jaccard index

To analyse the impact of the investigated variables (see paragraph 'Gradients in species composition') on the percentage cover of the life-forms graminoids, legumes, and non-legume forbs as well as on the Jaccard index of two vegetation records per parcel we used an Analysis of covariance (ANCOVA)-model with sequential sums of squares. The variables were introduced into the ANCOVA-model in the same order as in the CCA-model. We tested effects of culture against remaining variation among villages and of all other factors against variation due to remaining differences among parcels. Current landuse, which denotes current meadows and pastures, was nested within abandonment.

Species dominance

To assess species dominance within parcels, we calculated mean cover per parcel relative to total cover for each species within each landuse combination and ranked species in decreasing order of their relative cover values. We considered the ten species with the highest cover values within a landuse combination as dominant. We did not consider abandoned former meadows that had been fertilized for this analysis, as they were represented by only two parcels.

Species frequency

To assess species frequency, both for the total data set and per landuse combination, we counted the number of occurrences of each species in all parcels and within each landuse type. Then, we classified the species in five frequency classes (I: occurrence in ≤ 20% of the parcels of a land use combination, II: > 20-40%, III: > 40-60%, IV: > 60-80%, V: > 80%; Dierschke, 1994). For the analysis of species frequency per landuse type we did not consider the two abandoned meadows that had been fertilized.

Species pools per landuse type

Because not all landuse types were present for each combination of village and altitudinal level, we studied different numbers of parcels for each landuse type in the field. Therefore, we used a permutation procedure to compare the total number of species that occur in fertilized and unfertilized meadows and pastures (independent of traditional landuse). For each landuse type, we calculated species accumulation data with 1000 random permutations of parcels. We used least-squares fits to the non-linear species-area curve $S = cA^z$ (S = number of species, A = area in m^2, c and z = constants (Krebs, 1994)) to these data to estimate the values of c and z for fertilized and unfertilized meadows and pastures. Based on these values, we calculated the total number of species expected in vegetation records of 70 parcels (= 3500 m^2) of each type. With a χ^2-test we analyzed, whether total species numbers differed between fertilized and unfertilized meadows and pastures.

Effects of landuse changes of former unfertilized meadows

We were especially interested in the consequences of landuse changes of unfertilized meadows. Therefore, we prepared a list of characteristic species of unfertilized meadows (93 species), which we defined as species occurring in more than 20 %, but less than 80 % of all unfertilized meadows. This excluded both very rare species and species which are common regardless of the type of landuse. Our definition of characteristic species differs from the one of Braun-Blanquet (1964), which served different purposes. For these characteristic species, we calculated the relative difference in frequency between formerly unfertilized meadows that had been converted to pastures or that had been abandoned and unfertilized meadows that were still mown.

We did all statistical analyses with the program R (R Development Core Team, 2004) except for the CCA which we did with CANOCO (ter Braak & Smilauer, 2002). To calculate the species accumulation curves we used the R package vegan (Oksanen, 2005).

Results

Species composition in parcels of different landuse

In the 216 parcels, we recorded 561 plant species. Three hundred and seventeen species occurred in more than four parcels. Seventy-five of the 561 species occurred in ≥20% of the parcels, and 33 species occurred in ≥40% of the parcels. Only 72 of the 561 species occurred in all landuse types.

Per village we recorded between 176 and 284 (mean 229) plant species. The expected accumulated number of species in 70 parcels of unfertilized meadows was 431, in unfertilized pastures 477, in fertilized meadows 281, and in fertilized pastures 398, i.e.

the negative effect of fertilization on species richness was more pronounced in mown grasslands than in grazed ones (df = 1, $\chi^2 = 5.5673$, $p<0.05$).

Altogether, the investigated variables explained 19.7% of all observed variation in species composition in the CCA. Variance partitioning (Table 1) revealed that variation among villages explained most of the total explained variance (42.2%, including 8.9% among villages of different cultures), followed by landuse factors (in total 27.7%), abiotic factors (in total 16.8%), and altitude (13.1%). Among the landuse factors, current landuse (mowing or grazing) and fertilization had the strongest impact on species composition (Table 1). Of the variance explained by villages, 18.2% could be assigned to differences in the east-west- and 18.2% in the north-south-gradient of geographic location, while 17.3% could be assigned to differences in precipitation among villages.

Species composition of the two vegetation records per parcel was more similar to each other, i.e. had smaller Jaccard indices, in fertilized than in unfertilized parcels ($p<0.05$), in traditionally mown than in traditionally grazed parcels ($p<0.01$), and in used compared with abandoned parcels ($p<0.001$).

Table 1 Partitioning of explained variation in species composition of 216 grassland parcels in the Swiss Alps among different geographical and landuse characteristics of the parcels. The percentages of explained variation were obtained with Canonical Correspondence Analysis using a hierarchical model (see methods).

Variable	% of explained variation	*p*
Aspect	3.8	$p < 0.01$
Slope	7.2	$p < 0.01$
pH	5.8	$p < 0.01$
Culture	8.9	$p < 0.01$
Village	33.3	$p < 0.01$
Altitude	13.1	$p < 0.01$
Fertilization	8.2	$p < 0.01$
Traditional landuse (mowing or grazing)	4.2	$p < 0.01$
Abandonment	4.8	$p < 0.01$
Current landuse (mowing or grazing)	10.5	$p < 0.01$

Abiotic variables and landuse factors

Altitude was related to all landuse factors. Abandoned and currently grazed parcels were situated at higher altitudes than currently mown ones (Tukey's HSD, $p<0.05$ each), unfertilized parcels were situated at higher altitudes than fertilized ones, and traditionally grazed parcels tended to be situated at higher altitudes than traditionally mown ones (Table 2).

Currently mown parcels tended to be more south-oriented than grazed ones (Tukey's HSD, $p=0.055$) while traditionally mown parcels were more south-oriented than traditionally grazed ones (Table 2). Abandoned parcels were steeper than currently mown and grazed ones (Tukey's HSD, $p<0.01$ each), unfertilized ones steeper than fertilized ones, and traditionally mown parcels were steeper than traditionally grazed ones (Table 2). The pH-values of the soil showed no relation with any of the landuse factors (Table 2). Except for the relation between slope and fertilization, the relations between abiotic and landuse factors were only weak (Table 2).

Table 2 Matrix of relations between abiotic and landuse factors (coefficient of determination (r^2) and their significances from one-way ANOVAs) of 216 grassland parcels in the Swiss Alps. Current management includes abandoned, mown, and grazed parcels; traditional landuse includes mown and grazed parcels; fertilization includes fertilized and unfertilized parcels. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

	Current management	Traditional landuse	Fertilization
Altitude	0.044**	0.015	0.093***
Aspect	0.034*	0.086***	0.003
Slope	0.064***	0.033**	0.230**
pH	0.001	0.002	0.001

Landuse and dominant species

Anthoxanthum odoratum agg. and *Festuca rubra* agg. were among the ten most dominant species in all landuse combinations. Nevertheless, each landuse combination harbored a characteristic set of dominant species (Table 3). In unfertilized landuse types the ten most dominant species covered about 30% of the plot-area relative to total cover, while in fertilized parcels it was more than 40%. *Ranunculus acris*, *Taraxacum officinale* agg., and *Trifolium repens* were only among the dominant species in fertilized parcels, while *Carex sempervirens* and *Leontodon hispidus* s.l. were only among the dominant species in unfertilized parcels. *Alchemilla xanthochlora* agg. and *Poa alpina* were only

Table 3 Mean proportion of relative cover (%) in all types of landuse combination for each of 28 species dominant in at least one landuse combination. Species are listed in declining order of their overall frequency. The ten most dominant species within each landuse combination are marked in bold face. Nomenclature is according to Lauber & Wagner (2001) except for *Anthoxanthum* agg.

Fertilization	Unfertilized					Fertilized		
Traditional landuse	Mown			Grazed		Mown		Grazed
Current landuse	Mown	Grazed	Abandoned	Grazed	Abandoned	Mown	Grazed	Grazed
Festuca rubra agg.	**7.5**	**6.5**	**8.4**	**7.0**	**6.3**	**3.0**	**8.0**	**5.2**
Anthoxanthum odoratum agg.	**3.9**	**4.0**	**2.9**	**3.4**	**3.0**	**4.3**	**3.9**	**3.1**
Agrostis capillaris	**2.7**	**3.4**	**2.2**	**3.2**	**3.2**	1.6	**4.2**	**4.3**
Nardus stricta	1.7	**5.9**	**2.0**	**5.5**	**3.7**	0.1	0.8	2.6
Trifolium pratense ssp. *pratense*	**2.4**	**1.9**	0.4	**2.0**	0.8	**4.6**	**5.3**	**3.6**
Dactylis glomerata	1.5	1.2	**1.9**	1.1	2.1	**4.9**	**3.7**	**3.0**
Alchemilla xanthochlora agg.	1.3	1.4	0.4	**2.1**	0.1	2.7	**5.1**	**6.2**
Trifolium repens ssp. *repens*	0.9	0.7	0.0	1.1	0.6	**5.7**	**4.0**	**4.0**
Chaerophyllum villarsii	**2.3**	1.5	**2.9**	1.0	**2.6**	2.4	1.2	1.3
Phleum rhaeticum	0.7	1.5	1.4	1.7	**2.2**	1.1	1.7	**3.4**
Poa alpina	0.2	**1.8**	0.0	**2.1**	0.3	1.5	**3.4**	**3.8**
Taraxacum officinale agg.	0.1	0.5	0.0	0.2	0.1	**6.3**	2.5	**3.2**
Carex sempervirens	**2.1**	**2.4**	**4.8**	**1.8**	1.2	0.1	0.3	0.1
Potentilla erecta	**2.0**	**1.7**	**3.4**	1.5	**3.1**	0.1	0.8	0.1
Rumex alpestris	0.7	0.8	0.4	0.3	0.2	**4.0**	**3.0**	2.4
Leontodon hispidus s.l.	**2.7**	**1.7**	1.0	**1.9**	0.1	1.1	1.6	1.4
Ranunculus acris	0.5	0.9	0.2	0.4	0.0	**3.7**	**2.9**	2.5
Geranium sylvaticum	0.9	0.8	1.3	0.2	1.2	**3.8**	1.3	0.7
Trisetum flavescens	1.1	0.1	0.4	0.1	0.0	**4.4**	1.2	0.7
Brachypodium pinnatum	0.7	0.6	1.2	0.4	**4.6**	0.1	0.1	0.1
Potentilla aurea	1.1	**1.7**	0.6	**1.8**	0.3	0.5	0.9	0.9
Vaccinium myrtillus	0.9	1.1	**1.8**	1.3	1.8	0.0	0.1	0.2
Briza media	**1.9**	0.8	0.9	0.8	1.4	0.1	0.3	0.1
Calamagrostis villosa	0.0	0.0	1.3	0.0	**2.5**	0.0	0.0	0.0
Molinia caerulea	0.3	0.5	0.2	0.1	**2.6**	0.0	0.0	0.0
Poa trivialis ssp. *trivialis*	0.2	0.0	0.0	0.0	0.1	**3.0**	0.1	0.2
Laserpitium latifolium	**1.9**	0.2	0.5	0.1	0.0	0.0	0.0	0.0
Phleum hirsutum	0.4	0.4	**1.5**	0.1	0.1	0.1	0.2	0.0

dominant in pastures. Several species were only dominant in one specific landuse combination: *Briza media, Laserpitium latifolium* and *Rhinanthus alectorolophus* were only dominant in unfertilized meadows that had always been mown, and *Geranium sylvaticum* and *Heracleum sphondylium* s.l. were only dominant in fertilized meadows that had always been mown. These results clearly indicate that differences in species composition between parcels of different landuse were not just limited to relatively rare species.

Cover by plant species of different life-forms

The percentage cover of the different life-forms was mainly affected by differences in landuse. In fertilized parcels, the percentage legume cover was higher ($p<0.001$) and the percentage graminoid cover was lower ($p<0.05$) than in unfertilized parcels. In abandoned parcels, the percentage graminoid cover was higher than in agriculturally used parcels, and especially so in formerly grazed parcels (Fig. 3A, interaction traditional landuse by abandonment, $p<0.05$). Accordingly, the percentages forb and legume cover were lower in abandoned than in agriculturally used parcels ($p<0.001$ for both, Fig. 2B, C).

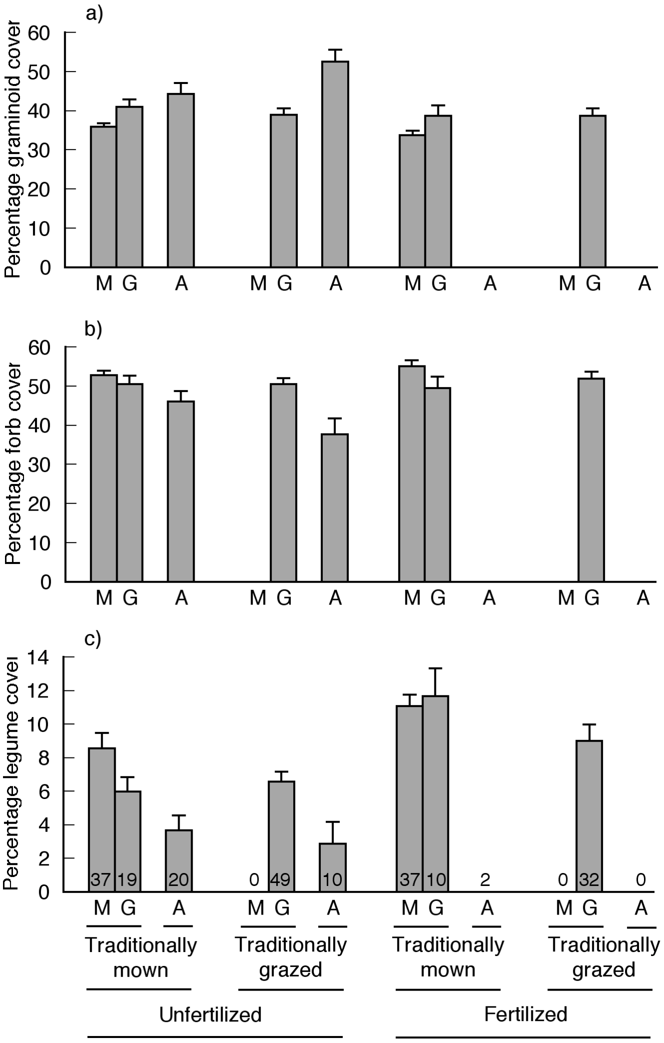

Fig. 2 Percentage cover (± SE) of the life-forms A) graminoids, B) forbs, and C) legumes in 216 grassland parcels in the Swiss Alps. Each landuse type is characterized by its combination of fertilization (unfertilized, fertilized), traditional landuse (mown, grazed), and current landuse (M =Mown, G = Grazed, A = Abandoned). Numbers in the columns indicate the replicate parcels.

In currently grazed parcels, the percentage graminoid cover was slightly higher and the percentage forb cover slightly lower than in currently mown parcels (Fig. 2A, B). The percentage legume cover was higher in traditionally mown than in traditionally grazed parcels, and especially so in fertilized parcels (interaction fertilization by traditional landuse, $p=0.08$). Among the unfertilized traditionally mown grasslands, the percentage legume cover was lower in currently grazed than in still mown ones, whereas among fertilized traditionally mown grasslands, it was higher in currently grazed than in still mown ones (Fig. 2C). These results indicate, that among the landuse variables, fertilization and abandonment caused stronger shifts in proportions of life-forms than the difference between mowing and grazing did.

Apart from landuse effects, we detected lower graminoid cover in parcels with steeper slopes ($p<0.001$) and a higher one in parcels with higher pH-values ($p<0.001$).

Effects of landuse change of unfertilized former meadows

The mean frequency of the 93 species occurring in more than 20%, but less than 80% of all unfertilized mown parcels was significantly lower in former meadow parcels that had been converted to pastures (-13.1%, Fig. 4) and were abandoned (-24.8%, Fig. 3) than in still mown grasslands.

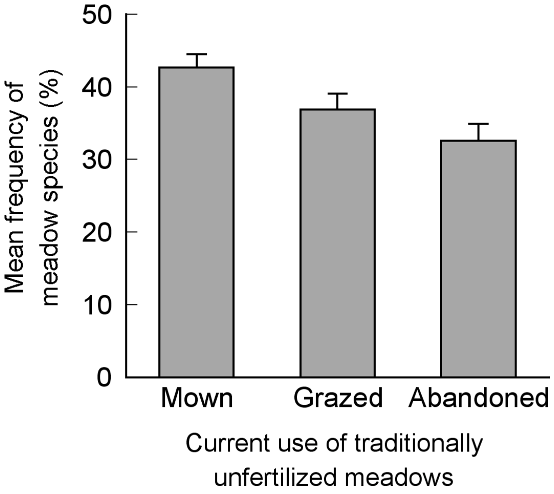

Fig. 3 Mean frequency (± SE) of 93 plant species characteristic for unfertilized meadows (occurring in more than 20 %, but less than 80 % of all unfertilized meadow parcels) in grassland parcels in the Swiss Alps that nowadays are still mown, are grazed, or abandoned.

In unfertilized formerly mown parcels that had been converted to pastures, 58 of the 93 species occurred on average 36.7% less frequently, while 35 occurred on average 25.3% more frequently than in those that were still mown. In unfertilized former meadows that had been converted to pastures, species generally considered to be rather common (*Achillea millefolium*, *Ajuga reptans*, *Dactylis glomerata*, *Veronica chamaedrys*), or indicating high nutrient levels (e.g., *Rumex alpestris*, *Taraxacum officinale* agg.) were more frequent than in still mown ones. In contrast, the orchid *Gymnadenia conopsea* was 74% less frequent, and *Listera ovata* was missing completely. *Pulsatilla alpina* s.l. was 18.9% less frequent, and *Paradisea liliastrum* was 21.7% more frequent in unfertilized parcels that had been converted to pastures than in those that were still mown.

In abandoned unfertilized former meadows, 67 of the 93 species were on average 46.0% less frequent, while 26 of the 93 species were on average 29.8% more frequent than in meadows that were still mown. Even typical meadow-species such as *Paradisea liliastrum* and *Pulsatilla alpina* s.l. were 61.9% and 115.8% more frequent, respectively, in abandoned parcels. However, this was an exception, as many of the species with higher frequency in abandoned unfertilized former meadows were common species of light forests and also included the dwarf shrub *Vaccinium vitis-idaea*.

In summary, grazing or abandonment of unfertilized former meadows caused strong shifts in species composition and generally reduced the frequency of characteristic species of unfertilized meadows.

Discussion

Effects of geographic location, cultural traditions and abiotic factors

Differences between the twelve villages covering an east-west gradient of 170 km and a north-south gradient of 70 km with different precipitation and between different altitudes from the valley bottom to the alp level had by far the strongest impact on the species composition of Alpine grasslands. Very likely, the detected differences in plant species composition between grasslands in villages of different cultural traditions were also mainly related to geographic differences. Germanic villages are located in the wetter northern part of the Swiss Alps because Alemannic people immigrated into the Alps from the North, while Walser and Romanic villages are located in southern parts with a drier climate (Bätzing, 2003). Accordingly, the vegetation of grasslands in Germanic villages indicated moister conditions than in Romanic and Walser villages (data not shown).

The independence of the percentage cover of the different life-forms from altitude implies that the observed altitudinal effects result from a replacement of montane by subalpine species, and of subalpine by alpine species within life-forms with increasing altitude. The abiotic factors pH, slope and aspect were of lower importance for species composition, but nevertheless explained a significant proportion of the variation.

Effects of landuse

Not surprisingly, landuse types were not distributed randomly in the landscape. The analyses of relations between abiotic and and use factors confirmed the suggestion that farmers in the Swiss Alps try to reduce their workload. Abandoned parcels were situated at higher altitudes or had steeper slopes, which implies a low accessibility, while only parcels at lower altitudes and with lower slopes were fertilized. However, the low values of the coefficients of determination show that among the effects of landuse factors on plant species composition only a small proportion is due to correlations with abiotic factors.

Effects of agricultural landuse, i.e. fertilization, mowing or grazing, and abandonment, on plant species composition accounted for almost 30% of the total explained variation.

Fertilization of grasslands is agriculturally important as it increases productivity. But it also alters interspecific competition, which causes changes in species composition and reduces species diversity in favor of dominant species that are limited in growth at lower nutrient levels (Tilman, 1997). Compared with the vegetation of unfertilized parcels, we found the vegetation of fertilized parcels more homogeneous harboring fewer but more abundant species, as indicated by a higher Jaccard index of vegetation similarity between two plots per parcel. The increase in the percentage legume cover and a reduced percentage graminoid cover in fertilized parcels suggest that not nitrogen, but rather phosphorus was the most limiting factor in unfertilized parcels. As nodule development, and therefore legume growth, requires adequate amounts of phosphorus (Bordeleau & Prévost, 1994) these findings suggest phosphorus limitation of the grassland soils (Bobbink, 1991; Stöcklin & Körner, 1999).

The higher total cover of graminoids, non-legume forbs, and legumes in fertilized parcels was accompanied by a lower cover of dwarf shrubs, as reported in other studies (Tasser & Tappeiner, 2002; Zoller & Bischof, 1980).

The investigated agriculturally used grasslands are either mown or grazed. Meadows are mown between one and four times per season, and usually the whole sward is cut at once. Depending on parcel size and weather conditions, pastures are grazed for periods of several days up to several weeks (Maurer et al., 2006) and their vegetation is affected by trampling and selective foraging (Bakker, Deleeuw & Vanwieren, 1984). This was reflected in the more heterogeneous species composition of traditionally grazed parcels than of mown parcels, as indicated by their lower Jaccard index of vegetation similarity between the two study plots per parcel.

Changes in plant species composition and cover values are also particularly strong after abandonment of grasslanduse in the Alps (e.g., Bischof, 1984; Tasser & Tappeiner, 2002; Zoller, Bischof, Erhardt & Kienzle, 1984). The increased graminoid cover and decreased legume and forb cover in abandoned parcels in our study reflect that graminoids effectively spread at the expense of other species (Stammel, Kiehl & Pfadenhauer, 2003), especially after landuse changes (Fischer & Wipf, 2002). This is most likely due to their ability of pronounced clonal growth (Stöcklin, 1992). The stronger increase in graminoid cover in abandoned pastures than in abandoned meadows is likely to be due to the more frequent occurrence of vegetation gaps, such as hoof prints, which promote clonally colonizing graminoids.

Consequences of grazing or abandonment of unfertilized meadows

Unfertilized meadows in general, and the ones addressed in our study in particular, harbor a very species-rich vegetation (Ellenberg, 1996; Maurer et al., 2006). Moreover, because mowing is very labor-intensive, many of these unfertilized grasslands either have been converted to pastures or completely abandoned. Unfertilized parcels converted to pastures had only slightly and non-significantly fewer plant species than parcels that are still mown (Maurer et al., 2006). However, conversion to pastures had considerable impacts on species composition. In many parcels, characteristic meadow-species were replaced by generalist plant species, supporting similar findings after landuse changes in South Tyrol (Tasser & Tappeiner, 2002). Moreover, although they were not additionally fertilized, parcels converted to pastures were colonized by some species indicating higher nutrient levels. This supports the idea that grazing animals create nutrient-rich patches and thereby open new niches in otherwise nutrient-poor grasslands (Vandenbos & Bakker, 1990; WallisDeVries, Bakker & van Wieren, 1998).

Many plant species characteristic for unfertilized meadows were less frequent in abandoned meadows. However, the more frequent occurrence of *Pulsatilla alpina* and *Paradisea liliastrum* in abandoned unfertilized meadows than in still used ones suggests that abandoned parcels also can serve as a refuge for certain species, at least for some time. Nowadays, unfertilized meadows have to be mown yearly to be subsidized, while earlier some of them were only mown every two or three years. Therefore, nowadays flowering stems of plants may be damaged, or seeds have not been mature and dispersed when parcels are mown, which could reduce survival and reproduction of sensitive species. We suggest that the function of a refuge of abandoned unfertilized meadows is limited to species whose requirements, such as the ones of *Paradisea* and *Pulsatilla* (Lauber & Wagner, 2001), do not completely correspond to the present conditions in mown parcels. Furthermore, species have to be tall enough not to be impaired by reduced light in abandoned parcels. To preserve the plant species that were reduced in frequency after landuse changes, parcels should be mown every few years.

Conclusions

Along with topographic factors, landuse plays an important role in shaping the plant species composition of grasslands in the Swiss Alps. Therefore, the current socio-economically motivated landuse changes in the Alps have strong impacts on the grassland species composition. These impacts strongly depend on the type of landuse change. The change from unfertilized meadows to pastures is less severe for vegetation composition than abandonment, and therefore low-intensity grazing should be considered a valuable alternative to abandonment. Abandoned grasslands may serve as refuge only for few characteristic grassland species. Most plants typical for unfertilized meadows decrease in frequency, when unfertilized meadows are converted to pastures or abandoned. Therefore, to preserve sensitive species in the long run, mowing unfertilized meadows every few years should be promoted as highly valuable conservation practice, as it can provide the seeds and niches to maintain a high plant species richness and a vegetation composition typical for unfertilized meadows.

As unfertilized parcels only produce a small yield, mowing will only be maintained if financial incentives are paid to farmers. Currently, the amount of subsidies granted for Swiss farmers mainly depends on the cultivated area, the number of livestock per farm, and the topography of the managed land. Furthermore, farmers have to comply with certain minimal ecological requirements, and extra efforts are additionally rewarded. Important criteria for these ecological payments are landuse intensity, including the date of first mowing or grazing per season. Because of the strong differences in plant species composition between differently used parcels of grassland, we conclude that effective financial incentives should also take the actual plant species composition into account. Moreover, because all landuse combinations are associated with their own characteristic set of species, maintaining a high diversity of plant species in the landscape requires a high diversity of landuse types. Therefore, at the scale of farms, municipalities, and valleys, subsidies should not favor only one particular type of grasslanduse, but a combination ultimately maintaining a high plant species diversity both within and among grasslands.

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Appendix 317 plant species occurring in at least five of the 216 grassland parcels studied in the Swiss Alps. Species are listed in declining order of frequency across all parcels. Frequency classes are indicated for land use combinations of fertilization, traditional land use, and current land use. I = occurrence in ≤ 20 % of parcels, II = occurrence in > 20-40 % of parcels, III = occurrence in > 40-60 % of parcels, IV = occurrence in > 60-80 % of parcels, V = occurrence in > 80 % of parcels.

Fertilization	Unfertilized					Fertilized			All parcels
Traditional land use	Mown			Grazed		Mown		Grazed	
Current land use	Mown	Grazed	Aband.	Grazed	Aband.	Mown	Grazed	Grazed	
Festuca rubra agg.	V	V	V	V	V	III	V	IV	V
Anthoxanthum odoratum agg.	V	V	IV	V	IV	IV	IV	IV	V
Trifolium pratense ssp. *pratense*	V	V	II	IV	III	V	V	V	V
Agrostis capillaris	IV	IV	IV	V	III	III	IV	V	IV
Lotus corniculatus agg.	V	V	IV	V	IV	II	III	IV	IV
Alchemilla xanthochlora agg.	IV	III	II	IV	I	V	IV	V	IV
Trifolium repens	IV	III		IV	II	V	V	V	IV
Potentilla aurea	IV	IV	IV	V	I	II	V	IV	IV
Campanula scheuchzeri	V	IV	IV	V	III	II	III	IV	IV
Dactylis glomerata.	III	IV	III	III	III	V	V	IV	IV
Rumex alpestris	III	IV	III	III	I	V	IV	V	IV
Silene vulgaris ssp. *vulgaris*	IV	IV	IV	III	III	III	IV	III	IV
Ranunculus montanus agg.	IV	IV	IV	IV	II	II	II	IV	III
Plantago lanceolata	IV	IV	I	III	II	III	IV	IV	III
Leucanthemum vulgare agg.	V	IV	III	III	I	III	IV	II	III
Veronica chamaedrys	III	III	III	II	I	V	IV	IV	III
Leontodon hispidus s.l.	IV	IV	III	IV	I	II	II	III	III
Achillea millefolium	II	IV	III	III	III	III	V	IV	III
Geranium sylvaticum	IV	IV	IV	II	II	IV	III	III	III
Chaerophyllum villarsii	IV	III	IV	III	III	IV	III	II	III
Nardus stricta	III	V	II	V	II	I	III	III	III
Galium pumilum	IV	IV	V	IV	III	I	I	II	III
Potentilla erecta	IV	IV	IV	IV	IV	I	II	I	III
Phleum rhaeticum	III	III	III	III	II	II	III	IV	III
Ranunculus acris	II	III	I	II	I	V	IV	IV	III
Cerastium fontanum s.l.	II	II	I	III	I	V	III	IV	III
Phyteuma betonicifolium	III	III	IV	III	III	II	II	II	III
Poa alpina	I	III	I	IV	I	III	IV	IV	III
Crocus albiflorus	III	III	II	III	I	III	II	III	III
Carlina acaulis	IV	III	IV	III	III	I	I	I	III
Luzula sudetica agg.	III	III	II	III	II	I	II	II	III
Carex sempervirens	IV	IV	IV	III	III	I	I	I	III
Taraxacum officinale agg.	II	II		II	I	V	III	IV	III
Briza media	IV	III	III	II	III	I	II	I	II
Vaccinium myrtillus	III	IV	III	III	III		I	I	II
Trisetum flavescens	III	II	I	I		V	III	II	II
Hypericum maculatum ssp. *maculatum*	II	II	I	III	I	II	II	III	II
Trollius europaeus	III	III	II	II	I	II	III	I	II
Plantago media	III	II	I	II	I	I	III	II	II
Silene nutans ssp. *nutans*	III	II	III	II	I	I	II	I	II
Solidago virgaurea	I	III	IV	III	III		II	I	II
Crepis aurea	II	III	I	III	I	I	II	III	II
Crepis pyrenaica	III	III	III	I	I	II	II	I	II
Geum montanum	III	III	III	II	I	I	I	II	II
Soldanella alpina	II	III	III	III	I	I	I	II	II
Gentiana acaulis	III	III	I	III	I	I	I	I	II
Helianthemum nummularium ssp. *obscurum*	III	III	III	II	II		I	I	II
Rhinanthus alectorolophus	IV	I	I	II	I	III	I	I	II

Fertilization	Unfertilized					Fertilized			All parcels
Traditional land use	Mown			Grazed		Mown		Grazed	
Current land use	Mown	Grazed	Aband.	Grazed	Aband.	Mown	Grazed	Grazed	
Arnica montana	II	III	III	II	II	I	I	I	II
Carum carvi	I	I	I	II	I	II	III	III	II
Carex pallescens	II	I	I	III	II	I	II	II	II
Heracleum sphondylium s.l.	II		I	I		V	II	II	II
Trifolium montanum	III	III	II	II	II	I	II		II
Polygonum viviparum	III	II	III	II	I	I	II	I	II
Scabiosa lucida	III	II	III	II	I	I	I	I	II
Silene dioica	I	II		I		V	I	III	II
Hippocrepis comosa	III	III	II	II	I	I	II	I	II
Prunella vulgaris	I	II		III	I	I	II	III	II
Bellis perennis	I	II		I		IV	II	III	II
Campanula barbata	III	III	III	II	I		I	I	II
Helictotrichon pubescens	III	II	II	I	I	II	I	I	II
Hieracium lactucella	II	II		III	I	I	I	II	II
Phyteuma orbiculare	III	I	III	II	II	I	I	I	II
Myosotis alpestris	II	II	I	I		II	II	II	II
Carex caryophyllea	II	III	I	II	I	I	I	II	II
Ranunculus tuberosus	II	I	I	II	I	I	II	II	II
Pimpinella major	III	II	II	II	I	I	I	I	II
Thymus pulegioides	III	II	I	II	I	I	II	I	II
Carduus defloratus	II	II	II	II	II			I	II
Poa annua agg.		I		I		III	II	III	II
Anthyllis vulneraria ssp. *alpestris*	III	II	II	II	I		I		II
Homogyne alpina	I	II	II	III	I		I	I	II
Thymus polytrichus	II	II	II	III	I	I		I	II
Trifolium badium	II	II	I	II		I		II	II
Hieracium pilosella	I	III	II	III	I			I	II
Ajuga reptans	II	II		I		II	I	II	I
Carex montana	III	II	I	II	II	I	I	I	I
Leontodon helveticus	I	II	I	III	I	I		II	I
Deschampsia cespitosa	I	I	I	II	II	I	I	III	I
Hieracium hoppeanum	II	II	II	I	I	I	I	I	I
Linum catharticum	III	II	I	II	I		I		I
Arabis ciliata	II	II	I	II	I	I	I	I	I
Dactylorhiza maculata	II	II	I	II	II	I		I	I
Acinos alpinus	II	II	I	II	I	I	I		I
Lolium perenne	I	I		I		III	I	II	I
Brachypodium pinnatum	II	I	II	I	II	I	I	I	I
Centaurea scabiosa s.l.	III	I	I	I	I	I	I		I
Gymnadenia conopsea	III	I	III	I	I	I	I		I
Veronica serpyllifolia	I	I		I		III	I	II	I
Botrychium lunaria	II	II	II	II	I	I	I	I	I
Luzula sylvatica	I	II	I	II	I	I	I	I	I
Thesium alpinum	II	II	I	II	I		I		I
Pulsatilla alpina	II	II	IV	I		I	I		I
Veratrum album	II	I	I	I	I	I	I	II	I
Euphorbia cyparissias	II	II	II	II	III		I	I	I
Polygala alpestris	I	II	I	II	I		I	I	I
Tragopogon pratensis	II	I	I	I	II	I	II	I	I
Alchemilla conjuncta agg.	I	I	II	II	I		I	I	I
Laserpitium latifolium	III	II	III	I	I	I	I		I
Poa chaixii	I	II	II	I	I	I	I	I	I
Vaccinium vitis-idaea	II	I	III	I	I			I	I
Trifolium alpinum	I	II	I	II	I	I		I	I

Fertilization	Unfertilized					Fertilized			All parcels
Traditional land use	Mown			Grazed		Mown		Grazed	
Current land use	Mown	Grazed	Aband.	Grazed	Aband.	Mown	Grazed	Grazed	
Euphrasia rostkoviana	II	I	I	II	I	I	II	I	I
Thymus serpyllum agg.	I	II	I	I	I		II	I	I
Koeleria pyramidata	II	II	I	I	II	I	I		I
Acer pseudoplatanus	I	I		I		I		II	I
Ajuga pyramidalis	I	II	I	II	I			I	I
Lysimachia nemorum	I	I	I	I		I	II	II	I
Vicia cracca ssp. *cracca*	I	I	I	I	I	II	II	I	I
Calluna vulgaris	I	I	II	II	I		I	I	I
Carex ornithopoda	II	II	I	II	I		I	I	I
Plantago serpentina	I	II		II	I	I	I	I	I
Polygala vulgaris ssp. *vulgaris*	II	II	I	I	I	I	I		I
Bartsia alpina	I	I	II	I	I	I	I		I
Gentiana verna	I	I		II		I		I	I
Cynosurus cristatus	I	I		I		I	II	I	I
Phleum hirsutum	II	I	III	I	I	I	I		I
Poa trivialis ssp. *trivialis*	I		I		I	III	I	I	I
Thalictrum minus ssp. *minus*	I	II	II	I	I	I	I		I
Ligusticum mutellina	I	I	I	II	I	I		II	I
Pimpinella saxifraga agg.	II	II	I	I	I		I		I
Helictotrichon versicolor	II	II	II	I					I
Vaccinium uliginosum agg.	I	I	I	II	II			I	I
Alchemilla hybrida agg.	II	II	I	I		I	I		I
Biscutella laevigata	I	II	I	I	I	I	I	I	I
Centaurea jacea ssp. *jacea*	I	I	I	I	I	I	I	I	I
Luzula campestris	I	I		I	I	I	I	I	I
Prunella grandiflora	I	I	I	I	I	I	I		I
Galium album	I	I	I	I		I	II	I	I
Lathyrus pratensis	I	II	I	I	I	I	I	I	I
Poa pratensis			I	I		II	I	I	I
Veronica officinalis	I	II	I	I	I		I	I	I
Paradisea liliastrum	II	II	II	I	I	I			I
Polygala chamaebuxus	I	II	I	I	I				I
Festuca pratensis	I			I	I	I	II	II	I
Leontodon autumnalis	I	I		I	I	I	I	II	I
Viola tricolor agg.	II	I	I	I	I	I	I	I	I
Carex flacca	I	I	I	II	I			I	I
Hypochaeris uniflora	II	II	II	I		I			I
Poa violacea	II	I	I	I	I	I			I
Rumex obtusifolius		I				II	I	I	I
Cerastium arvense ssp. *strictum*	I	II	I	II		I		I	I
Knautia dipsacifolia	I		III	I	I	I	I	I	I
Anthriscus sylvestris.	I			I		II	I	I	I
Pseudorchis albida	I	II	I	I	I				I
Sanguisorba minor ssp. *minor*	I	I	I	I	I		I	I	I
Centaurea nervosa	I	II	II	I	I	I		I	I
Coeloglossum viride	I	I	I	II					I
Hieracium murorum	I	I	I	I	I				I
Sesleria caerulea	I	I	II	I	I				I
Polygonum alpinum	I	II	I	I		I	I	I	I
Fragaria vesca	I	I	I	I	I	I	I		I
Plantago atrata	I	I	I	I	I		I	I	I
Aster bellidiastrum.	I	I	I	II					I
Rumex alpinus				I		I		III	I
Stellaria graminea	I			I	I	I	II	I	I

Fertilization	Unfertilized					Fertilized			All parcels
Traditional land use	Mown			Grazed		Mown		Grazed	
Current land use	Mown	Grazed	Aband.	Grazed	Aband.	Mown	Grazed	Grazed	
Antennaria dioica	I	I	I	I			I		I
Campanula rhomboidalis	I	I				I	I	I	I
Pedicularis tuberosa	I		I	I	I		I		I
Seseli libanotis	I	I	I	I	I		I	I	I
Cirsium acaule	I	I	I	I				I	I
Myosotis decumbens						II		I	I
Silene rupestris	I	I	I	II	I	I		I	I
Viola biflora	I		I	II	I	I			I
Plantago major ssp. *major*		I		I		I	II	II	I
Astrantia minor		I	I	II	II				I
Listera ovata	II		I	I	I				I
Molinia caerulea	I	I	I	I	II				I
Peucedanum ostruthium	I	I	I	I	I	I		I	I
Ranunculus repens		I	I	I	I	I		I	I
Selaginella selaginoides	I	I		II	I			I	I
Thesium pyrenaicum	II	I	I						I
Trifolium medium	I	I	I	I				I	I
Avenella flexuosa	I	I	I	I	II		I	I	I
Erica carnea	I		I	I	I		I		I
Galeopsis tetrahit		I	II	I	II		I	I	I
Colchicum autumnale	I		I	I		I		I	I
Potentilla crantzii	I	II	I	I	I			I	I
Sagina saginoides		I		I				I	I
Juniperus communis ssp. *nana*	I	I	I	I	II		I	I	I
Bromus erectus ssp. *erectus*	I	I	I	I	I	I			I
Carex leporina				I	I	I		I	I
Hypochaeris radicata	I	I		I		I		I	I
Melampyrum sylvaticum	I	I	II	I	II				I
Pedicularis foliosa	I	I	I	I		I			I
Primula elatior	I	I		I		I		I	I
Rhododendron ferrugineum	I	I	I	I	I				I
Veronica alpina		I		I		I		I	I
Alopecurus pratensis	I					II	I	I	I
Galium boreale	I	I	I	I	I	I	I		I
Potentilla grandiflora	I	I	I	I	I		I		I
Salvia pratensis	I	I	I	I	I	I	I	I	I
Anemone narcissiflora	I		II	I					I
Gentiana campestris	I	I	I	I					I
Globularia nudicaulis	I	I	I	I	I				I
Parnassia palustris	I		I	I					I
Galium verum ssp. *verum*	I	I	I	I	I		I	I	I
Hieracium lachenalii	I	I	I	I			I	I	I
Knautia arvensis	II			I			I	I	I
Ranunculus bulbosus	I	I	I			I	I	I	I
Ranunculus aconitifolius				I		I		I	I
Rhinanthus glacialis	I		I	I	I				I
Scabiosa columbaria	I	I	I	I				I	I
Viola hirta	I		I	I	I			I	I
Agrostis stolonifera	I		I	I	I		I		I
Carex nigra				I	I		I	I	I
Vicia sepium	I		I			I	I	I	I
Anemone nemorosa	I	I		I		I		I	I
Hieracium bifidum	I	I	I	I	I				I
Hieracium villosum	I	I	I	I	I				I

Fertilization	Unfertilized					Fertilized			All parcels
Traditional land use	Mown			Grazed		Mown		Grazed	
Current land use	Mown	Grazed	Aband.	Grazed	Aband.	Mown	Grazed	Grazed	
Phyteuma ovatum	I	I		I		I		I	I
Polygonatum verticillatum	I	I	I		I				I
Primula farinosa	I	I	I	I	I				I
Ranunculus ficaria	I	I		I		I		I	I
Rumex acetosella s.str.	I	I	I	I	I			I	I
Sempervivum montanum			I	I	I			I	I
Carex pilulifera		I	I	I			I	I	I
Crepis biennis	I	I				I	I		I
Medicago lupulina	I			I		I	I	I	I
Onobrychis montana	I	I	I	I			I		I
Arrhenatherum elatius	I		I	I		I		I	I
Cirsium spinosissimum		I		I				I	I
Huperzia selago	I	I		I	I				I
Laserpitium halleri		II	I	I	I				I
Luzula lutea	I	I	I	I					I
Myosotis nemorosa	I			I		I		I	I
Nigritella nigra	I		I	I					I
Phyteuma hemisphaericum	I	I		I					I
Primula veris s.l.	I		I	I	I	I		I	I
Rubus idaeus	I	I	I	I	I				I
Valeriana officinalis agg.	I		I	I	I			I	I
Platanthera bifolia	I		I	I	I		I		I
Polygonum bistorta		I				I	I	I	I
Allium carinatum ssp. *carinatum*	I	I	I	I	I				I
Cardamine pratensis		I				I		I	I
Carduus nutans	I	I		I		I		I	I
Carex sylvatica	I			I		I		I	I
Cuscuta epithymum	I		I	I					I
Festuca ovina agg.	I		I	I	I	I			I
Galium lucidum	I		I	I	I				I
Geum rivale	I	I	I	I		I		I	I
Holcus lanatus	I			I		I		I	I
Luzula spicata		I		I				I	I
Poa angustifolia	I		I	I	I	I			I
Rosa spec.	I	I	I	I	I				I
Rumex acetosa	I	I		I		I		I	I
Trifolium thalii		I		I				I	I
Veronica arvensis						I		I	I
Knautia drymeia	I	I	I			I	I		I
Phleum pratense			I	I		I	I	I	I
Adenostyles alliariae				I				I	I
Agrostis rupestris		I		I					I
Alnus viridis	I	I	I	I					I
Buphthalmum salicifolium	I	I	I	I					I
Calamagrostis villosa	I		I	I	I				I
Dianthus superbus	I	I	I						I
Euphrasia minima	I	I		I				I	I
Glechoma hederacea ssp. *hederacea*	I	I		I		I		I	I
Luzula luzuloides	I	I	I		I			I	I
Maianthemum bifolium	I	I	I	I	I			I	I
Picea abies	I	I	I	I	I				I
Pulmonaria australis		I	I	I	I				I
Salix retusa	I	I	I	I					I
Senecio alpinus								II	I

Fertilization	Unfertilized					Fertilized			All parcels
Traditional land use	Mown			Grazed		Mown		Grazed	
Current land use	Mown	Grazed	Aband.	Grazed	Aband.	Mown	Grazed	Grazed	
Succisa pratensis	I	I		I	I	I			I
Viola calcarata	I	I	I	I					I
Androsace chamaejasme			I	I			I		I
Anthyllis vulneraria ssp. *valesiaca*	I	I		I		I	I		I
Astragalus glycyphyllos	I	I	I	I			I		I
Equisetum arvense		I		I			I	I	I
Agrostis schraderiana			I	I	I				I
Calycocorsus stipitatus				I		I		I	I
Carex flava agg.				I	I			I	I
Carex panicea	I	I		I		I			I
Festuca pulchella	I		II						I
Helianthemum nummularium ssp. *grandiflorum*	I	I	I	I	I				I
Larix decidua			I	I	I				I
Onobrychis viciifolia	I		I		I	I			I
Sedum sexangulare	I	I		I	I				I
Senecio doronicum			I	I					I
Silene acaulis				I					I
Stachys officinalis	I	I	I	I					I
Tofieldia calyculata	I		I	I	I				I
Campanula glomerata ssp. *glomerata*		I	I	I		I	I	I	I
Chenopodium bonus-henricus				I		I	I	I	I
Festuca valesiaca s.l.	I	I		I	I		I		I
Medicago sativa		I	I			I	I		I
Rumex scutatus	I			I			I		I
Calamagrostis epigejos			II		I				I
Cirsium helenioides	I	I	I			I		I	I
Crepis conyzifolia	I		I	I					I
Danthonia decumbens		I	I	I	I				I
Gentiana purpurea		I	I		I				I
Hypericum perforatum	I	I		I					I
Orchis ustulata	I			I					I
Origanum vulgare			I	I	I			I	I
Potentilla sterilis	I	I		I				I	I
Rhinanthus minor	I			I		I			I
Traunsteinera globosa	I		I						I
Securigera varia	I	I	I		I		I		I
Achillea erba-rotta ssp. *moschata*				I					I
Aconitum napellus agg.			I	I					I
Aegopodium podagraria						I			I
Alchemilla alpina agg.				I					I
Anthyllis vulneraria ssp. *vulneraria*	I			I					I
Cardamine flexuosa		I						I	I
Carex echinata				I	I	I		I	I
Carex ferruginea	I			I				I	I
Daphne striata	I		I		I				I
Dianthus carthusianorum	I	I	I						I
Euphrasia hirtella	I			I					I
Festuca violacea agg.	I			I	I				I
Fraxinus excelsior	I	I		I		I			I
Galium rubrum	I			I					I
Gentiana lutea	I		I	I					I
Gypsophila repens			I	I	I				I
Medicago falcata			I	I	I			I	I

Chapter 4

Leaf herbivory and fungal pathogen infection in grassland in the Swiss Alps

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submitted

Summary

1. In the cultural landscape of the Alps, grassland contributes to high biodiversity not only by its plant species richness, but also by the diversity of biological interactions between plants, herbivores, and pathogens.
2. We recorded the leaf area damaged by ten types of herbivory and five types of fungal pathogen infection on 12’054 plant leaves of legumes, other forbs, and graminoids collected in 215 grassland parcels of different land use and altitude in the Swiss Alps when vegetation was best developed.
3. We asked how the extent of leaf damage by herbivory and fungal pathogen infection, and their diversity, are affected by plant functional group, land use, and altitude, and whether extent and diversity of leaf damage are related to each other, to plant species richness, and to standing crop.
4. With 83% of all leaves infested, herbivory and fungal pathogen infection were ubiquitous. However, only 2.7% leaf area was damaged by herbivory and 1.2% by fungal pathogens. Damage by herbivory was highest on legumes, and damage by fungal pathogens was highest on graminoids. More leaf damage by herbivory occurred in traditionally mown sites and at lower altitudes, while damage by fungal pathogen infection was independent of land use and altitude. Most types of herbivory were found on legumes and on leaves from fertilised sites, whereas the diversity of fungal pathogen types was highest on graminoids and in unfertilised sites. Larger standing crop was associated with higher leaf damage and diversity of herbivory types per leaf. In parcels with higher number of plant species per parcel the diversity of herbivory types was lower at the leaf level, but tended to be higher at the parcel level.
5. Our results highlight the omnipresence of plant-herbivore and plant-pathogen interactions. Moreover, they indicate neither a conflict between conservation goals for different taxa, nor a conflict between the conservation of biological interactions of plants and agricultural goals in the Alps. Therefore, we suggest maintaining the high diversity of mountain grassland by diverse low-intensity farming.

Key words: agricultural land use; biodiversity; biological interactions; conservation; forbs; fungi; grasses; herbivores; legumes; plant functional groups

Introduction

Meadows and pastures are an important element of the cultural landscape of the Alps and are famous for their characteristic and diverse flora and fauna (Ellenberg, 1996; Zoller & Bischof, 1980). The various landscapes of the Alps have been shaped by humans and their cattle over thousands of years (Ellenberg, 1996) and they are among the most plant species rich areas in Europe (Väre et al., 2003). Different cultural traditions (Germanic, Romanic, and Walser) with their specific farming practices have contributed to the high diversity of the alpine landscapes (Bätzing 1991). However, in the Alps land use is undergoing major changes (Bätzing, 2003) where both abandonment and intensification of land use constitute major threats to alpine plant species diversity (Fischer & Wipf, 2002; Olsson et al., 2000).

Biodiversity consists of diversity at several levels of biological integration (communities, species, genes), and includes biological interactions such as pollination, herbivory, and fungal disease (Primack, 1993). In addition to land use, biodiversity is also affected by geological, topographic, and climatic conditions. In the Alps, the altitudinal gradient is particularly important (Rahbek, 1995; Theurillat et al., 2003). Efforts to protect the diversity of alpine landscapes should be based on a comprehensive knowledge of biodiversity at all levels and spatial scales. However, while plant species richness is comparatively well studied in grasslands in the Alps, information about the diversity of herbivores and plant pathogens is very scarce.

Conflicts between different conservation goals arise if high diversity of herbivores or plant pathogens is associated with low diversity of the other group, or of plants. Moreover, if high diversity of herbivores and pathogens were associated with substantial plant biomass losses, their conservation would conflict with the agricultural goal of high biomass production. Currently, the relationships between plant species richness, diversity of herbivory and pathogens, and standing crop are not known for mountain grassland.

Several studies with herbivores and pathogens in grassland at lower altitudes suggest that degree and diversity of herbivory and pathogen infection are affected by land use (Kruess & Tscharntke, 2002; Wettstein & Schmid, 1999), site productivity (Mulder et al., 2002; Siemann, 1998), and plant species diversity (Knops et al., 1999; Mitchell et al., 2002). Moreover, altitudinal effects on the extent of herbivory have been reported for molluscs (Scheidel & Bruelheide, 2001). In wetlands in the pre-Alps, the diversity and abundance of grasshoppers and butterflies was negatively affected by management and declined with altitude (Wettstein and Schmid 1999). However, a comprehensive study of degree and diversity of herbivory and pathogen infection is missing for grassland at higher altitudes.

Different plant functional groups are likely to be differently affected by herbivores and pathogens (Symstad et al., 2000). Due to their high nitrogen contents, legumes are likely to be preferred by herbivores (Mattson, 1980). Pathogens are more likely and more abundant in larger host populations (Mitchell et al., 2002). Therefore, due to their usually high abundances, graminoids may be more likely to be infected by fungal pathogens than forbs or legumes are.

We measured the amount of leaf damage by herbivory and by fungal pathogen infection at three altitudinal levels around each of 12 villages across the Swiss Alps as an indicator for the intensity of plant-herbivore and plant-pathogen interactions in mountain grassland. We measured leaf damage once, whenever possible at the time of maximum standing crop, when the level of damage is most relevant for farmers. To measure diversity, we classified leaf damage according to different types of herbivory and fungal pathogen infection. This indirect approach to measure biological interactions allowed us to quantitatively assess leaf damage by different types of herbivory and fungal pathogen infection for 215 grassland parcels.

For scoring leaf damage by different herbivory types we followed Crawley (1983) who classified leaf damage by herbivores according to feeding habit. Because single damage types can be caused by several taxa, it is not possible to identify the species that caused a particular damage type. Nevertheless, Crawley's classification of damage types of herbivores allowed us to assess the diversity of herbivory. For fungal pathogen infection, the situation is more difficult. There is no such classification of leaf damage by different types of fungal pathogens. Therefore, for fungal pathogens we used broad taxonomic categories that could be distinguished with a binocular microscope.

We studied the degree of damage by different types of herbivory and fungal pathogen infection of leaves of 215 grassland parcels of different land uses at different altitudes in the Swiss Alps. To account for potential regional and cultural differences, these grassland parcels represented four villages of each of the three main cultural traditions in the Swiss Alps, Romanic, Germanic, and Walser. Our main questions were: (1) How large is the damage of plant leaves by herbivory and fungal pathogen infection, and does the extent of leaf damage depend on plant functional group, land use, and altitude? (2) Which, and how many, different types of herbivory and fungal pathogen infection are present on leaves of different origin, and do number and composition of types depend on plant functional group, land use, and altitude? (3) How are leaf damage and the diversity of types of herbivory and fungal pathogen infection related to each other, to plant species richness, and to the amount of standing crop per grassland parcel?

Materials and methods

Study sites

We studied grassland in 12 villages in the Swiss Alps, four of each of the three cultural traditions (named after the original settling tribes) Romanic, Germanic, and Walser (K.Maurer, A.Weyand, M.Fischer, J.Stöcklin, unpublished data). Each village belongs to a separate alpine valley, and the 12 villages are located along an east-west gradient of about 170 km. We selected villages from those whose agriculture has only changed modestly during the last 50 years, that are not very touristy, and that have less than 1500 inhabitants.

At three altitudinal levels per village (valley at about 1000 m asl, intermediate altitudes about 1500 m asl, and alp at about 2000 m asl), we selected parcels of land representing up to 12 different land use combinations (K.Maurer, A.Weyand, M.Fischer, J.Stöcklin, unpublished data). Parcels were fertilised or unfertilised, had traditionally been mown or grazed, and were currently mown, grazed, or abandoned. In total, we selected 216 grassland parcels, ranging from 12 to 24 per village.

Leaf sampling

We had taken vegetation records in two 5 m x 5 m plots separated by about 5 m in each grassland parcel in summer 2002 (69 parcels) and summer 2003 (147 parcels) to assess plant species composition and diversity (K.Maurer, A.Weyand, M.Fischer, J.Stöcklin, unpublished data). As close as logistically possible, we did this when the vegetation was best developed, shortly before managed parcels were mown or grazed. At this time, leaf damage is most relevant for farmers. Additionally, in each plot we harvested biomass 4 cm above ground in a randomly selected area of 0.5 m x 0.5 m to assess standing crop. At the same time, in 215 of the 216 parcels we collected one leaf of each of ten plants of the three functional plant groups graminoids (Poaceae, *Carex,* and *Luzula*), legumes, and non-legume forbs (except for thistles and orchids). In each plot, we collected leaves of plants closest to positions defined by a walking grid. This procedure resulted in a sample of 30 randomly selected leaves per plot, i.e. 60 leaves per parcel, except for parcels without or with only few legume plants. We transported the leaves in plastic bags, pressed them in plant presses for up to five days, and dried them in a drying oven at 40°C for at least two days.

Scoring of herbivory and fungal pathogen infection

In winter 2003/2004, we visually screened all 12’054 leaves for damage by herbivory and fungal pathogen infection with a binocular microscope. To classify different types of leaf damage, we distinguished ten categories of infestation by herbivory and five categories of infestation by fungal pathogen infection (Table 1).

For herbivory, we distinguished between clipping, holing, rasping, mining, skeletonising, and spinning according to Crawley (1983). Additionally, we considered the categories windowing, puncturing, eggs, and larvae/pupae. We considered eggs and larvae/pupae after realising their frequent presence and large numbers on the leaves. We are aware that scored eggs and larvae/pupae would have developed into one of the other categories. Nevertheless, introducing the classes of eggs and larvae/pupae were not only due to practicability, because the simultaneous occurrence of different stages of development also constitutes diversity.

We scored fungal pathogen infection according to taxonomical categories and distinguished between rust fungi, powdery mildew, hyphomycetes, and ascomycetes. Because it was not possible to unambiguously classify all infestations by fungal pathogens, we also introduced the category of unknown fungi, which may however to some degree include infection by the groups mentioned above. Finally, we classified any leaf damage as unknown, which could not be ascribed to any of these categories. We did not include such unknown damage into the analyses.

For each leaf, we estimated the leaf area damaged by herbivory and fungal pathogen infection separately in percent. Then, we recorded the presence and absence of all categories of leaf damage. Additionally, we noted the category that caused the largest area of leaf damage, both for herbivory and fungal pathogen infection. To estimate the proportion of damage per leaf we compared the leaves with leaf templates with known proportions of damaged area. For damage amounting to less than one percent, we used the value 0.5 for the calculations.

Table 1 Ten types of leaf damage by herbivory and five types of leaf damage by fungal pathogens and their characteristics scored on 12'054 leaves of plants from 215 grassland parcels in the Swiss Alps.

Category	Characteristics
Herbivory	
Clipping	Damage of leaf margins; usually sharp edges
Holing	Damage within leaf; continuous hole in the leaf
Windowing	Damage within leaf, but with remaining single cell layer
Rasping	Removal of single cell layers from surface; often white short dashes
Mining	Continuous mine in the leaf, visible from both sides
Skeletonising	Damage by continuous hole along the leaf veins
Spinning	Cocoons or cocoon-like structures
Puncturing	Fine punctured stitch-like pattern
Eggs	Mostly accumulated, but also single ones
Larvae/pupae	Mostly single
Fungal pathogens	
Rust fungus	Greyish sporangia with brown rusty spores, crumb-like on leaf surface or veins
Hyphomycetes	Small, black, cloud-like structures on leaf surface
Powdery mildew	Patches of fine white mycelium, mostly on upper leaf surface
Ascomycetes	Patches with pustule-like structure in the centre
Unknown Fungi	Undefined damage (patches with parchment-like inner part, holes with red margin, mine visible from only one side, other concentric coloured patches with light centre/dark margin)

Data analysis

We included all 12'054 leaves and assessed damage by herbivory and by fungal pathogen infection per leaf, per functional group and parcel, and per parcel. We analysed the effects of land use and altitude on damaged leaf area and diversity of types of herbivory and fungal pathogen infection with analyses of variance (ANOVA) using a hierarchical mixed model including the factors culture, village, altitude, fertilisation, abandonment, current land use, traditional land use, parcel and all interactions (Table 2). Villages were nested within cultures, and current land use was nested within abandonment, because in our study current land use is meant to refer to currently mown and grazed grassland parcels. To analyse differences between functional groups, we added the factor functional group and all its interactions to the model. In all analyses, we treated village and parcel as random factors.

Table 2 ANOVA model of leaf damage by, and diversity of types of, herbivory and fungal pathogen infection on 12’054 leaves of plants from 215 grassland parcels of different altitude and land use in 12 villages of 3 different cultural traditions in the Swiss Alps.

Source of variation	Mean Squares	Variance ratios (F-values)
Culture	ms_{cult}	$ms_{\text{cult}} / ms_{\text{vill}}$
Village	ms_{vill}	$ms_{\text{vill}} / ms_{\text{residual}}$
Altitude	ms_{alt}	$ms_{\text{alt}} / ms_{\text{residual}}$
Fertilisation	ms_{fert}	$ms_{\text{fert}} / ms_{\text{residual}}$
Traditional land use	ms_{trad}	$ms_{\text{trad}} / ms_{\text{residual}}$
Abandonment	ms_{aband}	$ms_{\text{aband}} / ms_{\text{curr}}$
Current land use	ms_{curr}	$ms_{\text{curr}} / ms_{\text{residual}}$
Traditional land use x Fertilisation	$ms_{\text{trad x fert}}$	$ms_{\text{trad x fert}} / ms_{\text{residual}}$
Abandonment x Fertilisation	$ms_{\text{aband x fert}}$	$ms_{\text{aband x fert}} / ms_{\text{curr x fert}}$
Current land use x Fertilisation	$ms_{\text{curr x fert}}$	$ms_{\text{curr x fert}} / ms_{\text{residual}}$
Abandonment x Traditional land use	$ms_{\text{aband x trad}}$	$ms_{\text{aband x trad}} / ms_{\text{residual}}$
Culture x Altitude	$ms_{\text{cult x alt}}$	$ms_{\text{cult x alt}} / ms_{\text{vill x alt}}$
Village x Altitude	$ms_{\text{vill x alt}}$	$ms_{\text{vill x alt}} / ms_{\text{residual}}$
Culture x Fertilisation	$ms_{\text{cult x fert}}$	$ms_{\text{cult x fert}} / ms_{\text{vill x fert}}$
Culture x Traditional land use	$ms_{\text{cult x trad}}$	$ms_{\text{cult x trad}} / ms_{\text{vill x trad}}$
Culture x Abandonment	$ms_{\text{cult x aband}}$	$ms_{\text{cult x aband}} / ms_{\text{vill x aband}}$
Culture x Current land use	$ms_{\text{cult x curr}}$	$ms_{\text{cult x curr}} / ms_{\text{vill x curr}}$
Village x Fertilisation	$ms_{\text{vill x fert}}$	$ms_{\text{vill x fert}} / ms_{\text{residual}}$
Village x Traditional land use	$ms_{\text{vill x trad}}$	$ms_{\text{vill x trad}} / ms_{\text{residual}}$
Village x Abandonment	$ms_{\text{vill x aband}}$	$ms_{\text{vill x aband}} / ms_{\text{residual}}$
Village x Current land use	$ms_{\text{vill x curr}}$	$ms_{\text{vill x curr}} / ms_{\text{residual}}$
Fertilisation x Altitude	$ms_{\text{fert x alt}}$	$ms_{\text{fert x alt}} / ms_{\text{residual}}$
Traditional land use x Altitude	$ms_{\text{trad x alt}}$	$ms_{\text{trad x alt}} / ms_{\text{residual}}$
Abandonment x Altitude	$ms_{\text{aband x alt}}$	$ms_{\text{aband x alt}} / ms_{\text{curr x alt}}$
Current land use x Altitude	$ms_{\text{curr x alt}}$	$ms_{\text{curr x alt}} / ms_{\text{residual}}$
Culture x Altitude x Fertilisation	$ms_{\text{cult x alt x fert}}$	$ms_{\text{cult x alt fert}} / ms_{\text{vill x alt x fert}}$
Culture x Altitude x Traditional land use	$ms_{\text{cult x alt x trad}}$	$ms_{\text{cult x alt x trad}} / ms_{\text{vill x alt x trad}}$
Culture x Altitude x Abandonment	$ms_{\text{cult x alt x aband}}$	$ms_{\text{cult x alt x aband}} / ms_{\text{vill x alt x aband}}$
Culture x Altitude x Current land use	$ms_{\text{cult x alt x curr}}$	$ms_{\text{cult x alt x curr}} / ms_{\text{vill x alt x curr}}$
Village x Altitude x Fertilisation	$ms_{\text{vill x alt x fert}}$	$ms_{\text{vill x alt x fert}} / ms_{\text{residual}}$
Village x Altitude x Traditional land use	$ms_{\text{vill x alt x trad}}$	$ms_{\text{vill x alt x trad}} / ms_{\text{residual}}$
Village x Altitude x Abandonment	$ms_{\text{vill x alt x curr}}$	$ms_{\text{vill x alt x aband}} / ms_{\text{residual}}$
Village x Altitude x Current land use	$ms_{\text{vill x alt x aband}}$	$ms_{\text{vill x alt x curr}} / ms_{\text{residual}}$
Residual	ms_{residual}	

Aspect and slope of a parcel, and yearly differences could affect herbivores and fungal pathogens. However, including aspect, slope, and year of leaf harvest as covariates turned out not to change levels of significance. Therefore, we present results of the model without covariates.

To analyse differences in the composition of damage types between parcels, first, we conducted a principal component analysis (PCA) of the occurrence of different categories of herbivory and fungal pathogen infection per leaf. Then, to test for effects of altitude and land use on the composition of herbivory and fungal pathogen diversity we did an ANOVA of the four principal components with the same model as mentioned above.

Results

Leaf damage by herbivory and fungal pathogen infection

82.8% of the 12’054 leaves were damaged by at least one category of herbivory or fungal pathogen infection. The most frequent categories of herbivory were, in declining order, clipping, holing, rasping, and windowing (Table 3). Moreover, eggs were present on 49% of all leaves and dominant on 25.1% of them, indicating that damage by and number of occurring herbivory types would presumably increase after egg development. For fungal pathogen infection, the most frequent categories, in declining order, were rust fungi (3.5%), hyphomycetes (2.1%), powdery mildew (1.2%), and ascomycetes (0.4%). However, these numbers were small compared to the 18.9% of leaves with unknown fungi (Table 3). 58.7% of the leaves were infested by herbivory only, 6.0% by fungal pathogens only, and 18.0% by both.

We recorded 2.7 ± 0.13% (mean ± SE) leaf damage by herbivory, and 1.2 ± 0.09% by fungal pathogen infection. Parcels with higher damage of leaf area by herbivory also had higher damage by fungal pathogen infection ($N = 215$, $R = 0.18$, $p < 0.01$, Fig. 1A). Herbivory was most pronounced on legumes (4.5 ± 0.3% damage per leaf), followed by non-legume forbs (2.6 ± 0.1%), and graminoids (1.3 ± 0.1%, Fig. 2A). In contrast, fungal pathogen infection was most pronounced on graminoids (1.5 ± 0.1%), followed by non-legume forbs (1.2 ± 0.1%), and legumes (0.7 ± 0.1%, Fig. 2A).

The lower the altitude, the more leaf area was damaged by herbivory (valley 3.3 ± 0.2%, intermediate altitudes 2.6 ± 0.2%, alp 2.0 ± 0.2%; $N = 215$, $F_{1,98} = 34.1$, $p < 0.001$). The damaged leaf area of legumes was higher in the valley than at intermediate altitudes, where it was higher than at the alp level ($N = 638$, $F_{2,199} = 14.1$, $p < 0.001$, Fig. 3A). On graminoids, damage was also higher in the valley grassland, but did not differ between grassland at intermediate altitudes and at the alp level. Leaf damage of forbs was independent of altitude. Moreover, altitude did not affect damage by fungal pathogen infection.

Table 3 Percentage of leaves of legumes, non-legume forbs, and graminoids damaged by 10 different herbivory types, by 5 different fungal pathogen types, and by unknown causes in 215 grassland parcels in the Swiss Alps. Also included are the percentage of each damage category present per parcel, and the percentage of leaves for which the particular category was the dominant cause of damage.

Type of leaf damage	**Present in x% of**					**Dominant in % leaves**
Herbivory	**Leaves of legumes**	**Leaves of non-legume forbs**	**Leaves of graminoids**	**Leaves of all groups**	**Parcels**	
Clipping	38.29	23.12	8.09	22.43	100.0	16.9
Holing	38.23	20.84	4.15	20.24	99.5	10.0
Windowing	28.34	15.59	8.97	17.11	99.5	7.5
Rasping	27.45	12.63	20.56	19.86	94.9	14.3
Puncturing	2.51	2.56	0.24	1.73	45.6	0.4
Skeletonising	2.32	0.19	0.47	0.93	28.8	0.3
Mining	0.94	0.52	1.12	0.85	29.8	0.4
Spinning	0.52	1.00	0.14	0.56	16.3	0.2
Eggs	54.74	53.10	39.95	49.00	100.0	25.1
Larvae/pupae	2.48	5.91	1.83	3.45	74.4	1.5
Fungal pathogens						
Rust fungi	14.44	19.23	22.43	18.91	65.1	3.1
Hyphomycetes	4.05	2.49	4.11	3.53	43.7	1.1
Powdery mildew	2.34	1.38	2.52	2.07	23.3	1.1
Ascomycetes	1.71	1.33	0.57	1.18	16.7	0.4
Unknown Fungi	0.69	0.52	0.14	0.44	97.7	18.3
Unknown	8.3	9.2	5.7	23.3	100.0	-

In traditionally mown grassland, more leaf area was damaged by herbivory on legumes and forbs than in those that had always been grazed, whereas there was no such effect for graminoids (N = 638, $F_{2,199} = 5.6$, $p < 0.01$, Fig. 3B). Moreover, land use did not affect leaf damage by fungal pathogens.

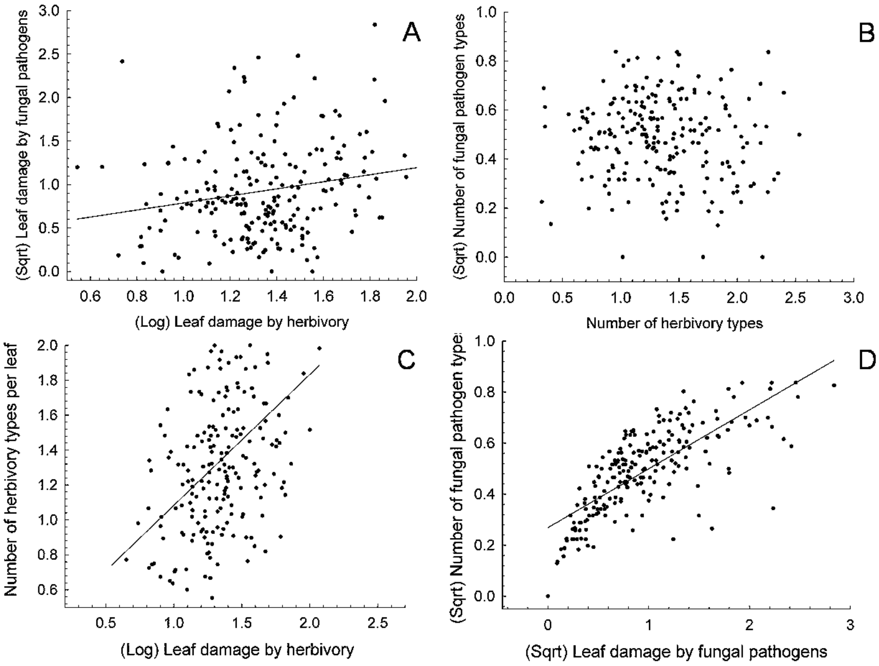

Fig. 1 Relationships between A) leaf damage by herbivory and by fungal pathogen infection, B) diversity of types of herbivory and fungal pathogen infection, C) leaf damage and the diversity of types of herbivory and D) leaf damage and diversity of types of fungal pathogen infection on leaves from 215 grassland parcels in the Swiss Alps. Sqrt denotes square root transformation, log denotes logarithm to the base of ten.

Diversity of types of herbivory

Per leaf, we recorded 1.36 ± 0.01 of the ten types of herbivory. Per parcel, we recorded 6.89 ± 0.07 types of herbivory. The number of herbivory types per leaf was higher in parcels with higher average leaf damage by herbivory (N = 215, R = 0.40, $p < 0.001$, Fig. 1C).

More herbivory types occurred on legumes (1.90 ± 0.05 types per leaf) than on non-legume forbs (1.35 ± 0.03), and graminoids (0.86 ± 0.03, Fig. 4). The lower the altitude, the more types of herbivory occurred per leaf (valley 1.53 ± 0.05 types per leaf, intermediate altitudes 1.40 ± 0.04, alp 1.01 ± 0.04; N = 215, $F_{1,98} = 137.8$, $p < 0.001$).

This was more pronounced for legumes than it was for graminoids and non-legume forbs (functional group by altitude interaction; N = 638, $F_{2,199} = 3.2$, $p < 0.05$, Fig. 4A).

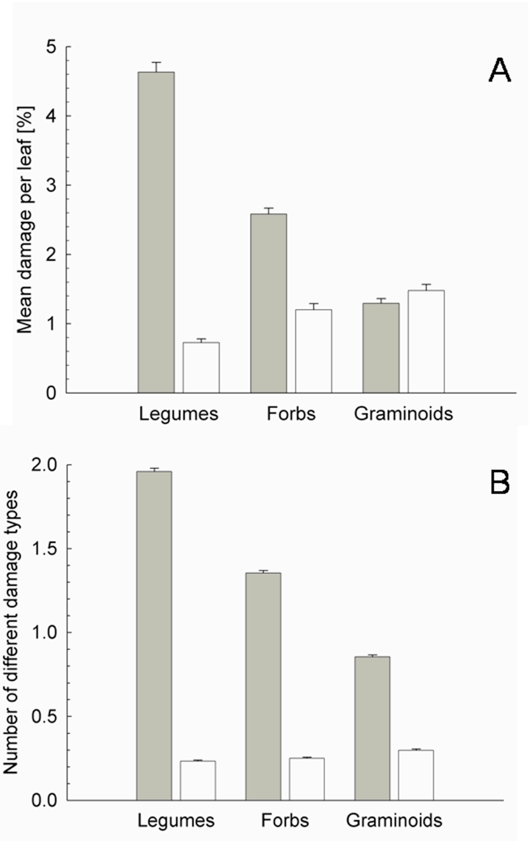

Fig. 2 A) Mean damage per leaf by herbivory (shaded) and fungal pathogen infection (open), and B) mean number of different damage types by herbivory (shaded) and fungal pathogen infection (open) recorded on leaves of the three functional groups (legumes, non-legume forbs, graminoids) from 215 grassland parcels in the Swiss Alps. Error bars denote 1 standard error.

More types of herbivory occurred in traditionally mown grassland parcels (1.45 ± 0.04 types per leaf) than in grazed ones (1.20 ± 0.04, N = 215, $F_{1,98} = 18.3$, $p < 0.001$, Fig. 4B). Moreover, more types of herbivory occurred in fertilised (1.62 types per leaf ± 0.03) than in unfertilised grassland (1.18 ± 0.05, N = 215, $F_{1,98} = 42.4$, $p < 0.001$, Fig. 4C). The differences in the numbers of types of herbivory per leaf, which were highest on legumes, intermediate on non-legume forbs, and lowest on graminoids, were more pronounced in traditionally mown grassland parcels than in traditionally grazed ones (functional group by traditional land use interaction, N = 638, $F_{2,199} = 8.8$, $p < 0.001$, Fig. 4B).

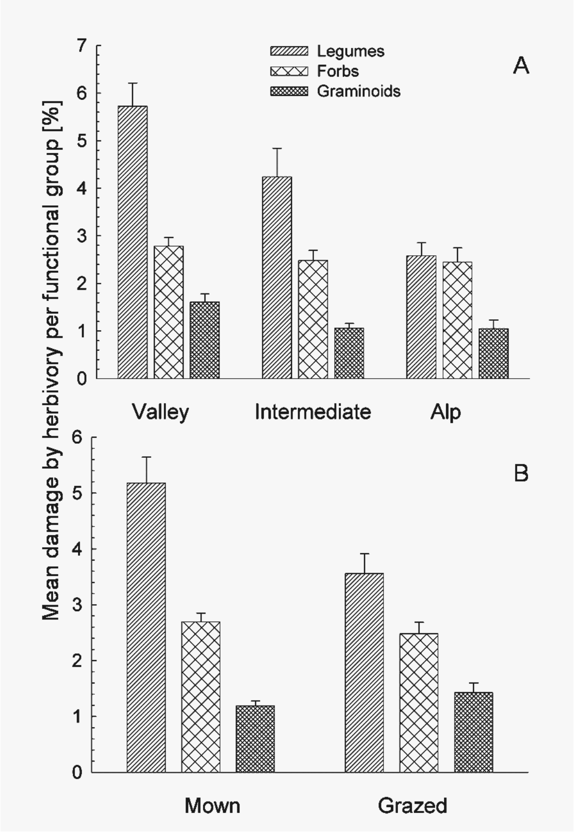

Fig. 3 The relationship between mean leaf damage by herbivory per functional group (legumes, non-legume forbs, graminoids) in 215 grassland parcels in the Swiss Alps and A) the altitudinal belt where the parcel is situated, and B) traditional land use. Error bars denote 1 standard error.

Diversity of types of fungal pathogen infection

Per leaf, we recorded 0.26 ± 0.004 of the five types of fungal pathogen infection, and per parcel, we recorded 2.47 ± 0.07. The number of types of fungal pathogen infection per leaf was higher in parcels with higher average leaf damage by fungal pathogen infection ($N = 215$, $R = 0.675$, $p < 0.001$, Fig. 1D). Fungal pathogen diversity was highest for graminoids (0.30 ± 0.01 types per leaf), followed by forbs (0.25 ± 0.01) and legumes (0.22 ± 0.01, Fig. 2B).

While the diversity of fungal pathogen types did not differ among altitudes, it was affected by land use. On graminoids in unfertilised sites, we found more types of fungal pathogen infection (0.34 ± 0.02 per leaf) than in fertilised ones (0.23 ± 0.03; functional group by fertilisation interaction, $N = 638$, $F_{2,199} = 13.1$, $p < 0.001$). Moreover, we recorded more types of fungal pathogen infection on graminoids in abandoned sites (0.45 ± 0.05) than in managed ones (0.27 ± 0.01; functional group by abandonment interaction, $N = 638$, $F_{2,199} = 11.8$, $p = 0.078$).

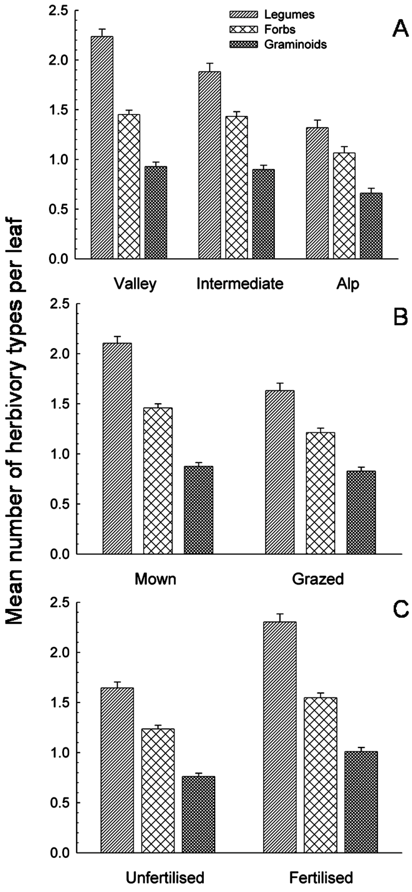

Figure 4 The relationship between the diversity of herbivory per leaf and functional group (legumes, non-legume forbs, graminoids) in 215 grassland parcels in the Swiss Alps and A) the altitudinal belt where the parcel is situated, B) traditional land use, and C) fertilisation. Error bars denote 1 standard error.

Composition of types of damage by herbivory and fungal pathogen infection

Analysis of variance of the principal components of types of damage by herbivory demonstrated significant effects of altitude (PC1, N = 215, $F_{1,101} = 99.0$, $p < 0.001$) and traditional land use (PC1, N = 215, $F_{1,101} = 18.5$, $p < 0.001$) on the composition of these types. This reflects the higher number of eggs on leaves from valleys and intermediate altitudes than from high altitudes (N = 215, $F_{2,212} = 13.8$, $p < 0.001$), and the larger damage by clipping (N = 215, $F_{1,213} = 16.8$, $p < 0.001$), holing (N = 214, $F_{1,101}= 7.8$, $p < 0.01$), and windowing (N = 214, $F_{1,212} = 13.6$, $p < 0.001$) in traditionally mown than in grazed parcels. In contrast to herbivory, the composition of types of fungal pathogen infection was independent of altitude and land use.

Relationships of herbivory and fungal pathogen infection with standing crop and plant species diversity

Standing crop per parcel was positively correlated with the leaf area damaged by herbivory (N = 209, R = 0.184, p < 0.001, Fig. 5A), the number of herbivory types per leaf (N = 209, R = 0.174, p < 0.05, Fig. 5C), the leaf area damaged by fungal pathogen infection (N = 209, R = 0.277, p < 0.001, Fig. 5E), and the number of fungal pathogen infection types per leaf (N = 209, R = 0.433, p < 0.001, Fig. 5G). With increasing number of plant species per parcel the number of herbivory types per leaf decreased (N = 215, R = -0.288, p < 0.001, Fig. 5D).

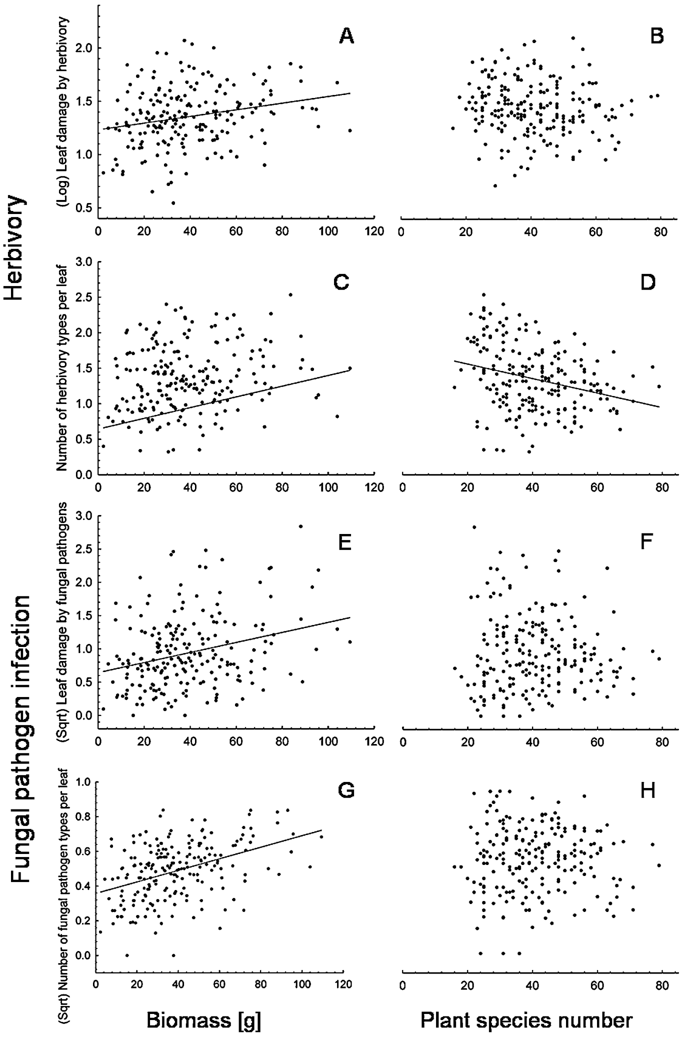

Fig. 5 Relationships between leaf damage by herbivory and A) standing crop, B) plant species number, between the diversity of herbivory types per leaf and C) standing crop, D) plant species number, between leaf damage by fungal pathogen infection and E) standing crop, F) plant species number, between the diversity of types of fungal pathogens per leaf and G) standing crop and H) plant species number in 215 grassland parcels in the Swiss Alps. Sqrt denotes square root transformation, log denotes logarithm to the base of ten.

Discussion

Leaf damage by herbivory and fungal pathogen infection

The infestation of 83% of all sampled leaves demonstrates the ubiquity of plant-herbivore and plant-pathogen interactions in mountain grassland. According to the damaged leaf area, herbivory plays a greater role than fungal pathogens do. However, the low extent of damage by herbivores and fungal pathogens indicates that biomass losses are not substantial. The 2.7% average leaf damage by herbivory is in line with other results showing that leaf damage in 10 herbaceous species in early successional habitats rarely exceeded 3% (Carson & Root, 1999). Such low amounts of leaf damage due to herbivory seem to be quite common (Landsberg & Ohmart, 1989). The observed percentage of leaf damage by fungal pathogens of 1.2% is much higher than the 0.05% damaged leaf area recorded in two tree species (Siemann 2003). However, for pathogens comparable data for grassland are lacking.

The highest leaf damage by herbivory on legumes is in line with the idea that legumes are an important source of nutrient supply for herbivores, and that nitrogen is a limiting nutrient for herbivores (Mattson, 1980). The dominant leaf damage of fungal pathogens on graminoids suggests that factors other than nitrogen affect the preference of fungal pathogens. Possibly, fungal pathogens are dominant on graminoids, because these are more abundant in grassland. Correspondingly, rust species, the most frequent fungal pathogens in our study, are common on pasture grass species (Braverman, 1986).

The positive correlation of leaf damage by herbivory and leaf damage by fungal pathogen infection suggests that herbivore damage favours fungal pathogen infection. Insect herbivores can serve as vectors for plant pathogens (Kluth et al., 2002). It was suggested that herbivores affect plants more as vectors of disease than as biomass consumers (Ohnesorge, 1976). However, in our study leaf damage by fungal pathogens was even lower than the one by herbivores. Alternatively, the positive correlation of leaf damage by herbivory and by fungal pathogen infection may reflect that leaf damage by herbivores can facilitate pathogen infection. Whereas the leaf damage by both groups was positively correlated, the diversities of types of herbivory and fungal pathogen infection were independent of each other. This indicates no conflict between the conservation goals of high diversity of herbivores and high diversity of fungal pathogens. Apparently, both groups contribute independently to biodiversity of mountain grassland.

At higher altitudes herbivory damage decreased, while damage by fungal pathogens was not affected by altitude. Lower temperature and shorter season seem to restrict herbivores more than they restrict fungal pathogens. More pronounced herbivory at lower altitudes was also found for grasshoppers (Wettstein & Schmid, 1999) and molluscs (Baur & Raboud, 1988; Scheidel & Bruelheide, 2001). Moreover, in our study especially legume leaves were more damaged by herbivory at lower altitudes, emphasising the importance of this functional group as food supply for herbivores.

The observed higher herbivory damage in traditionally used meadows indicates a preference of herbivores for mown sites, most likely due to frequent disturbances of pastures by trampling by cattle. Accordingly, disturbances in grazed sites were found to negatively influence the presence and establishment of herbivores (Kerley et al., 1993), and intensive grazing was reported to disturb plant-insect interactions (Kruess & Tscharntke, 2002). Thus, in cases where formerly mown grasslands now have to be grazed to reduce labour, the number of cattle and the duration of grazing need to be limited. Microclimatic conditions could also play a role for herbivores, but they were probably not much different between pastures and meadows as this would most likely have affected the level of fungal pathogen infections, which did not differ between pastures and meadows. Finally, as far as damage was caused by flower-visiting insects or their larvae, in the Alps herbivores may prefer mown over grazed grassland, because here mown grassland parcels are usually more flower-rich than grazed ones are (Fischer & Wipf, 2002).

Diversity and composition of types of herbivory and fungal pathogen infection

More types of herbivory on legume leaves, more fungal pathogen types on grass leaves, and more types of herbivory at lower altitudes correspond with the results on leaf damage, and with the positive correlation of the number of types of both herbivory and fungal pathogen infection with leaf damage. Possibly, this indicates a simple mass effect, where a higher number of single plant-parasite interactions is associated both with more severe leaf damage and with a higher likelihood, that a higher number of different herbivore and fungal pathogen groups is involved. Alternatively, it could indicate a direct relationship between higher diversity of interactions and more severe leaf damage. In any case, the damage by single types of herbivory or fungal pathogen infection remained small. Therefore, we conclude that in mountain grassland herbivores and fungal pathogens do not develop the disease-like character described for crop monocultures (Oerke, 1994).

The higher number of types of herbivory at low altitudes is in line with the higher amount of leaf damage there. Most likely it is due to climatic conditions, which may also explain the altitudinal effect on the composition of herbivory types. The above-average number of eggs on leaves from low altitudes reflects the higher reproduction rates of insects under more advantageous climatic conditions (Virtanen & Neuvonen, 1999).

Herbivory was more diverse in mown than in grazed parcels, and the categories with the highest overall frequencies, clipping, holing, and windowing, were also more frequent on mown sites. We conclude that herbivores with these feeding habits prefer mown over grazed grassland. However, the preference of herbivores to specific land use regimes may differ between taxonomic groups. E.g., a more diverse grasshopper community was reported from grazed wetland sites in the pre-Alps, whereas in mown sites the diversity of butterflies was higher (Wettstein & Schmid, 1999). Jeanneret et al. (2003) suggested that there are no general rules relating species diversity to habitat and landscape features, because such relationships strongly depend on the specific organism and study region. Nevertheless, in the Alps mown grassland may be likely to be preferred by herbivores, because meadows are often richer in attractive flowers than pastures (Fischer & Wipf, 2002) and many insect herbivores are flower-visitors.

Increased herbivore diversity may be related to increased productivity of plants via an increase of overall herbivore abundance (Siemann, 1998). Moreover, leaves are generally of higher quality and palatability after nitrogen fertilisation (Davidson & Potter, 1995). Accordingly, we found more different types of herbivory per leaf in fertilised than in unfertilised parcels. In contrast, per parcel the number of types of herbivory did not differ between fertilised and unfertilised parcels. Most likely in fertilised parcels the higher diversity of herbivory per leaf was counterbalanced by a lower diversity of herbivory between species, because fertilised parcels are less rich in plant species. Accordingly, in experimental grassland the diversity of herbivores increased with the number of weed species (Dyer & Stireman, 2003).

The higher diversity of types of fungal pathogen infection on graminoids in unfertilised grassland, both per leaf and per parcel, most likely is due to an indirect effect of plant species abundance, as in unfertilised sites overall plant species richness is high whereas grass abundance is low. As species specificity restricts the majority of fungal pathogens to one or few closely related host-species (Brandenburger, 1985; Burdon, 1994), we suggest that this specificity might play a greater role for the diversity of fungal pathogen infection than fertilisation does.

Relationship between herbivory, fungal pathogen infection, and standing crop

The positive correlation between standing crop per parcel and leaf damage by, and diversity of types of, herbivory is in line with the preference of herbivores for higher-quality leaves. In unproductive alpine grassland dominated by a single sedge two specialized and very abundant grasshopper species caused heavy biomass losses (Blumer & Diemer, 1996). If, however, plant and herbivore communities are highly diverse, effects of herbivory are found to be dispersed across plant structures and species (Hunter & Price, 1992; Tilman, 1982) and little biomass is consumed. In our grassland parcels, biomass losses remained low even in the fertilised and more productive sites. Possibly, this is due to the still relatively high plant species diversity of these grasslands in the Alps, where the specificity of many herbivores does not allow for large biomass losses. Alternatively, it might reflect positive effects of herbivory on plant growth, as reported for herbivory by insects (McNaughton, 1983) and mammals (Paige, 1992).

Standing crop was positively correlated with the amount of leaf damage by fungal pathogen infection and the diversity of different types per leaf. Possibly, infection is more pronounced due to the higher quality of leaves in more productive sites. Moreover, higher standing crop could promote fungal pathogens because it is associated with higher levels of moisture (Kochy & Wilson, 2004). Furthermore, as higher standing crop was associated with lower plant species diversity and therefore higher abundance per plant species, higher pathogen damage per leaf could also have been due to higher host plant abundance.

At the parcel level, we found no significant relationships between the diversity of different types of herbivory and biomass, and between the diversity of fungal pathogen infection and biomass. This indicates no conflicts between the agricultural goal of high biomass production and the conservation goals of high diversity of fungal pathogens and herbivores.

Relationship between herbivory, fungal pathogen infection, and plant species diversity

At first glance, more types of herbivory per leaf in plant species poor parcels appear to suggest a conservation conflict between plant species diversity and herbivore diversity. However, this relationship disappeared when the diversity of types was considered at the parcel level. Pathogen diversity was also independent of plant species diversity. Therefore, we suggest promoting low-intensity land use where high plant, herbivore, and pathogen diversity contribute to high overall community biodiversity and where biomass losses by herbivores and pathogens are low.

Conclusions

Our comprehensive study across 12 villages from several regions in the Swiss Alps demonstrates the omnipresence of herbivores and fungal pathogens in mountain grassland and indicates that plant-herbivore and plant-pathogen interactions contribute largely to community diversity. At the same time, leaf damage by herbivores and fungal pathogens was very moderate, even in the case of legumes and at lower altitudes where leaf damage was highest. Therefore, conserving the diversity of plant-herbivore and plant-pathogen interactions will not be at the expense of dramatic plant biomass losses.

Our indirect approach of measuring biological interactions allowed us to distinguish between eight categories of leaf damage by herbivores and four frequent taxonomical categories of fungal pathogen infection. This approach enabled us to assess herbivore and fungal pathogen diversity for many field sites at large distances within a short period of time. This worked especially well for plant-herbivore interactions. Because some damage by fungal pathogens could not be classified, the method can be improved for the indirect assessment of plant-pathogen interactions.

Currently, much grassland in the Alps is undergoing land use changes. An increasing proportion of grassland is fertilised and formerly mown grasslands are grazed or abandoned. We found low altitudes and traditional mowing to promote plant-herbivore interactions, while fertilisation negatively affected fungal pathogens. This implies that the current land use changes in the Alps from mowing to grazing or to abandonment decrease the diversity of plant-herbivore interactions, and fertilisation decreases pathogen diversity. As our results did not reveal conservation conflicts between plants, herbivores, and fungal pathogens, we suggest conserving and maintaining a great diversity of plant-herbivore and plant-pathogen interactions by maintaining diverse low-intensity land use.

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Chapter 5

Isolation and characterization of microsatellite DNA markers in the grass *Poa alpina* L.

Katrin Maurer, Barbara Gautschi, Anne Weyand, Jürg Stöcklin, and Markus Fischer

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Abstract

The important fodder grass *Poa alpina* L. occurs at several ploidy levels with common aneuploidy. We isolated and characterized five polymorphic microsatellite markers for the study of molecular genetic variation of this species. As first examples of the value of the developed markers for population genetic analyses we show that plants with more chromosomes have more microsatellite bands and that isolation by distance plays a small role in shaping microsatellite diversity of *P. alpina* in the Swiss Alps**.**

Keywords: Microsatellite, *Poa alpina*, population genetics

The Alpine meadow-grass, *Poa alpina* L., is a common species of subalpine and alpine grasslands. The species constitutes an aggregate of phenotypically diverse polyploid ecotypes with common aneuploidy (Müntzing 1980). Chromosome numbers range from 2n = 22 to 46 in Switzerland (Duckert-Henriod & Favarger 1987) to more than 60 in Scotland (Müntzing 1980). Some plants of *P. alpina* reproduce via seeds, while others reproduce vegetatively forming bulbils in the panicles instead of seeds. It is not completely clear to which degree the mode of reproduction is genetically determined or phenotypically plastic.

We extracted DNA from leaf samples of eight plants originating from a natural population in a pasture at 1950 m asl in Pany, Switzerland. Total genomic DNA was extracted using a phenol-chloroform extraction procedure (Rogers & Bendich 1994) modified by Steinger *et al.* (1996), except that we incubated the samples mixed with cetyltrimethyl ammonium bromide (CTAB) buffer and mercaptoethanol at 65°C.

In the lab of ecogenics GmbH (Zurich, Switzerland), we produced an enriched DNA library from size-selected genomic DNA ligated into TSPAD-linker (Tenzer *et al.* 1999) and enriched by magnetic beads selection with biotin-labelled (CA)13 and (GA)13 oligonucleotide repeats (Gautschi *et al.* 2000a; Gautschi *et al.* 2000b). Out of the 384 screened recombinant colonies, 98 gave a positive signal after hybridization. We sequenced plasmids from 80 positive clones as described in Gautschi *et al.* (2000a) and designed primers for 15 microsatellite inserts.

We amplified DNA on a PTC-100 Programmable Thermo Controller (MJ Research Inc.) with 10 µl reaction volumes containing 10 ng genomic DNA, 0.5 µl each of the forward and reverse primers, 5 µl Hotstar Taq Mastermix (Qiagen, Hombrechtikon, Switzerland), and 3 µl of sterilized H_2O. We performed multiplex-PCR with fluorescent labelled primers Poa CA1D4 (label: 6-FAM) and Poa CA1F4 (HEX). We labelled the forward primers at all loci. After a preliminary denaturation step at 95 °C for 15 min., we performed polymerase chain reaction (PCR) amplification as follows: 30 cycles at 95 °C for 30 s, 30 s at locus-specific annealing temperature (Table 1), 30 s at 72 °C, and a final 8 minute extension step at 72 °C. We mixed 1 µl of the PCR product with 10 µl of a 75:1 solution of formamide and GeneScan-500(ROX) size standard (Applied Biosystems, Foster City, USA). We determined fragment lengths on an ABI PRISM 310 Genetic Analyzer using GeneScan 2.1 and Genotyper 2.1 (Applied Biosystems, Foster City, USA). We analyzed all clearly detectable bands after careful verification. Of the 15 microsatellite inserts, five turned out polymorphic among the eight plants (Table 1).

We used these five microsatellite markers to study the genetic diversity of 415 plants of *P. alpina* originating from 54 agriculturally used parcels of land, representing a 170 km gradient across the Swiss Alps. To test whether the number of bands per plant was correlated with the number of chromosomes, we counted chromosomes in the root tips of 25 plants. Plants with more chromosomes had more bands and this relationship was stronger for smaller chromosome numbers (N=25, Linear regression model, linear term t=6.02, p<0.0001, quadratic term t=-6.09, p<0.0001, R^2=0.66). The number of chromosomes per plant was unknown for the other plants, and plants could show more than two bands per locus. Of course, these data do not conform to standard statistics for codominant microsatellite markers of diploid organisms, such as observed and expected heterozygosity and tests for deviation from Hardy-Weinberg equilibrium. Therefore, we prepared a presence-absence matrix for each band across all plants. As measures of genetic diversity per parcel of land we used mean number of bands per plant and total number of bands per parcel. Moreover, as measure of pairwise genetic distance between parcels of land, we calculated pairwise Euclidian distances based on the relative abundances of each band. Genetic distances between pairs of parcels of land were weakly positively correlated with pairwise geographical distances (r_M=0.12, p=0.057), suggesting that isolation by distance plays a small role in shaping microsatellite diversity of *P. alpina* in the Swiss Alps.

Our results show that a presence-absence matrix of bands allows deriving valuable population genetic information with our markers. Moreover, if ploidy levels are known the markers will also allow determining heterozygosity and F-statistics.

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Table 1 Genetic characteristics of 5 *Poa alpina* L. microsatellite loci based on 8 individuals genotyped from one population in the Swiss Alps. The characteristics of repeat motifs are based on the sequenced clones. T_a = locus-specific annealing temperature.

Locus	Primer Sequences (5'-3')	Repeat motif	T_a [°C]	Size range [bp]	No. of bands	Mean number of bands per plant ± SE	Accession no.
Poa CA1D4	F: AACTTTGGTGCAAGCAGCTC*	$(GT)_{14}AT(GT)_2$	60	239-301	12	3.13 ± 0.44	AY942201
	R: ACAGGGAGCTACACAAAGCAG						
Poa GAC1	F: GAAGAACAAGAGGAACAAGATAGC*	$(GA)_6\ (CA)_5(GA)_3GGGA\ (GA)_3AA(GA)_5G\ (GA)_{31}$	60	92-182	20	5.00 ± 0.33	AY942202
	R: AAAAGAGGGCTACGCGATTC						
Poa GA1C3	F: AAGGAGGAACAACTCGAAAGC*	$(GA)_{37}$	64	195-293	12	2.00 ± 0.19	AY942203
	R: AGTGTTTCCCTCCTCGATAGTG						
Poa CA1F4	F: ACTGGTTTGTCATCGCACTG*	$(GA)_{16}$	60	199-232	12	4.13 ± 0.23	AY942204
	R: TTCCTCATCTAGCGGAGACC						
Poa CAB12	F: TCATCTTGGCCATCATCTTG*	$(CA)_{18}$	62	125-172	10	2.63 ± 0.32	AY942205
	R: TTCTGTACGTTGACCTGACTGG						

* Fluorescent labelled primer

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Chapter 6

Microsatellite diversity of the agriculturally important *Poa alpina* L. in the Swiss Alps

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submitted

Abstract

Genetic diversity within a species is important for its ecological amplitude and is a prerequisite for future evolutionary change. We studied effects of natural factors and agricultural land use on genetic diversity in the Alpine Meadow Grass *Poa alpina,* which is common in subalpine and alpine natural sites and agriculturally used land, where it is an important fodder grass. The species comprises seed-producing and bulbil-producing plants. We assessed genetic diversity with five microsatellite markers for 569 *P. alpina* plants originating from 20 natural sites and 54 grassland parcels of different cultural tradition, land use, and altitude in the Swiss Alps. Due to poly- and frequent aneuploidy of *P. alpina,* data analyses were based on a presence/absence matrix of microsatellite bands. We found a low but significant differentiation in the presence/absence of microsatellite bands between natural sites and agriculturally used parcels, while their microsatellite band diversity within populations did not differ. We found an increased differentiation in microsatellite bands with increasing geographic distance among parcels, a differentiation among grazed and mown parcels, and among sexually and vegetatively reproducing populations. Band richness of sampled plants per village was higher where sampled plants represented parcels of more different land use types. Within populations microsatellite band diversity was higher in grazed than in mown parcels. We conclude that the diversity of human land use in the Alps has increased the genetic diversity in *Poa alpina.* Therefore, the ongoing socio-economically motivated land use changes, which reduce the number of different land use types, will affect genetic diversity of *P. alpina* negatively.

Introduction

A high genetic diversity is necessary for plant populations to adapt to a continuously changing environment (Frankham *et al.* 2002). Genetic diversity comprises genetic diversity within individuals, genetic differences among individuals within a population, and genetic differences among populations (Meffe & Carroll 1997), and it is shaped by the balance between genetic drift, inbreeding, recombination, gene flow, mutation, and selection (Loveless & Hamrick 1984; Hartl & Clark 1997). This balance depends on important life history traits, such as the mode of reproduction or life form (Loveless & Hamrick 1984; Hamrick & Godt 1989; Hamrick & Godt 1997).

Selection can be exerted by both natural and anthropogenic factors. Such potential natural determinants of genetic diversity include abiotic parameters, such as altitude or soil conditions. Moreover, genetic diversity may be affected by the diversity of the surrounding community. Higher plant species richness was suggested to increase genetic diversity if it increases the diversity of available niches (Odat *et al.* 2004; Vellend & Geber 2005). Potential anthropogenic determinants of genetic diversity include land use diversity if different land management creates genetic differentiation among populations. For instance, frequent fertilization and defoliation led to a reduction of genetic diversity in natural populations of *Festuca pratensis* (Kölliker *et al.* 1998). We studied genetic diversity and its determinants for a common and important fodder plant which occurs over a large altitudinal range at natural sites and in agriculturally used grassland, the Alpine Meadow grass *Poa alpina* L., in the Swiss Alps.

The species can reproduce via seeds and vegetatively by producing bulbils. In an accompanying common garden study the proportion of genotypes reproducing vegetatively via bulbils was higher among samples from higher altitudes (Weyand 2005), in line with the hypothesis of an adaptive advantage of vegetative reproduction in the harsher conditions at higher altitudes (Bauert 1993; Pluess & Stöcklin 2005; Weppler & Stöcklin 2005). This differentiation in the reproductive mode may affect both within-population diversity and population differentiation. Furthermore, as *P. alpina* occurs across a wide geographical range, isolation by distance is likely to have shaped the distribution of genetic diversity among populations of different regions (Wright 1943). Genetic diversity within and differentiation among populations of *P. alpina* is probably also enhanced due to the highly variable polyploidy and frequent aneuploidy within the species (Duckert-Henriod & Favarger 1987), which presumably restricts gene flow among individuals and populations and is likely to increase the ecological amplitude of the species (Briggs & Walters 1997; Brochmann *et al.* 2004; Soltis *et al.* 2004).

For about 5000 years, the Alpine landscapes and in particular their grasslands have been shaped by human land use (Bätzing 2003). In the European Alps, *P. alpina* is one of the most important fodder grasses for cattle (Conert 1998). Therefore, *P. alpina* has been under agricultural selection pressure for hundreds of years. Plants of *P. alpina* showed adaptation to anthropogenic land use variation in a common garden experiment (Weyand 2005). There, plants from pastures allocated more biomass to reproduction than plants from natural sites did, while plants from meadows allocated less biomass to reproduction than plants from natural sites did, suggesting divergent selection between parcels of different land use. Higher allocation to reproduction in pastures may suggest that genetic diversity is higher in plants from pastures. Higher genetic diversity in pastures could also be maintained by the spatially more heterogeneous conditions created by grazing animals. In the Alps the relationship between land use and genetic diversity within a species is of particular interest, as due to land use changes during the last decades many meadows have been converted to pastures (Bätzing 2003) and the diversity of land use types in the landscape has decreased.

In the Swiss Alps, the cultural traditions Romanic, Germanic, and Walser contributed to a high landscape diversity through their different agricultural practices (Bätzing 2003). If differences in land use lead to genetic divergence between plants, villages with higher land use diversity may harbour higher genetic diversity of *P. alpina* than villages with lower land use diversity.

We studied the effects of natural factors and agricultural land use on genetic diversity of *P. alpina* within and among 12 villages in the Swiss Alps. Each of the three cultural traditions Romanic, Germanic, and Walser was represented by four villages. At the parcel level we studied genetic diversity within and among populations from 20 natural sites and from 54 agriculturally used grasslands at different altitudes in these 12 villages. The agriculturally used parcels were either mown or grazed and they were either additionally fertilized or unfertilized. Plant species diversity was known for all parcels from a previous study (Maurer *et al.* 2006).

As molecular markers we used five polymorphic microsatellite loci (Maurer *et al.* 2005). Microsatellites are generally assumed to be selectively neutral (Scribner & Pearce 2000) and they offer high resolution (Schlötterer 1998). Therefore, they are ideal to investigate gene flow and genetic drift. Natural selection is highly unlikely to act on the investigated microsatellite loci themselves, and could affect their diversity only if they were linked to loci under selection (Hartl & Clark 1997; Till-Bottraud & Gaudeul 2002).

We asked the following questions: (1) Are *Poa alpina* populations from agriculturally used grassland genetically differentiated from natural populations? (2) Is genetic differentiation among villages and among populations related to geographical distances, to differences in land use, and to differences in reproductive modes? (3) Is genetic diversity within villages related to cultural traditions and to land use diversity? (4) Is genetic diversity within grassland parcels related to altitude, land use, and reproductive mode?

Methods

Study species

Poa alpina L. (Poaceae) is a common grass at subalpine and alpine levels in the northern hemisphere. Its presence indicates high levels of nutrients and soil moisture (Conert 1998). Accordingly, it occurs in pastures and nutrient rich meadows, but also as a pioneer species in scree slopes and in snowbeds. In the European Alps, *P. alpina* is among the most important fodder grasses due to its high contents of fats and proteins (Bachmann 1980; Conert 1998). Similar to other species in the genus *Poa* and the Poideae (Brysting *et al.* 2004), *Poa alpina* constitutes a polyploid complex. Because of frequent aneuploidy and the presence of multiple B-Chromosomes, chromosome numbers of ploidy levels are highly variable (Müntzing 1980; Steiner & Heidenreich 1997). In Switzerland reported chromosome numbers range from 2n = 22 to 46 (Duckert-Henriod & Favarger 1987), and more than 60 chromosomes were found in Scotch plants (Müntzing 1980). Chromosome numbers counted in the root tips of 25 plants of this study varied between 22 and 61 per plant. Presumably, variable polyploidy adds to genetic diversity in *Poa alpina*, as heterozygosity increases strongly with ploidy level (Brochmann *et al.* 2004).

Some plants of *P. alpina* produce seeds, while others reproduce vegetatively by forming bulbils in the panicles instead of seeds (Müntzing 1980). Such bulbils grow into little plantlets on the maternal plants, which therefore are called pseudoviviparous. Eventually, the plantlets may dehisce from the maternal plant and root (Pierce 1998). Usually, pseudoviviparous plants also develop a sexual floret at the basis of the plantlets

(Philipson 1934; Müntzing 1980; Pierce *et al.* 2003). It is not known whether these sexual florets produce fertile pollen and viable seeds and whether there is gene flow between such florets and sexually reproducing plants. The mode of reproduction appears largely genetically determined, while phenotypic plasticity in the mode of reproduction plays a minor role (Schwarzenbach 1953; Schwarzenbach 1956; Heide 1989).

Study area

The study area comprises 12 villages in the Swiss Alps, four of each of the three cultural traditions Romanic, Germanic, and Walser (Fig. 1). The villages are situated along a 170 km east-west gradient. Each village belongs to a separate alpine valley. To represent typical agricultural villages, the study villages were randomly selected with the restriction that their agricultural character had only changed modestly during the last 50 years, and that they were not very touristy and did not have more than about 1500 inhabitants.

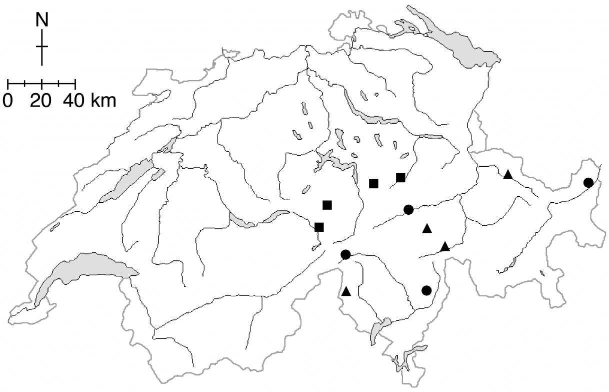

Fig. 1. Map of Switzerland with the 12 study villages and their cultural traditions.
● = Romanic
■ = Germanic
▲ = Walser

Study design and sampling

We searched for *P. alpina* in parcels of land chosen for a vegetational survey of grasslands (Maurer *et al.* 2006). In each village these grassland parcels were situated at three altitudinal levels, at the valley bottom (c. 1000 m asl), at intermediate altitudes (c. 1500 m asl), and at the alp level (c. 2000 m asl). We searched for *P. alpina* L. in those of 216 grassland parcels where, according to local farmers, the type of land use had never changed. These parcels were characterised by a combination of land use (mown or grazed) and fertilisation (fertilised or unfertilised). In each parcel where *P. alpina* occurred, we sampled eight plants at interdistances of five meters to minimize the probability of sampling the same genotype more than once. Altogether, we sampled plants from 54 agriculturally used grassland parcels, 13 meadows and 41 pastures, of which 19 were additionally fertilised and 35 were not. Because *P. alpina* did not occur in all parcels and not all land use types were applied in each village, we could not find plants of all combinations of land use and fertilisation per village. However, there was no geographical pattern in the presence and absence of certain land use types that could have confounded differences in land use with differences due to geographic distance. In the same way as in the agricultural parcels we sampled eight plants from each of 20 natural sites above tree line that had never been used agriculturally. The sampled plants were also used for a common garden experiment (Weyand 2005). We obtained single genotypes by separating collected plants into four single tillers which we planted in the corners of 7 cm x 7 cm pots. After two months growth in a greenhouse we discarded three of the four plants and kept one randomly selected plant per genotype. After another two months we collected leaf samples of each plant. Some plants turned out to be other species than *P. alpina* and some of the plants died. Thus, finally, we analysed 415 plants of *P. alpina* from 54 agriculturally used parcels and 154 plants from 20 natural sites, usually eight plants per parcel, and a few times only seven or six.

Microsatellite analysis

We dried collected leaf samples immediately with silica gel. Then, we ground about 30 mg of the material in Eppendorf tubes with a glass bead in a shaking mill. We extracted DNA according to a Rogers & Bendich (1994) protocol modified by Steinger (1996), except that we incubated samples with CTAB buffer and mercaptoethanol at 65° C.

We screened all plants for variation at five polymorphic microsatellite loci (Maurer *et al.* 2005). We amplified DNA with 10 µl reaction volumes containing 10 ng genomic DNA, 0.5 µl each of the fluorescence-labelled forward primer and of the reverse primer, 5 µl Hotstar *Taq* Mastermix (Qiagen, Hombrechtikon, Switzerland), and 3 µl of sterilized H_2O. After a preliminary denaturation step at 95° C for 15 min., we amplified DNA with polymerase chain reaction (PCR) on a PTC-100 Programmable Thermo Controller (MJ Research Inc.) for 30 cycles of 30 s denaturing at 95° C, 30 s of annealing at locus-specific temperatures (Maurer *et al.* 2005) and 30 s of extension at 72° C, with a final 8 minute extension step at 72° C. We mixed 1 µl of the PCR product with 10 µl of a 75:1 solution of formamide and GeneScan-500 (ROX) size standard

(Applied Biosystems, Foster City, CA, USA). We determined fragment lengths by capillary gel electrophoresis with an ABI PRISM 310 Genetic Analyser using GeneScan 2.1. (Applied Biosystems, Foster City, CA, USA). Plants were combined in random order for PCR and the sequencer-runs. Microsatellite bands were binned using Genotyper 2.1 (Applied Biosystems, Foster City, CA, USA) by correcting peaks manually after automated scoring, and we controlled the assignment of each peak to the corresponding band. In each PCR-run at least one blank sample was added to control for a possible contamination of the samples. Preliminary tests of repeated PCR with a sample of eight plants showed a very high accuracy of the produced band-pattern. Thus, band-scoring appeared to be the most possible source of a potential genotyping error. Therefore, we independently scored a sample of 50 plants (8.8% of all analyzed individuals) twice for each of the five primers and obtained a genotyping error of 1.48%.

Due to their polyploidy, plants could show more than two microsatellite bands per locus. In a subset of 25 analyzed plants the number of bands was positively correlated with the number of chromosomes, but variable chromosome number due to aneuploidy and frequent B-chromosomes do not allow to assess ploidy level (Maurer *et al.* 2005). Therefore, our data does not conform to standard statistics for codominant microsatellite markers of diploid organisms, such as observed and expected heterozygosities, and tests for deviation from Hardy-Weinberg equilibrium. Consequently, all analyses in this study are based on a presence-absence matrix for each band across all plants.

Analysis of differences between natural sites and agriculturally used grassland parcels

To test for differentiation between natural and agricultural sites, we partitioned molecular variation between natural sites and agriculturally used parcels, among parcels, and within parcels with analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) based on the pairwise Euclidean distance matrix of the presence and absence of microsatellite bands in individuals of all 74 parcels. Then, to test whether regional differentiation was similar for natural and agricultural sites, we partitioned molecular variation among villages, among parcels, and within parcels separately for the plants of the 54 agriculturally used parcels and for those of the 20 natural sites.

Analysis of differences among villages and among agriculturally used grassland parcels

To identify potential determinants of genetic differentiation among villages, we used Mantel tests (Mantel 1967; Manly 1997). We tested the relationship of the matrix of Euclidean genetic distances among villages, based on the relative abundance of each band occurring in agriculturally used parcels per village, with the matrix of geographical distances. Moreover, we tested the relationship of the matrix of genetic distances and the matrix of cultural distances, where pairs of villages of the same cultural tradition were assigned the cultural distance 0, and pairs of villages of different traditions 1, with a Partial Mantel test (Legendre & Legendre 1998, n = 1000 permutations), where we controlled for geographical distances. To examine potential differentiation among parcels of grassland of different land use (mown vs. grazed) and fertilisation (fertilised vs. unfertilised), we partitioned molecular variation among these groups, among parcels within these groups, and within parcels with AMOVA including plants of the 54 agriculturally used parcels. Furthermore, we used AMOVA to examine potential differentiation between 10 parcels from which all sampled plants exclusively reproduced pseudoviviparously in the common garden (Weyand 2005), and 22 parcels from which all sampled plants exclusively produced seeds.

Additional to AMOVA, we measured among-parcel differentiation as differentiation for microsatellite band richness $\rho_{ST(n)}$ among all 54 agriculturally used populations and also among meadows and pastures separately following El Mousadik & Petit (1996) and Petit *et al.* (1998). To obtain $\rho_{ST(n)}$, first we calculated the expected band richness $r'_{T(n)}$ of a random sample of n=6 plants out of all 415 plants and $r'_{S(n)}$ of a random sample of n=6 plants for each parcel. Then, we calculated the differentiation for band richness as $\rho_{ST(n)} = 1 - r'_{S(n)} / r'_{T(n)}$ (where S represents the single parcels and T the total population of all sampled plants) for each locus separately and the mean across the five loci.

To identify potential determinants of genetic differentiation among agriculturally used parcels we used Mantel tests (Mantel 1967; Manly 1997). We tested the relationship of the matrix of Euclidean genetic distances between parcels, based on the relative abundance of each allele per parcel, with the matrix of geographical distances among parcels. Furthermore, we obtained the matrix of altitudinal differences among parcels, the matrix of land use distances, where pairs of parcels of the same land use (mowing or grazing) were assigned the land use distance 0, and pairs of parcels of different land use 1, and similarly, the matrix of fertilisation distances (fertilised vs. unfertilised). Controlling for effects of geographic distance because of geographical clustering of villages we did Partial Mantel tests (Legendre & Legendre 1998, n = 1000 permutations) of the relationships between the matrix of genetic distances and these other matrices using geographical distance as covariate. Similarly, we tested the relationship between genetic distance among parcels and distances in reproductive mode with a partial Mantel test using geographical distance as covariate considering the 32 grassland parcels with samples with a uniform reproductive mode as described above. In the matrix of distances in reproductive mode, pairs of parcels of the same reproductive mode were assigned the value 0 and pairs with different modes the value 1.

Analysis of genetic diversity within villages and within parcels

We measured genetic diversity within villages and within parcels as band richness $r'_{(n)}$ for each locus following El Mousadik & Petit (1996), except that we used plants as sample units instead of genes. We used the rarefaction procedure of Hurlbert (1971) to estimate band richness for a standardized sample size of *n* plants. As rarefaction sample size we used the smallest one available, *n* = 16 for villages and *n* = 6 for parcels. For each locus, according to the formula $\hat{r}_{(n)} = \sum_i \left[1 - \binom{N - N_i}{n} \Big/ \binom{N}{n} \right]$ (where N_i represents the number of occurrences of the i^{th} band among the N sampled plants of a population and n the standardized sample size), we calculated the expected number of different bands $r_{(n)}$ in a sample of n plants. We obtained the corrected band richness $r'_{(n)}$ by subtracting one from the band richness $r_{(n)}$, because a village or a parcel with only one single band is considered to be monomorphic. Then, we calculated mean band richness over all five loci, for simplicity further on called band richness.

We tested potential effects of the village characteristics latitude and longitude, ratio of the numbers of meadows and pastures in a village, altitude, number of land use combinations (combination of land use and fertilisation) investigated in a village, and culture on band richness per village with linear models and analysis of covariance (ANCOVA). For the significant variables number of land use combinations and culture we calculated ANCOVAs with sequential sums of squares including both variables. For these analyses only plants from agriculturally used parcels in each village were included. Furthermore, we tested whether band richness per village was correlated with the accumulated number of plant species occurring in the corresponding parcels of each village with a Pearson's product moment correlation.

To analyse within-parcel genetic diversity, we investigated effects of cultural tradition, altitude, land use, and fertilisation on band richness per parcel and on the mean number of bands per plant for each parcel with hierarchical analysis of covariance (ANCOVA) with sequential sums of squares. We tested effects of culture against remaining variation among villages and of all other factors against variation due to remaining differences among parcels. To account for differences among parcels because of different soil conditions or solar radiation, we used pH values and aspect of each parcel as covariates. However, as these covariates did not qualitatively change the results, we present results without covariates. We tested the distribution of the residuals using diagnostic plots. Furthermore, we tested whether there was a difference in within-parcel diversity between populations from agriculturally used parcels and populations from natural sites. As there was no difference we present results including only the agriculturally used parcels.

Furthermore, we tested whether microsatellite diversity was affected by the abundance of *P. alpina* in the parcels. We calculated Spearman's rank correlations with the mean abundance of *P. alpina* of two plots (5 m x 5 m) per parcel and the measures of microsatellite diversity mean number of bands, band richness, and mean Euclidean genetic distance of each parcel to all other parcels.

To test whether genetic diversity of *P. alpina* was correlated with plant species diversity, we calculated Spearman's rank correlations between mean plant species richness of two plots (5 m x 5 m) per parcel and mean number of bands per plant and parcel, and between total plant species richness of two plots per parcel and band richness per parcel.

We did all statistical analyses with the software R (R Development Core Team 2004). For Mantel tests and Partial Mantel tests we used the R-package vegan (Oksanen 2005) and for AMOVAs the R-package ade4 (Thioulouse *et al.* 2004).

Results

Overall microsatellite diversity and differentiation between natural and agriculturally used grassland parcels

Among the 569 plants of *Poa alpina* altogether we detected 209 bands at the five microsatellite loci, between 25 and 61 per locus. We detected between one and eight bands per plant and locus, with a mean of 3.35. In total we detected 531 multilocus-microsatellite phenotypes among all 569 plants and 386 multi-locus microsatellite phenotypes among the 415 plants from agriculturally used parcels.

We found a genetic differentiation between natural and agriculturally used grassland parcels as low, but highly significant 1.1 % of the variation in the presence and absence of microsatellite bands resided between natural and agriculturally used grassland parcels (AMOVA, $P < 0.004$).

Table 1 Summary of analysis of molecular variance (AMOVA) of microsatellite phenotypes of plants of *Poa alpina* from 54 agriculturally used parcels and from 20 natural sites grouped in 12 villages. AMOVA was based on the matrix of pairwise Euclidean distance between individuals in the presence and absence of microsatellite bands. % = Percentage of total variation

	Variance component			
Source of variation	df	Absolute	%	*P*
54 populations from agriculturally used parcels				
Among villages	11	0.7981	6.76	< 0.001
Among parcels within villages	42	2.1790	18.39	< 0.001
Within parcels	361	8.8422	74.86	< 0.001
Total	414	11.8122	100.00	
20 populations from natural sites				
Among villages	10	1.0184	8.42	< 0.001
Among parcels within villages	9	2.2502	18.61	< 0.001
Within parcels	134	8.8203	72.96	< 0.001
Total	153	12.0889	100.00	

Genetic diversity among villages

Regional differentiation was slightly higher in natural sites, as 8.4 % of the variation in the presence and absence of bands in natural sites resided among villages, while the corresponding proportion was 6.8 % for agriculturally used sites (AMOVA, for both $P < 0.001$, Table 1). We found no statistically significant relationship of genetic distances with geographic distances (Mantel test with plants from agriculturally used parcels, $r_M = 0.22$, $P = 0.16$) or cultural differences (Partial Mantel test with plants from agriculturally used parcels and with geographic distance as covariate, $r_M = 0.02$, $P = 0.28$) among pairs of the 12 villages.

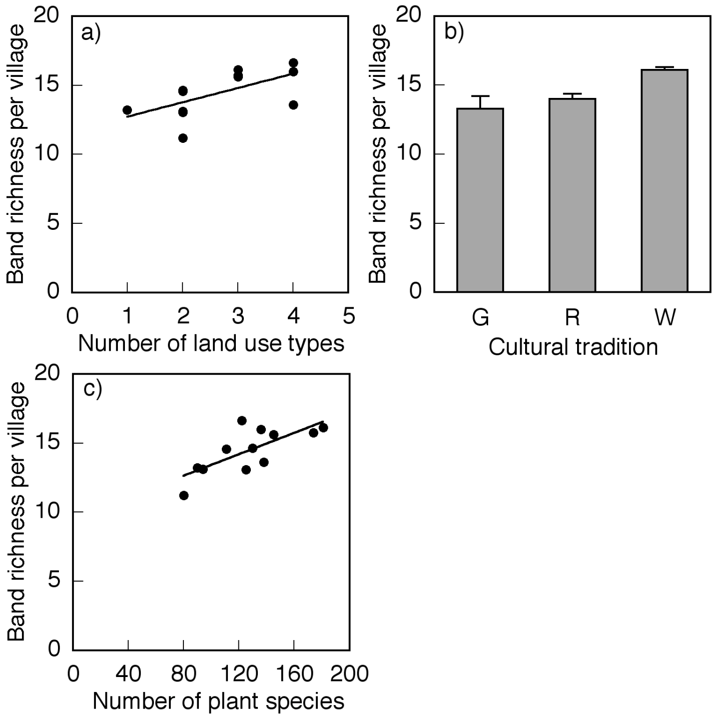

Fig. 2. Relationship between microsatellite band richness of *Poa alpina* per village sample and a) number of land use types investigated per village (ANOVA-model with sequential sums of squares, $P < 0.05$), b) cultural tradition of 12 villages in the Swiss Alps (Tukey-HSD, $P < 0.05$), and c) number of plant species recorded in the same parcels per village. Band richness is based on a standardized sample size of 16 plants. G = Germanic tradition, R = Romanic, W = Walser. In Fig. 2a three data points are hidden by others.

Genetic diversity within villages

Band richness per village, based on a standardized sample size of 16 plants, was between 11.2 and 16.6 bands, with a mean of 14.5. We found a strong impact of land use diversity on genetic diversity per village, as band richness was higher in villages with higher numbers of land use combinations among the sampled parcels ($P < 0.05$, Fig. 2 a). Furthermore, we found an effect of cultural traditions on genetic diversity within villages because in villages with Walser tradition, band richness was significantly higher than in Germanic villages (Tukey HSD, $P < 0.05$, Fig. 2 b). When both these significant variables were fitted simultaneously, the effect of cultural tradition only was significant when introduced into the model before the number of land use combinations (introduced first: $P < 0.05$, second: $P = 0.15$), while the significant effect of the number of land use combinations was independent of the fitting sequence. Moreover, band richness per village was significantly positively correlated with the accumulated number of plant species occurring in the parcels with *P. alpina* per village ($r = 0.73$, $P < 0.01$, Fig. 2c).

Genetic diversity among agriculturally used parcels

25.1 % of the variation in the presence and absence of bands resided among parcels (AMOVA, $P < 0.001$) and 18.4% resided among parcels within villages (AMOVA; $P < 0.001$, Table 1). Parcel differentiation for band richness $\rho_{ST(n)}$ was 0.25 across all five loci (Table 2), in line with the AMOVA result. Differentiation for band richness was higher among the 13 meadows than among the 41 pastures (0.32 and 0.23, respectively Table 2).

We found a tendency to isolation by distance, as geographically more distant parcels tended to be genetically more distant (Mantel test, $r_M = 0.12$, $P = 0.057$, Fig. 3 a), while genetic distances were independent of altitudinal differences among parcels (Partial Mantel test with geographic distance as covariate, $r_M = -0.03$, $P = 0.65$). Samples of *P. alpina* from parcels of the same land use (mowing or grazing) were genetically more similar than those from parcels with different land use (Partial Mantel test with geographic distance as covariate, $r_M = 0.15$, $P < 0.05$, Fig. 3 b) indicating genetic differentiation between land use types, while we found no indication for genetic differentiation among fertilised and unfertilised grassland parcels (Partial Mantel test with geographic distance as covariate, $r_M = 0.07$, $P = 0.095$). Accordingly, when we partitioned variation among parcels according to land use, we found 1.18 % to reside between mown and grazed grassland parcels (AMOVA, $P < 0.02$), and only 0.02 % between fertilised and unfertilised grassland parcels (AMOVA, $P > 0.42$).

Table 2 Population genetic measures for 54 populations from agriculturally used parcels of grassland in the Swiss Alps.

Microsatellite locus	Mean number of bands per plant and population	$r'_{S(6)}$	$r'_{T(6)}$	$\rho_{ST(6)}$	$\rho_{ST(6)}$ among meadows	$\rho_{ST(6)}$ among pastures
Poa CA1D4	2.88	7.19	9.03	0.20	0.29	0.18
Poa GAC1	4.44	11.62	16.35	0.29	0.36	0.27
Poa GA1C3	2.23	5.12	6.75	0.24	0.34	0.21
Poa CA1F4	3.87	8.39	10.57	0.21	0.25	0.19
Poa CAB12	3.37	7.29	10.33	0.29	0.34	0.28
Mean	**3.36**	**7.92**	**10.61**	**0.25**	**0.32**	**0.23**

$r'_{S(6)}$ = Band richness per population with a standardized sample size of six plants
$r'_{T(6)}$ = Band richness of the hypothetical total population with a sample size of six plants
$\rho_{ST(6)}$ = Differentiation for band richness among populations
$\rho_{ST(6)}$among meadows = Differentiation for band richness among 13 meadows
$\rho_{ST(6)}$ among pastures = Differentiation for band richness among 41 pastures

Samples of parcels with sexual and those with vegetative reproduction were differentiated in microsatellite bands, as genetic distances between samples of parcels with different modes of reproduction were significantly larger than between samples of parcels with the same mode of reproduction (Partial Mantel test, $r_M = 0.17$, $P < 0.05$, Fig. 3 c). Accordingly, 4.2 % of the variation resided between the 22 parcels with exclusively seed producing samples of *P. alpina* and the 10 parcels with exclusively pseudoviviparous ones (AMOVA, $p < 0.001$).

Overall, the results of this section indicate substantial regional genetic differentiation of *P. alpina* among different villages and genetic differentation among parcels with different reproductive modes of *P. alpina*. Genetic differentiation among mown and grazed parcels was less pronounced.

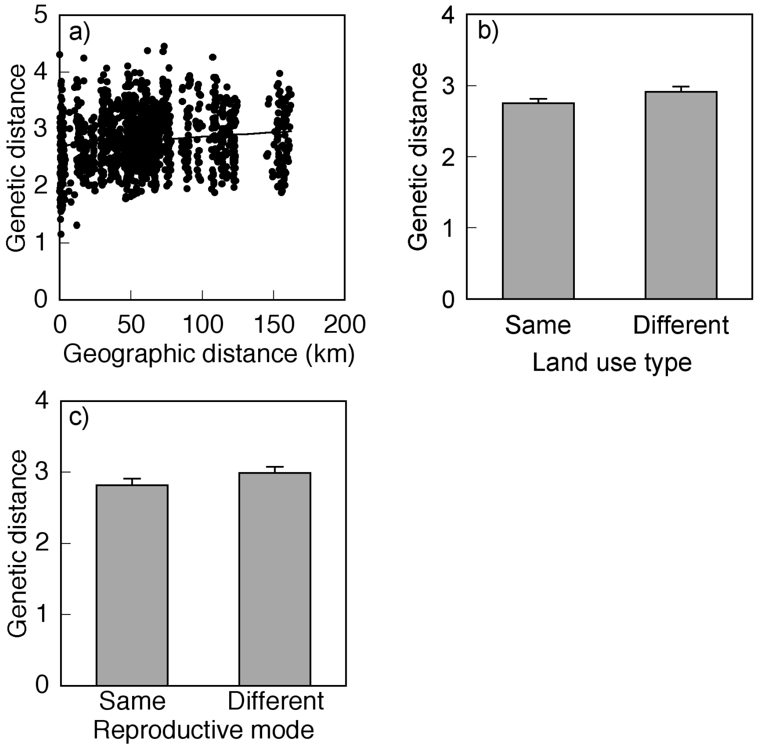

Fig. 3. Relationships of pairwise Euclidean genetic distances between populations of *Poa alpina* from agriculturally used parcels of grassland in the Swiss Alps a) with pairwise geographic distances for all 54 parcels(Mantel test, $r_M = 0.12$, $P = 0.057$, b) with pairwise correspondence or difference in land use for the 41 pastures and 13 meadows (Partial Mantel test with geographic distance as covariate, $r_M = 0.15$, $P < 0.05$), and c) with pairwise correspondence or difference in reproductive mode for 10 parcels with exclusively pseudoviviparously reproducing samples of *Poa alpina* and 22 exclusively seed-producing samples (Partial Mantel test with geographic distance as covariate, $r_M = 0.17$, $P < 0.05$).

Genetic diversity within agriculturally used parcels

74.9 % of the variation in the presence and absence of bands resided within parcels (AMOVA; p < 0.001; Table 1). Band richness, based on a sample size of six plants, varied between 5.12 and 11.62 per parcel and locus (Table 2), and the mean number of bands per plant was between 2.88 and 4.44 per locus (Table 2) and 16.8 across all loci .

Band richness increased with increasing parcel altitude, while the mean number of bands per plant was independent of altitude (Table 3). Cultural traditions did not affect genetic diversity within parcels (Table 3).

Table 3 Summary of analyses of the mean number of microsatellite bands per plant (MB) and band richness per parcel (BR) in 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps. Culture denotes the Romanic, Germanic, and Walser cultural traditions. Village denotes the 12 study villages. Fertilisation denotes the difference between unfertilised and fertilised parcels. Land use denotes differences between mown and grazed grasslands. In the sequential sums of squares ANCOVA, effects of culture were tested against remaining variation among villages. n.s. denotes values of p > 0.1

Source of variation	df	SS_{MB}	F_{MB}	p_{MB}	SS_{BR}	F_{BR}	p_{BR}
Culture	2	4.34	0.24	n.s.	7.72	2.04	n.s.
Village[Culture]	9	81.38	7.54	p<0.01	17.01	1.12	n.s.
Altitude	1	3.94	3.28	p<0.1	9.60	5.67	p<0.05
Fertilisation	1	0.25	0.21	n.s.	0.05	0.03	n.s.
Land use	1	8.78	7.32	p<0.05	6.83	4.04	p<0.1
Fertilisation*land use	1	0.60	0.50	n.s.	0.55	0.33	n.s.
Culture*altitude	2	4.00	1.00	n.s.	1.45	0.31	n.s.
Village[Culture]*altitude	9	18.05	1.67	n.s.	20.80	1.37	n.s.
Culture*fertilisation	2	1.34	0.39	n.s.	3.90	1.16	n.s.
Culture*land use	2	5.47	1.16	n.s.	0.98	0.08	n.s.
Village[Culture]*fertilisation	7	12.12	1.44	n.s.	11.78	0.99	n.s.
Village[Culture]*land use	3	7.09	1.97	n.s.	18.10	3.57	p<0.1
Altitude*fertilisation	1	0.11	0.09	n.s.	0.00	0.00	n.s.
Altitude*land use	1	0.40	0.33	n.s.	6.26	3.70	p<0.1
Residuals	11	13.19			18.61		

We found an effect of land use on within-parcel microsatellite diversity as the mean number of bands per plant was 3.0 % higher in pastures than in meadows ($F_{1,53}$ = 7.32; $P < 0.05$, Table 3, Fig. 4). Moreover, band richness was marginally significantly higher in pastures than in meadows ($F_{1,53}$ = 4.04; $P = 0.07$, Table 3).

Within-parcel microsatellite band diversity did not appear to be affected by more pronounced genetic drift in smaller populations of *P. alpina,* because the mean number of bands per plant ($P = 0.3714$), band richness per parcel ($P = 0.6486$), and mean Euclidean genetic distance of each parcel to all other parcels ($P = 0.6599$) were independent of mean abundance of *P. alpina*

Correlations between mean plant species richness in two plots per parcel and the mean number of bands or total plant species richness in two plots per parcel and band richness were not statistically significant ($P = 0.1875$ and $P = 0.9031$, respectively), indicating that genetic diversity of *P. alpina* was not affected by plant species diversity of the 54 agriculturally used grassland parcels.

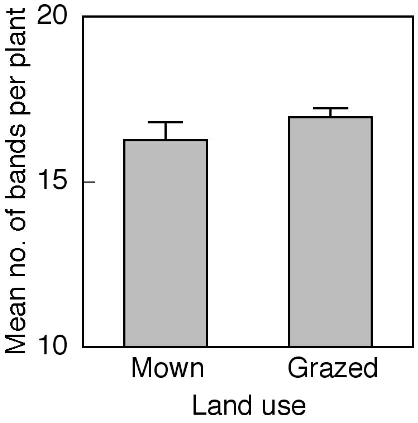

Fig. 4. Relationship between number of bands per plant and type of land use among 54 populations of *Poa alpina* from agriculturally used parcels of land in the Swiss Alps (ANCOVA-model with sequential sums of squares, $P < 0.05$).

Discussion

Genetic differentiation between natural and agriculturally used parcels

The small but highly significant microsatellite band differentiation between natural sites and agriculturally used parcels suggests that land use not only caused a divergence in genetic diversity between populations of *P. alpina* from mown and grazed grasslands, but also differentiation from natural habitats. This is in line with an accompanying quantitative genetic experiment, where plants from pastures and from meadows both differed from plants from natural sites in biomass allocation to reproduction (Weyand 2005). To our knowledge, only one study considering populations from natural sites and different agriculturally used parcels of land has been done, which investigated *Sesleria albicans* (Reisch *et al.* 2003). Unfortunately, the authors did not separate natural from agricultural populations but rather analysed, whether there was general variation in genetic diversity among habitat types, which prevents a comparison with their results.

Genetic differentiation among villages and among populations

Probably because of the large 170 km range comprising different Alpine valleys, genetic differentiation between grasslands was pronounced and explained 25% of the variation, and isolation by distance was apparent as marginally significantly positive relationship between pairwise geographical and genetic distances among parcels. Previously, in *P. alpina* isolation by distance had been studied and observed only within a pseudoviviparous population in Norway (Bjørnstad *et al.* 1995). In addition to geographic distance, variable polyploidy, which can restrict gene flow (Briggs & Walters 1997), may have contributed to population differentiation in *P. alpina.* Plants of *Poa alpina* were highly variable in chromosome number (between 22 and 61 in this study) due to frequent aneuploidy and multiple B-chromosomes (Müntzing 1980; Duckert-Henriod & Favarger 1987; Maurer *et al.* 2005).

Differentiation due to land use

Different habitat conditions can lead to genetically based ecotypic differentiation in grass species (Stapledon 1928). In our study, mowing and grazing over hundreds of years apparently led to genetic differentiation between mown and grazed populations of *P. alpina,* while fertilisation had no effect. This corresponds with the results of a common garden experiment using the same genotypes, which showed a divergence in biomass allocation between plants from mown and grazed parcels, but no difference between plants from fertilised and unfertilised parcels (Weyand 2005). Although the effect of mowing and grazing on genetic divergence between parcels was smaller than effects of isolation by distance or reproductive mode, it adds to the evidence that land use not only affects biodiversity at the plant community level, but also at the level of selectively neutral molecular diversity within species (Odat *et al.* 2004). The result is especially remarkable, as it suggests that land use affects biodiversity independently of regional differences. Land-use induced ecotypes have also been reported for the grassland forbs *Rhinanthus alectorolophus* (Zopfi 1993) and *Euphrasia rostkoviana* (Zopfi 1998).

For the observed higher population differentiation among populations of meadows than among populations of pastures there are two mutually not exclusive explanations. Firstly, endozoochorous or exozoochorous seed transport by cattle could increase gene flow among pastures. Secondly, the result could indicate more diverse habitat conditions among meadows than among pastures. Land use intensity of the investigated meadows with *P. alpina* varies between mowing every second year to twice per season and may thus be responsible for differentiation among mown parcels as different mowing intensity was reported to exert differential selection in *Festuca pratensis* (Kölliker *et al.* 1998). In contrast, among pastures land use intensity rather varies among seasons than among parcels, which results in more uniform selection, and may thus contribute to the weaker genetic differentiation among pastures than among meadows. A third alternative explanation of higher genetic drift among meadows than among pastures can be ruled out, because abundance of *P. alpina* was neither correlated with within-parcel genetic diversity nor with genetic distance of a parcel to all other parcels.

Differentiation due to reproductive mode

The observed substantial genetic differentiation among parcels with exclusively seed-producing samples of *P. alpina* and those with exclusively pseudoviviparous ones suggests that gene flow between seed-producing plants of *P. alpina* and pseudoviviparous ones, which produce a sexual floret at the basis of their inflorescence (Philipson 1934; Müntzing 1980), is rather low.

Genetic diversity within villages

In Walser villages more different land use combinations with *P. alpina* tended to be present than in Romanic and Germanic villages (result not shown). Most likely, this is due to the combination of the alpine to subalpine altitudinal distribution of *P. alpina* with the higher altitudes of Walser villages than of villages of the other traditions. Due to settlement history, the later arriving Walser people had to settle at higher altitudes than Romanic and Germanic people (Bätzing 2003). Accordingly, at the valley bottom of villages at lower altitudes, *P. alpina* was not present in all types of parcels. This reduced the number of investigated land use combinations in Romanic and Germanic villages and probably caused the observed dependence of the statistical significance of cultural tradition on band richness of *P. alpina* within villages on the fitting sequence. The higher genetic diversity of *P. alpina* in villages where *P. alpina* occurred in a larger number of different land use combinations is in line with the observed microsatellite differentiation among parcels of different land use. Moreover, it corresponds to the result of a study of plant species diversity of the same 12 villages, which revealed a significantly positive relationship between plant species richness per village and the number of different land use combinations present in the villages (Maurer *et al.* 2006). Accordingly, genetic diversity of *Poa alpina* was also positively correlated with the total number of plant species recorded in all the parcels per village where the *Poa* plants had been sampled.

Genetic diversity within populations

Agricultural land use significantly affected within-population genetic diversity of *P. alpina*. Populations originating from pastures were genetically more diverse with significantly more bands per plant and marginally significantly greater band richness than meadow populations. Because samples of *P. alpina* from meadows and those from pastures did not differ significantly in their reproductive modes (Weyand 2005), variation in reproductive mode cannot be responsible for these effects of land use on genetic diversity. Rather, this may be due to higher recruitment in grazed sites, either because of the higher biomass allocation of plants from pastures to reproduction (Weyand 2005), or because of the higher probability of establishment of seedlings and pseudoviviparous plantlets in pastures, which offer more vegetation gaps as safe sites for establishment (Grubb 1977). Moreover, selection in mown sites may be more uniform than in spatially more heterogeneous grazed sites, which may reduce genetic diversity more strongly in meadows than in pastures. Accordingly, in *Festuca pratensis* molecular genetic diversity was the lower, the more intense the cutting regime was (Kölliker *et al.* 1998). Furthermore, genetic diversity could have been enhanced in pastures because of higher gene flow due to seed transport by cattle. In contrast to our findings with *P. alpina*, genetic diversity of *F. pratensis* populations was also affected by fertilisation (Kölliker *et al.* 1998).

Parcels from higher altitudes had significantly higher band richness. In contrast, the number of bands per plant, which was not significantly different between exclusively pseudoviviparous and exclusively seed-producing samples, did not increase with altitude and neither did the number of chromosomes per plant (data not shown). A potential biological explanation for this set of results could be the differentiation between pseudoviviparous and seed-producing samples of *P. alpina* in combination with an increase in pseudoviviparous reproduction from 20 % in valley genotypes to 50 % in alpine genotypes observed in the common garden (Weyand 2005) and a resulting accumulation of bands typical for the two reproductive modes with increasing altitude.

Correlations between genetic diversity of Poa alpina and plant species richness

A high niche diversity has been suggested to maintain higher genetic diversity in plant species (Odat *et al.* 2004; Vellend & Geber 2005) and can also increase species richness, at least in nutrient-rich habitats (Gigon & Leutert 1996; Proulx & Mazumder

1998; Austrheim & Eriksson 2001; Maurer *et al.* 2006). Therefore, in contrast to our result, higher genetic diversity of *P. alpina* in parcels with higher plant species richness might have been expected. However, in our study, structural heterogeneity was higher in pastures than in meadows, which was also reflected by the higher genetic diversity in populations from pastures, but this was independent from species diversity.

Conclusions

Genetic diversity of *P. alpina* turned out to be affected by land use diversity in villages and by the specific land use within parcels. Higher genetic diversity within pastures than within meadows, genetic differentiation between populations from meadows and pastures, and higher genetic diversity within villages with more diverse land use imply two important conclusions. First, they demonstrate that the ongoing socio-economically motivated land use changes in the Swiss Alps do not only affect biodiversity at the landscape and community levels, but they may also change biodiversity within species when they decrease land use diversity. Moreover, promoting genetic diversity cannot be achieved by just maintaining the single type of land use associated with highest within-population diversity, but requires the maintenance of a high diversity of land use types.

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Chapter 7

Adaptation of the Alpine Meadow Grass *Poa alpina* to altitude and land use in the Swiss Alps

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submitted

Abstract

Plants can be adapted to natural and anthropogenic habitat differentiation. However, experimental demonstrations of the adaptiveness of intraspecific diversity are very scarce. Moreover, studies are missing, which simultaneously address several potential drivers of adaptation. We address these issues in the Alpine Meadow Grass *Poa alpina.* The species is widespread in subalpine and alpine habitats in natural sites and on agriculturally used land. Its plants reproduce via seed or via vegetative bulbils. We asked how land use and altitude affect the occurrence of *P. alpina* in the field and whether its performance in the common garden suggests adaptation to conditions at plant origin. First, we screened 216 parcels of land of different land use and altitude (1000-2400 m asl) around 12 municipalities in the Swiss Alps for the presence of *P. alpina*. *Poa alpina* occurred most frequently in fertilized and grazed sites and at higher elevations. Then, we set up a common garden experiment at about 1500 m asl with two plants of each of 615 genotypes originating from 57 grassland parcels and 21 natural sites. One plant per genotype was treated by clipping and one served as control. After two years, by the end of the experiment, nearly 90% of all plants had reproduced. In line with adaptive advantages of vegetative reproduction at higher altitudes, 77 % of reproductive plants from lower altitudes reproduced via seeds, while only 45% of plants from higher altitudes did so. The number of reproductive shoots produced throughout the experiment was highest for genotypes from grazed parcels, whereas clipped and final vegetative biomasses were highest for plants from mown parcels. Accordingly, in line with adaptive advantages of reproduction in grazed sites, which offer more gaps for establishment, allocation to reproductive biomass was higher in plants from grazed parcels than from mown parcels. The intraspecific differences between plants originating from parcels of different altitude and land use indicate that phenotypic differentiation of *P. alpina* in the field has a genetic component, which is shaped by adaptation to both natural and anthropogenic influences.

Key words: common garden experiment; genetic diversity; plasticity; reproduction; grassland;

Introduction

While it is theoretically clear that plants can be adapted to natural and anthropogenic habitat differentiation, experimental demonstrations of the adaptiveness of intraspecific diversity are very scarce (Callaway et al. 2003, Pigliucci 2003, Santamaria et al. 2003). Moreover, studies are missing, which simultaneously address several potential drivers of adaptation. Effects of natural and anthropogenic habitat differentiation on genetic within-species diversity should be especially important and apparent for species that are distributed across wide altitudinal and land use ranges. Such a species is the Alpine Meadow Grass *Poa alpina* L. (Poaceae) which is among the most frequent grasses in the European Alps (Conert 1998). It is widespread in mountain meadows and pastures and at natural sites such as rock fans, snow beds, and riverbeds. Moreover, it is one of the most important fodder grasses of the European Alps (Conert 1998).

The heterogeneous landscape of the Alps is characterized by pronounced natural, especially altitudinal, gradients (Theurillat et al. 2003). For thousands of years, this landscape and especially its grassland has also been shaped by different cultural and agricultural traditions of human land use (Ellenberg 1996, Bätzing 2003). This natural and anthropogenic heterogeneity is reflected in high landscape and community diversity within and between sites, and some studies have been directed towards explaining effects of this heterogeneity for vegetation composition (Guido and Gianelle 2001, Grabherr 2003). In the Swiss Alps, old cultural traditions, namely the Romanic, Germanic, and Walser ones, as well as recent land use changes affect vegetation diversity and composition (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). Such land use changes are particularly important, as in the Alps anthropogenic land use is undergoing large socio-economically motivated change (Bätzing 1991, 1993) involving both abandonment of land and intensification of agriculture (Olsson et al. 2000, Fischer and Wipf 2002). In contrast to between-species diversity and despite the presumably strong selection factors that natural and anthropogenic drivers constitute, their effects on within-species diversity received little attention.

Selection by different types of land use over hundreds of years is likely to have caused adaptive variation of growth and reproduction of *P. alpina*. Compared with natural and grazed sites, vegetative growth is likely to be favored under high levels of competition in mown grassland with their dense homogeneous swards (Abrahamson

1980). Alternatively, in very dense vegetation, such as the one in abandoned parcels, selection may favor reproduction as means of producing propagules for escape (Abrahamson 1980, Fischer and van Kleunen 2001).

Higher allocation to reproduction may have evolved in grazed grassland, where gaps in the vegetation favour recruitment more than homogeneous mown grassland does. *Poa alpina* occurs in two reproductive forms. Seminiferous plants produce seeds, while pseudoviviparous plants produce bulbils vegetatively, which develop into little plantlets on the parental plant (Pierce et al. 2000). Vegetative reproduction has been suggested to be of adaptive advantage at higher altitudes, where climatic conditions do not favour seedling establishment (Körner 2003).

While it is likely that long-term selection has led to genetic differentiation, phenotypic variation can additionally be shaped by plastic responses to land use. To some degree the mode of reproduction appears to be phenotypically plastic, as was demonstrated in experiments where plants turned pseudoviviparous at short day conditions and cold temperatures (Schwarzenbach 1953, Heide 1989). Moreover, plasticity can be adaptive in heterogeneous environments, where we expect plants to be more plastic, e.g. in response to disturbance brought about by land use, than plants from more uniform conditions (Fischer and van Kleunen 2001). Consequently, as grazed grasslands are more heterogeneous than mown ones, we expect higher such plasticity in plants from grazed sites, particularly for reproductive characters.

A powerful tool to partition phenotypic variation into genetic and environmental components is provided by experimentation in the common garden, where plant growth, plant response to simulated land use, and interactions between plant origin and simulated land use can be studied under uniform conditions (Silvertown and Charlesworth 2001).

We studied the effects of altitude and land use on the occurrence of *P. alpina* in more than 200 grassland parcels and on its growth and reproduction in a common garden experiment. To also study effects of origin on plasticity, we included a clipping treatment simulating land use. For the experiment, we used clonally derived plants from 615 genotypes from 21 natural sites and 57 grassland parcels. These sites represent different altitudes and land use around 12 municipalities along an east-west gradient of about 170 km. To account for potential regional and cultural differences, we selected four villages of each of the three main cultural traditions in the Swiss Alps, Romanic, Germanic, and Walser. We asked the following specific questions: (i) In the field, how does the occurrence of *P. alpina* depend on altitude and land use? In the experiment, (ii) do plants from higher altitudes rather reproduce via bulbils than via seeds, (iii) do plants from mown parcels allocate more to vegetative growth, (iv) do plants from grazed parcels allocate more to reproduction, and (v) are plants from grazed grassland more phenotypically plastic in response to experimental clipping than plants from more homogeneous abandoned or mown grassland are?

Methods

Species

The Alpine Meadow Grass *Poa alpina* L. (Poaceae) is widespread in upland regions in the Northern hemisphere, and locally at low altitudes in the North, in exposed grasslands and snow beds (Conert 1998). It occurs mainly on rich soils or alluvial plains and indicates high levels of nutrients and moisture. As a pioneer species, it also colonizes rock fans, other stony and rocky places of calcareous and siliceous rocks, and exposed slopes and ridges. In the Swiss Alps, it occurs in natural sites up to 4200 m asl and in agriculturally used land between 550 and 2500 m asl (Conert 1998). Because of its high fat and protein contents *P. alpina* is one of the most important fodder grasses in mountain meadows and pastures (Conert 1998).

The species is perennial and occurs in seminiferous and pseudoviviparous forms. The latter ones reproduce by proliferation of the spikelet axis and production of bulbils instead of seeds. Some seminiferous plants produce seeds sexually, others via apomixis (Müntzing 1933).

Occurrence of the species

We studied the occurrence of *P. alpina* in agricultural grassland around 12 villages in the Swiss Alps, four of each of the three cultural traditions (named after the original settling tribes) Romanic, Germanic, and Walser. Each village is part of a separate alpine valley, and the 12 villages are located along an east-west gradient of about 170 km. To exclude regions with extreme landscape changes we selected villages whose agriculture has only changed modestly during the last 50 years, that are not very touristy, and that have not grown to more than 1500 inhabitants. At three altitudinal levels per village (valley at about 1000 m asl, intermediate altitudes about 1500 m asl, and alp at about 2000 m asl), we had selected parcels of land representing different land use combinations

(K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). These combinations comprised parcels that were fertilized or unfertilized, had traditionally been mown or grazed, and were currently mown, grazed, or abandoned. In close collaboration with local farmers, we had found 147 out of 432 possible combinations of village, altitude, and traditional and current land use. At some altitudes in some villages were no parcels whose current land use differed from the traditional one. In these cases, we selected two parcels of the concerned type of traditional land use to increase sample size. In total, we selected 216 grassland parcels, between 12 and 24 per village, and screened them for the presence of *P. alpina*.

Plant material

For our common garden experiment, we used plants of *P. alpina* from 65 grassland parcels where land use had not changed. In each parcel, we sampled eight plants, plus two extra plants for potential replacement, at interdistances of at least 5 m. In addition, we sampled the same number of plants of *P. alpina* from 24 natural sites, two per village. We considered sites as natural, when they were located within or adjacent to natural plant communities such as the *Caricetum curvulae* or the *Caricetum firmae* (Ellenberg 1996), and when they were at least 200 meters separated from agriculturally used grassland.

Plant propagation

Because plant samples consisted of several tillers, we isolated single genotypes from each plant sample. To this end first we separated each field-collected plant into four single tillers, which we planted into the corners of 7 cm x 7 cm pots. As substrate, we used a 1:2 mix of sand and standard potting turf soil. We grew the plants in a greenhouse. After two months, in October 2002, we repotted one randomly selected plant grown out of the four single tillers, and discarded of the others. Then, after another 5 months, we divided the plants to receive two replicates per genotype and planted them each into new pots. After leaving the plants for 2 weeks in the greenhouse, we transferred them to the university garden (450 m asl) for outdoor acclimatization before planting them to the experimental site in the Alps. Four weeks later, in May 2003, immediately before transferring the plants to the experimental site, we measured initial size as number of tillers, diameter of the thickest tiller, and, if applicable, we recorded the mode of reproduction for each plant. At the same time, we clipped the plants to a standard height of 12 cm above ground to further reduce potential carry-over effects.

Experimental design

We used the plants to set up a common garden experiment in a formerly agriculturally used field at about 1500 m asl at Davos in the Swiss Alps in May 2003. In total, we planted 1380 plants (two plants of each of 690 genotypes) in a grid pattern in eight blocks, each consisting of four rows with up to 44 plants. The space available for each plant was 20 cm x 20 cm. For convenient application of a clipping treatment simulating land use to one of the plants per genotype, we planted block-wise in four blocks per treatment. The other plant per genotype in the remaining four blocks (alternating with the clipping-treatment blocks) served as control. Within blocks, plants were randomly assigned to planting positions in the grid. Monthly weeding prevented other plants – including *P. alpina* plants growing from seed or bulbils – from establishing within the experiment.

Measurements

In the clipping-treatment blocks, we harvested vegetative biomass (later on called clipped biomass) by clipping all leaves 5 cm above ground, but leaving out emerging reproductive shoots. In 2003, we clipped twice, in July and in October. In 2004, we clipped and harvested the vegetative biomass of the plants in the clipping-treatment blocks once at the end of June. One month later, we finished the experiment by harvesting the aboveground vegetative biomass of all plants, including the control plants, at 3 cm above ground (later on called final vegetative biomass). After the harvest, we dried the biomass at 80 °C and weighed it.

We harvested reproductive biomass of each plant for 2 years. In 2003, we harvested monthly from July to October. Of each reproducing plant, we cut off the reproductive shoots 5 cm above ground, counted them, and collected them in paper bags. Moreover, we noted the reproductive mode as seminiferous or pseudoviviparous. The reproductive shoots were only taken when at least a few anthers were visible (then noted as seminiferous), or when bulbils were well developed and about to dehisce (then noted as pseudoviviparous). Less developed shoots were left for the next harvest. For each plant, we harvested all reproductive biomass into the same paper bag to cumulatively receive the total amount of reproductive biomass. After drying the reproductive biomass at 80 °C, we weighed it and summed up the number of shoots. In 2004, we harvested reproductive biomass at the end of June and at the time of final harvest.

Statistical analysis

We analyzed the effects of land use and altitude on the occurrence of *P. alpina* in the field by logistic regression based on binomial distribution. The full hierarchical mixed model included the factors culture (referred to as cultural tradition), village (nested in culture), altitude, fertilization, abandonment, current land use, and all twofold interactions.

For the analyses of the experiment, 75 plants were not taken into account due to wrong sampling information. Moreover, independent of the experimental factors, another 75 plants died during the experiment and were not taken into account. Finally, of the originally 1380 plants, we included 1230 plants in the analyses, 905 from 57 grassland parcels and 325 from 21 natural sites.

For the clipping-treatment plants, we analyzed cumulative clipped vegetative biomass, cumulative clipped reproductive biomass, and cumulative number of clipped reproductive shoots. These cumulative numbers did not include the final harvest. The full hierarchical mixed ANOVA model included the factors culture, village (nested in culture), altitude, natural (referred to as natural sites), fertilization, abandonment, current land use, including all interactions and parcel (Table 1). From this full model, we selected reduced best models for each variable using Akaike's Information Criterion (AIC) -values (Burnham and Anderson 2002). This model selection procedure revealed as best model the one comprising the main seven factors culture, village, altitude, natural, fertilization, current land use, parcel, without any interactions (Table 1).

For all plants, we analyzed cumulative reproductive biomass and cumulative number of reproductive shoots, final vegetative biomass, total vegetative biomass (i.e. sum of final harvest and clipped biomass), and percentages of reproductive and vegetative biomass (relative to the sum of vegetative and reproductive biomass) with the same model as above, but adding the factors genotype and treatment. Again, we selected reduced best models for each variable starting with the full hierarchical model including all interactions. Finally, the best model included the main seven factors culture, village, altitude, natural, fertilization, current land use, parcel, and their two-way interactions

Table 1 Selected best ANOVA models for the analysis of common-garden variation in A) clipped vegetative biomass, B) reproductive biomass, number of reproductive shoots, final vegetative biomass, percentage of reproductive biomass, and total biomass. The analyses involved 1230 *Poa alpina* plants from 57 grassland parcels of different altitude and land use and 21 natural sites in 12 villages of three different cultural traditions in the Swiss Alp, except for the analysis of clipped biomass, which involved 612 such plants. Note: MS = mean square, F = variance ratio.

A Source of variation	MS	F
Culture	ms_{cult}	ms_{cult} / ms_{vill}
Village	ms_{vill}	ms_{vill} / ms_{parcel}
Altitude	ms_{alt}	ms_{alt} / ms_{parcel}
Natural	ms_{nat}	ms_{nat} / ms_{parcel}
Fertilization	ms_{fert}	ms_{fert} / ms_{parcel}
Current land use	ms_{curr}	$ms\ c_{urr} / ms_{parcel}$
Parcel	ms_{parcel}	$ms_{parcel} / ms_{residual}$
Residual = Plant	$ms_{residual}$	

B Source of variation	MS	F
Culture	ms_{cult}	ms_{cult} / ms_{vill}
Village	ms_{vill}	ms_{vill} / ms_{parcel}
Altitude	ms_{alt}	ms_{alt} / ms_{parcel}
Natural	ms_{nat}	ms_{nat} / ms_{parcel}
Fertilization	ms_{fert}	ms_{fert} / ms_{parcel}
Current land use	ms_{curr}	ms_{curr} / ms_{parcel}
Parcel	ms_{parcel}	$ms_{parcel} / ms_{genotype}$
Genotype	ms_{geno}	$ms_{geno} / ms_{residual}$
Clipping	ms_{clip}	$ms_{clip} / ms_{residual}$
Clipping x Culture	$ms_{clip\ x\ cult}$	$ms_{clip\ x\ cult} / ms_{clip\ x\ vill}$
Clipping x Village	$ms_{clip\ x\ vill}$	$ms\ clip\ x_{vill} / ms_{clip\ x\ parcel}$
Clipping x Altitude	$ms_{clip\ x\ alt}$	$ms\ clip\ x_{alt} / ms_{clip\ x\ parcel}$
Clipping x Natural	$ms_{clip\ x\ nat}$	$ms\ clip\ x_{nat} / ms_{clip\ x\ parcel}$
Clipping x Fertilization	$ms_{clip\ x\ fert}$	$ms\ clip\ x_{fert} / ms_{clip\ x\ parcel}$
Clipping x Current land use	$ms_{clip\ x\ curr}$	$ms\ clip\ x_{curr} / ms_{clip\ x\ parcel}$
Clipping x Parcel	$ms_{clip\ x\ parcel}$	$ms\ clip\ x_{parcel} / ms_{residual}$
Residual = Clipping x Genotype	$ms_{residual}$	

with treatment (Table 1). Final vegetative biomass and total vegetative biomass were log-transformed, and reproductive biomass and number of reproductive shoots were square root transformed prior to analysis of variance (ANOVA) to meet ANOVA assumptions.

To test variation in whether plants reproduced at all, we used logistic regressions based on binomial distribution. To analyze which factors affect the mode of reproduction we summed the number of reproducing plants per treatment and parcel and calculated the percentage of seminiferously reproducing plants, arcsin transformed it and fitted an ANOVA model including effects of culture, village, altitude, natural, fertilization, current land use, parcel, treatment, and all interactions of the treatment.

We analyzed all data separately for each year (by summing both harvests of clipped biomass and all harvests of reproductive biomass respectively number of reproductive shoots per year) and for both years combined. In all analyses, we treated village, parcel, and genotype as random factors. Including initial tiller diameter, number of tiller, and row and position in the experiment as covariates did not change levels of significance. Therefore, we report the results of models without covariates. We calculated all logistic regressions and ANOVAs with the program GENSTAT (Version 6.1, Lawes Agricultural Trust, 2002).

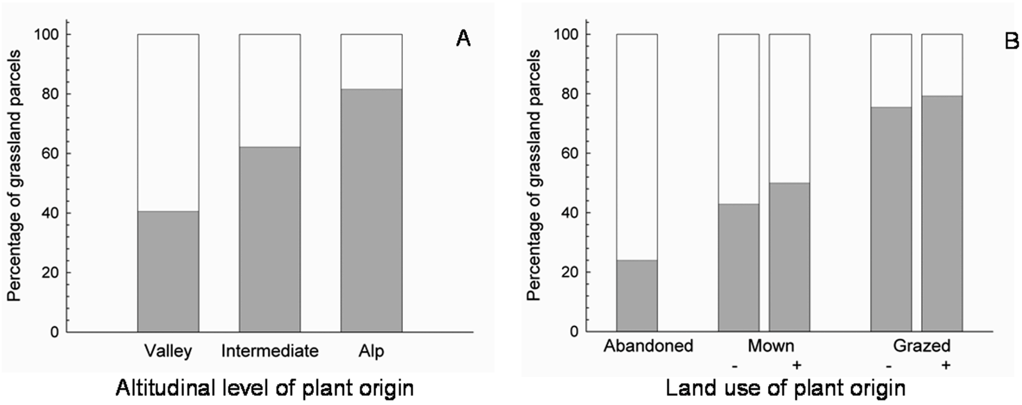

Fig. 1 Proportion of studied grassland parcels in the Swiss Alps in which *Poa alpina* occurred according to A) three altitudinal levels where the parcels were situated, and B) land use of the parcels. Fertilized parcels indicated by +, unfertilized by –. Shaded = *P. alpina* present, open = *P. alpina* absent.

Results

Occurrence of **Poa alpina** *in the field*

Poa alpina occurred in parcels representing 85 out of the 147 combinations of village, altitude, and land use. It occurred more frequently in parcels at higher altitudes than at lower ones (N = 147, $F_{2,130} = 12.57$, $p < 0.001$, Fig. 1A). Moreover, it occurred more frequently in fertilized parcels than in unfertilized ones (N = 147, $F_{1,130} = 12.88$, $p < 0.001$, Fig. 1B). Finally, *P. alpina* occurred most frequently in grazed parcels, followed by mown and abandoned ones (N = 147, $F_{2,130} = 25.87$, $p < 0.001$, Fig. 1B). In summary, higher altitude, fertilization, and grazing favored the occurrence of *P. alpina*.

Vegetative growth in the common garden

In 2003 and 2004 and for both years combined, clipped vegetative biomass was highest for plants from mown sites (ANOVA of combined clipped biomass: N = 612, $F_{2, 61} = 5.31$, $p < 0.01$) and lowest for plants from grazed and abandoned sites. Whereas in 2003 and for both years combined, clipped plants from natural sites produced the second highest vegetative biomass, they produced the least in 2004. While the above-mentioned differences between plants from mown and grazed sites were significant, those between plants from natural, used, and abandoned ones were not. Altitude of origin did not affect vegetative growth in the common garden.

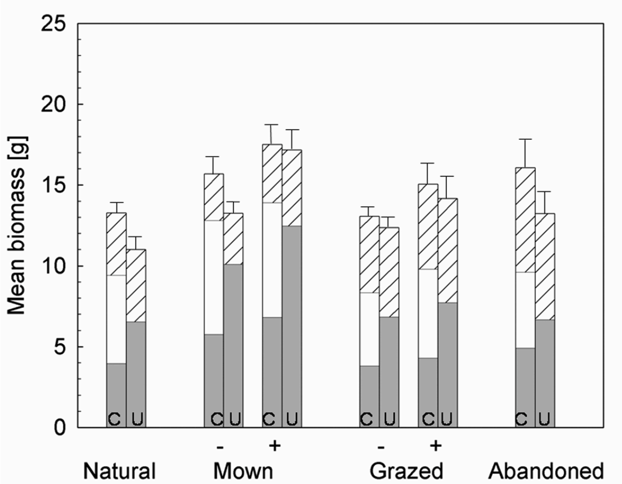

Fig. 2 Overview of biomass produced in the com-mon garden experiment with 1230 plants of *Poa alpina* originating from grassland parcels of different land use. Final vegetative biomass is indicated by shaded portions of bars, clipped biomass by open portions of bars, and reproductive biomass by hatched portions of bars. C denotes clipped plants, and U unclipped plants. Error bars denote 1 S.E. of total biomass.

At final harvest, control plants yielded more final vegetative biomass than clipped plants (N = 1230, $F_{1,536}$ = 180.26, $p < 0.001$, Fig. 2), although the percentage of vegetative biomass of total biomass was higher in clipped plants than in control plants (N = 1210, $F_{1,516}$ = 130.18, $p < 0.001$). Final vegetative biomass was higher in plants from mown grasslands (N = 1230, $F_{2,61}$ = 5.74, $p < 0.01$, Fig. 2) than in plants from grazed, natural, and abandoned ones. Moreover, at final harvest, plants originating from fertilized parcels yielded more final vegetative biomass than those from unfertilized ones did (N = 1230, $F_{1,61}$ = 4.26, $p < 0.05$). In summary, mowing and fertilization of the parcel of origin enhanced the common garden yield of final vegetative biomass.

Reproduction in the common garden

By the end of the experiment, 89.3% of the 1230 plants had reproduced. In 2003, 52.2% of all plants reproduced, and in 2004 82.9% reproduced. Overall, unclipped plants produced more reproductive biomass (5.12 g ± 0.23) than clipped ones did (4.43 g ± 0.19; N = 1097, $F_{1,61}$ = 14.43, $p < 0.001$).

In 2003, plants from grazed sites were more likely to reproduce than plants from mown and abandoned ones (N = 1230, $F_{2,61}$ = 5.80, $p < 0.01$). In 2004 and for the two years combined, land use of the parcel of origin did not affect the likelihood of reproduction.

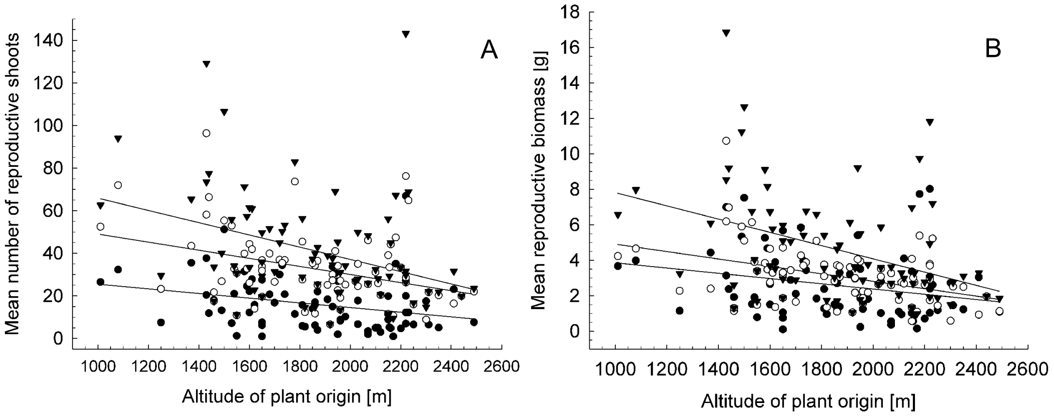

Fig. 3 A) Mean number of reproductive shoots, and B) mean reproductive biomass of *Poa alpina* plants originating from grassland parcels at different altitude grown in the common garden. Filled circles denote plants reproducing in 2003, open circles in 2004, and triangles the combination over both years (i.e. plants reproducing in both or either year).

Table 2 Vegetative and reproductive biomass in the common garden, and number of reproductive shoots and their means ± SE and number N of *Poa alpina* plants originating from grassland parcels of different land use in the Swiss Alps, combined over both years of the experiment and for the years 2003 and 2004 separately. Total biomass is the sum of vegetative and reproductive biomass. Numbers in brackets denote number of parcels of plant origin, and fertilized +, unfertilized -. Note: As not every plant reproduced every year, for the reproductive characters numbers and means of 2003 and 2004 do not add up to both years combined.

	Year	Natural (21)		Mown - (8)		Mown + (6)		Grazed - (29)		Grazed + (11)		Abandoned (4)	
		Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
Total biomass [g]		12.87 ± 0.52	325	14.92 ± 0.68	128	17.74 ± 0.9	93	12.97 ± 0.42	448	15.09 ± 0.94	172	15.01 ± 1.15	64
Final vegetative biomass [g]		5.25 ± 0.32	325	7.93 ± 0.344	128	9.68 ± 0.61	93	5.32 ± 0.24	448	6.02 ± 0.45	172	5.79 ± 0.54	64
Clipped vegetative biomass [g]	combined	5.45 ± 0.28	162	7.05 ± 0.47	64	7.08 ± 0.40	46	4.52 ± 0.22	224	5.51 ± 0.46	85	4.69 ± 0.59	32
	2004	0.92 ± 0.06	162	1.78 ± 0.18	64	1.78 ± 0.14	46	1.11 ± 0.06	224	1.36 ± 0.14	85	1.15 ± 0.10	32
	2003	4.53 ± 0.25	162	5.27 ± 0.35	64	5.30 ± 0.34	46	3.41 ± 0.19	224	4.15 ± 0.36	85	3.54 ± 0.54	32
Reproductive biomass [g]	combined	6.50 ± 0.60	274	3.01 ± 0.31	108	4.19 ± 0.57	85	5.13 ± 0.23	419	5.80 ± 0.47	151	4.17 ± 0.30	60
	2004	4.07 ± 0.42	241	2.66 ± 0.25	106	3.18 ± 0.43	85	3.38 ± 0.17	387	3.91 ± 0.36	141	2.77 ± 0.21	60
	2003	3.65 ± 0.41	174	2.43 ± 0.54	18	3.41 ± 0.63	25	2.95 ± 0.16	286	3.45 ± 0.30	94	2.73 ± 0.25	40
Number of reproductive shoots	combined	55.7 ± 5.1	274	26.3 ± 2.9	108	24.9 ± 2.6	85	47.4 ± 2.1	419	48.8 ± 3.7	151	40.7 ± 2.8	60

The number of reproductive shoots was closely positively correlated with reproductive biomass ($N = 1097$, $R = 0.862$, $p < 0.001$). Both the number of reproductive shoots (for both years combined: $N = 1097$, $F_{1,61} = 12.97$, $p < 0.001$ Fig. 3A) and reproductive biomass decreased with increasing altitude of origin (for both years combined: $N = 1097$, $F_{1,61} = 13.20$, $p < 0.001$, Fig. 3B). Moreover, the percentage of reproductive biomass decreased with increasing altitude ($N = 1097$, $F_{1,61} = 25.96$, $p < 0.001$).

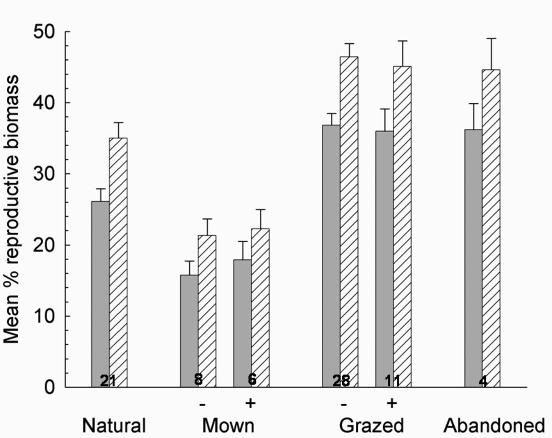

Fig. 4 Mean percentage of reproductive biomass relative to total biomass of clipping treatment plants (shaded) and control (hatched) plants of *Poa alpina* originating from grassland parcels of different land use grown in the common garden. Numbers within bars denote number of parcels of origin of the types of land use. Fertilized parcels indicated by +, unfertilized ones by –. Error bars denote 1 S.E.

More reproductive shoots were produced by plants from grazed sites than by plants from mown and abandoned ones ($N = 1097$, $F_{2,61} = 4.81$, $p < 0.05$, Table 2). Land use did not have any effects on the amount of reproductive biomass, whereas it affected the percentage of reproductive biomass. In both treatments, compared with plants from natural sites, allocation towards reproduction was significantly increased in plants from grazed sites and decreased in plants from mown ones ($N = 1210$, $F_{2,61} = 7.53$, $p = 0.001$, Fig. 4).

More plants per parcel originating from low altitudes reproduced via seed, whereas pseudoviviparous reproduction dominated in plants from high altitudes ($N = 156$, $F_{1,61} = 8.37$, $p < 0.01$, Fig. 5). Land use of the parcel of plant origin did not affect the mode of reproduction. We recorded only two plants that changed their reproductive mode from one year to another (one from seminiferous to pseudoviviparous, one vice versa). Different reproductive modes between the two plants of the same genotype occurred six times.

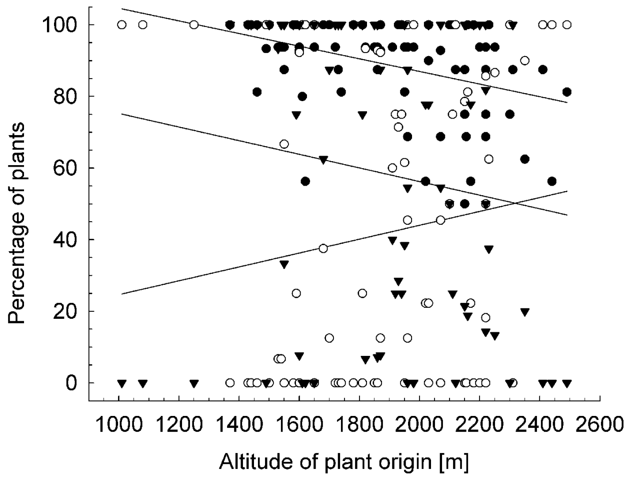

Fig. 5 Total proportion of reproducing *Poa alpina* plants and proportion of plants per reproductive mode (filled circles = reproducing, open circles = reproducing by bulbils, triangles = reproducing by seeds) in the common garden in relationship to the altitude of the parcel of plant origin.

Discussion

Habitats of Poa alpina

The higher likelihood of occurrence of *P. alpina* in grazed sites suggests a preference for more disturbed sites where vegetation gaps facilitate establishment of seedlings (Grubb 1977) or plantlets derived from bulbils. This corresponds to the results that plants in the common garden originating from grazed parcels produced more reproductive shoots and higher reproductive biomass than plants from other parcels (see below).

The higher likelihood of occurrence of *P. alpina* at higher altitudes corresponds well with its described distribution in subalpine habitats (Conert 1998). Our finding of higher likelihood of occurrence of *P. alpina* in fertilized grassland also confirms its preference for nutrient-rich habitats, which is also indicated by a by high nutrient indicator value of the species (Ellenberg et al. 1992).

Evidence for adaptation of vegetative growth

In our experiment, plants from mown grassland produced more vegetative biomass than plants from grazed sites did, suggesting divergent selection between mown and grazed sites. We suggest that, as hypothesized, high vegetative growth rates are competitively favorable in mown grassland where biomass is removed regularly. On the other hand, plants with high vegetative growth rates would be more conspicuous and therefore run higher risks of being consumed in grazed sites, which reduces the potential competitive advantage of fast growth. These differences in common-garden growth of plants from mown and grazed parcels suggest adaptation of *P. alpina* to land use. Similarly, intraspecific variation of the grass *Sesleria albicans* in a common garden was related to habitat quality and land use of its anthropogenic habitats of origin (Reisch and Poschlod 2003).

The absence of interacting effects on vegetative growth of clipping treatment and land use at the site of origin indicates that *P. alpina* did not adapt to different land use regimes by differences in plastic response to biomass removal.

Some previous studies of grassland plants report overcompensation of biomass removal by increased growth (Lennartsson et al. 1998, van Kleunen et al. 2004). Correspondingly, in our experiment previously clipped plants yielded a higher total biomass than previously unclipped plants indicating that compensatory growth also plays a role in *P. alpina*.

Evidence for adaptation of reproduction

Lower allocation to reproduction of plants from higher altitudes is in line with the hypothesis, that harsher conditions for seedling and bulbil establishment at higher altitudes cause an adaptive advantage of allocation to vegetative growth. In general, higher rates of plant establishment are expected in pastures due to their spatial heterogeneity and higher density of regeneration gaps (Grubb 1977). The higher likelihood of plants from grazed parcels than from mown parcels to reproduce and their higher biomass allocation to reproduction are likely to constitute adaptations to take advantage of the increased availability of open sites for establishment created by grazers and may explain part of the association of *P. alpina* with grazed habitats.

The absence of interacting effects on reproduction of clipping treatment and land use at the site of origin indicate that adaptation of reproduction of *P. alpina* to land use does not involve phenotypic plasticity.

Higher rates of reproduction of plants from grazed parcels do not only have demographic consequences, but are also likely to increase genetic diversity (Machon et al. 2001) due to increased seedling recruitment and higher numbers of dispersed propagules. In the case of *P. alpina*, genetic diversity measured with microsatellite markers (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data) and measured as broad-sense heritabilities of several quantitative traits (A. Weyand, K.

Maurer, J. Stöcklin, M. Fischer, unpublished data) was indeed higher for grazed parcels than it was for mown parcels. An alternative explanation for higher genetic diversity among genotypes from grazed parcels could be more monotonic selection in more homogeneous mown parcels than in more heterogeneous grazed parcels. Accordingly, an intensive cutting regime reduced genetic variability for *Festuca pratensis* (Kölliker et al. 1998).

Seed production and establishment of seedlings can be a rather risky mode of propagation at high altitudes (Bliss 1972, Billings 1974), which may be even more risky than establishing from bulbils. Therefore, the higher percentage of pseudoviviparously reproducing plants from higher altitudes may indicate an adaptation to climatic limitations endangering seed recruitment. The shorter growth periods at higher altitudes are more suitable for pseudoviviparously reproduced offspring, as bulbil-derived plantlets are supported by the maternal plant longer than seeds are and therefore can grow into mature plants much faster than seedlings can (Harmer and Lee 1978).

Apart from being adapted to altitudinal conditions, the mode of reproduction could also respond plastically to changing environments. Previous studies with *P. alpina* reported plastic responses by switching from pseudoviviparous to seminiferous reproduction after transplanting pseudoviviparous plants from cold to moderate temperatures (Schwarzenbach 1953, Bachmann 1980). However, in these studies, the majority of plants did not switch their mode of reproduction at all. Such switches seem to be the exception, as in our study, only two plants (0.16%) switched their reproductive mode between 2003 and 2004 respectively only six genotypes (0.98%) between the clipped and control replicate plant. Therefore, as far as these test environments are representative for natural variation, the mode of reproduction appears largely determined genetically in *P. alpina.*

In abandoned parcels with their high levels of standing crop, high reproductive effort is less likely to reflect an adaptation taking advantage of comparatively low competition. Therefore, the highest number of reproductive shoots in plants from abandoned sites might reflect an adaptation according to the alternative strategy, where increased production of propagules under high competition enables escape (Abrahamson 1980, Fischer and van Kleunen 2001, van Kleunen et al. 2001).

Some studies on manipulated losses of reproductive organs reported at least compensatory responses in reproductive traits (Gedge and Maun 1994, Gomez and

Fuentes 2001). In our study, the lower reproductive biomass in clipped plants indicates that the removal of vegetative biomass also reduced reproduction. Thus, there is no evidence for compensatory reproduction following biomass removal in *P. alpina*.

Conclusions

Our study demonstrates that in the heterogeneous landscape of the Alps, *P. alpina* is adapted not only to the natural altitudinal gradient, but also to anthropogenic land use variation. This implies that the ongoing socio-economically motivated change of agricultural land use in grassland of the Swiss Alps will not only change vegetation composition and diversity, but also adaptive intraspecific diversity of the widespread grass *P. alpina,* and most likely also of other species.

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Chapter 8

Biotic and abiotic determinants of quantitative genetic variation of the common grass *Poa alpina* L. in grasslands in the Swiss Alps

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Abstract

Multi-site studies of heritabilities and their potential drivers are very scarce. We studied broad-sense heritability H^2 in an important fodder grass, the clonal *Poa alpina*. We estimated H^2 of several reproductive and vegetative characters in a common garden experiment with 825 *P. alpina* plants originating from 53 grassland parcels of different cultural tradition, land use, and altitude along a 170 km east-west stretch in the Swiss Alps. Estimates of H^2 were significant for all traits and ranged from 0.363 to 0.572. H^2s were larger for parcels more rich in potassium, twice as high for grazed than for mown ones, and lower in parcels with higher diversity of herbivory. Moreover, and most likely associated with landscape diversity, H^2s were higher for parcels from villages of the old Romanic cultural tradition than for those of the Germanic and Walser traditions. Overall, land use was the most important determinant of H^2. We suggest promoting diverse land use regimes to conserve not only landscape and plant species diversity, but also heritable genetic diversity of *P. alpina*.

Key words: intraspecific diversity, broad-sense heritability, genetic variation, biodiversity, land use, agricultural land use, conservation

Introduction

The heritable genetic component of phenotypic variation is prerequisite for evolution and adaptation (Stearns, 1992). Potential natural drivers of heritable variation include abiotic parameters, such as topography and soil conditions, and biotic parameters, such as plant species diversity and the diversity of biological interactions. Potential anthropogenic drivers of heritable variation include variation in land use. However, heritable variation and the relative importance of its drivers are not even known for many widespread plant species. Therefore, we assessed heritable genetic variation and its drivers for the common Alpine Meadow grass *Poa alpina*, which in the Alps occurs over a large altitudinal range at natural sites and in agriculturally used grassland, where it is among the most important fodder species (Conert, 1998).

Heritability can be assessed in several ways. Narrow-sense heritability, which measures the proportion of additive genetic variation relative to total phenotypic variation (Falconer & MacKay, 1996), is considered the best predictor of response to selection for sexually reproducing plants. Broad-sense heritability measures the proportion of genotypic variation relative to total phenotypic variation (Falconer & MacKay, 1996). In sexually reproducing organisms it generally overestimates realized response to selection, because variation between genotypes, even in the absence of maternal carry-over effects, may not only include additive genetic variation, but also variation due to dominance or epistasis (Stearns, 1992). However, in clonal plants vegetative reproduction contributes largely to offspring reproduction, and therefore broad-sense heritability constitutes a better estimate for realized response to selection in clonal plants than in exclusively sexually reproducing plants (Fischer et al., 2004). A powerful tool to partition phenotypic variation into genotypic and environmental components is provided by experimentation in the common garden, where plant growth and plant response to simulated land use can be studied under uniform conditions (Silvertown & Charlesworth, 2001).

Heritable genetic variation is especially important for traits closely associated with fitness. Therefore, we studied heritable variation in growth and reproduction. Under stabilizing selection, such traits are likely to have low heritabilities (Stearns, 1980). Nevertheless, in heterogeneous environments substantial heritabilities of fitness-relevant traits may be maintained.

The landscapes of the Alps have been co-shaped by humans and their cattle over thousands of years (Ellenberg, 1996). Therefore, in the cultural landscape of the Alps, *P. alpina* has been under agricultural selection pressure for hundreds of years. In an accompanying study, we found *P. alpina* to be adapted to the natural altitudinal gradient (A.Weyand, K. Maurer, J. Stöcklin, M. Fischer, unpublished data). While plants originating from different altitudes did not differ in vegetative growth, the proportion of genotypes reproducing vegetatively via bulbils rather than via seed was higher among genotypes from higher altitudes, in line with the hypothesis of adaptive advantage of vegetative reproduction in the harsher conditions at higher altitudes (Klime_ et al., 1997). Moreover, plants of *P. alpina* turned out to be adapted to anthropogenic land use variation (A. Weyand, K. Maurer, J. Stöcklin, M. Fischer, unpublished data). In the common garden, plants from meadows had higher vegetative growth than plants from pastures, while plants from pastures allocated more biomass to reproduction than plants from meadows did. Plants from natural sites were intermediate, suggesting that the differences between plants from meadows and pastures constitute the adaptive result of divergent selection between parcels of different land use. These findings of adaptation to altitude and land use matter for the present study, because adaptive evolution may have depleted heritable variation within grasslands. However, the higher allocation to reproduction of genotypes from pastures and the higher frequency of sexually reproducing plants at lower altitudes may suggest that heritable variation is higher among genotypes from pasture parcels than from meadow parcels, and among genotypes from parcels at lower altitudes. Higher heritable variation could also be maintained in grazed parcels than in mown parcels because of the spatially more heterogeneous selection by grazing animals. The relationship between land use and heritable genetic variation is of particular interest in the Alps, because land use is undergoing major changes there and many meadows are converted to pastures (Bätzing, 2003).

In Switzerland three different cultural traditions, the Germanic, Romanic, and Walser ones, are found in the Alps. With their specific farming practices these cultural traditions have contributed to the high diversity of the alpine landscapes (Bätzing, 1991). In the valleys of Romanic regions, more diverse types of land use are still found than in the valleys of the other cultural traditions (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). If plants disperse between differently used parcels of grassland, the higher land use diversity in Romanic regions may suggest that heritable genetic variation of *P. alpina* is also higher among genotypes from grassland parcels from Romanic regions.

Heritable genetic variation may also be affected by soil conditions. Spatial or temporal heterogeneity of soil nutrients may cause heterogeneous selection, which in turn maintains heritable genetic variation (Stearns, 1992). Higher amplitudes of such heterogeneity is possible in grassland parcels with higher nutrient levels. Moreover, in grassland with higher nutrient availability the nutrient-indicator *P. alpina* (Landolt, 1977; Ellenberg et al., 1992) is likely to be more abundant. Higher abundance is likely to increase heritability because it reduces the likelihood of genetic drift (Fischer & Matthies, 1998).

Heritable genetic variation may also be affected by the diversity of the surrounding community. Higher plant species richness may increase heritable variation in *P. alpina*, if it increases the diversity of available niches (Odat et al., 2004; Vellend & Geber, 2005). However, relationships between plant species diversity and quantitative genetic diversity have not been studied. Moreover, a more diverse community of parasites may impose more heterogeneous selection than a less diverse one, and may thus maintain higher heritable variation in plants. However, the relationships between the diversity of herbivores and pathogens and heritable variation in plants are not known.

We estimated broad-sense heritability of several vegetative and reproductive characters of *P. alpina* in a common garden experiment for 53 grassland parcels that represent different altitudes and land use around 12 villages along a 170 km east-west gradient in the Swiss Alps. To account for potential cultural differences we had selected four villages of each of the three main cultural traditions in the Swiss Alps, Romanic, Germanic, and Walser. The parcels were either mown or grazed and they were either additionally fertilized or unfertilized. Plant species diversity, the diversity of herbivory and fungal pathogen infection, and microsatellite diversity of *Poa alpina* were known for all parcels from previous studies.

We addressed the following questions: (1) Is there heritable genetic variation in vegetative and reproductive traits in *P. alpina*? Is such heritable genetic variation related to (2) topographical and soils parameters, (3) land use and cultural traditions, and (4) the diversity of surrounding vegetation, of herbivory, and fungal pathogens, and of microsatellite markers at plant origin?

Materials and methods

Study species

The Alpine Meadow Grass *Poa alpina* L. (Poaceae) is widespread in mountain regions in the Northern hemisphere (Conert, 1998). It occurs mainly on rich soils or alluvial plains and indicates high levels of nutrients and moisture. As a pioneer species, it also colonizes stony and rocky places of calcareous and silicate rocks, and exposed slopes and ridges. In the Swiss Alps, it occurs in natural sites up to 4200 m asl and in agriculturally used land between 550 and 2500 m asl (Conert, 1998). Due to its high fat and protein contents *P. alpina* is one of the most important fodder grasses in mountain meadows and pastures (Conert, 1998).

The perennial species occurs in seminiferous and pseudoviviparous forms. The latter ones reproduce by proliferation of the spikelet axis and production of bulbils instead of seeds. Some seminiferous plants produce seeds sexually, others via apomixis (Müntzing, 1933). Moreover, *Poa alpina* is a polyploid complex with common aneuploidy (Müntzing, 1980) and highly variable chromosome numbers (Steiner et al., 1997). In Switzerland chromosome numbers ranging from 2n = 22 to 46 have been reported (Duckert-Henriod & Favarger, 1987), whereas more than 60 chromosomes are known from Scotch plants (Müntzing, 1980).

Study sites

Study plants of *P. alpina* originated from 12 villages in the Swiss Alps, four of each of the three cultural traditions (named after the original settling tribes) Romanic, Germanic, and Walser (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). The plants for this study were sampled in those 53 of a larger set of 216 grassland parcels in the Swiss Alps, where *P. alpina* occurred, and which represented unique combinations of village, altitudinal belt, and consistent land use. The 53 grassland parcels were situated at three altitudinal levels, 11 at the valley bottom (about 1000 m asl), 22 at intermediate altitudes (about 1500 m asl), and 20 at the alp level (about 2000 m asl). Parcels were fertilized (with liquid manure or dung) or unfertilized (i.e. never fertilized according to the farmer), and were currently mown or grazed. The unfertilized meadows were usually cut once a year whereas the fertilized meadows were cut between two and four times. Altogether, the parcels comprised 13 meadows, of which 6 were fertilized and 7 were not, and 40 pastures, of which 12 were fertilized and 28 were not.

Abiotic environment

From June to September 2002, we recorded the coordinates and altitude of the grassland parcels with a GPS, aspect as deviation from south in degrees, and slope in degrees to account for possible effects of topographic factors on the quantitative genetic diversity of *P. alpina*. To obtain concentrations of plant-available soil nutrients (potassium, calcium, magnesium, and phosphorous) we had soil samples from each parcel analyzed with the ammonium acetate EDTA method (AAEDTA 1:10) by Sol Conseil, Nyon, Switzerland. Additionally, we measured soil pH with a Hellige set (AVM Analyseverfahren, Freiburg, Germany).

Biodiversity measurements

As measure of landscape diversity around a village, we used the mean number of different land use types per village (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). As measure of plant species diversity, we used the mean number of plant species of two vegetation records per parcel, and Shannon's index of diversity and Evenness based on mean cover values of the two records (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). As measure of the diversity of biological interactions per parcel, we used the number of different damage types by herbivory and by fungal pathogen infection per leaf and per parcel (A. Weyand, K. Maurer, J. Stöcklin, M. Fischer, unpublished data). We measured molecular genetic diversity of *P. alpina* L. for 6-8 plants per parcel at five polymorphic microsatellite loci (Maurer et al., 2005). We used the mean number of microsatellite alleles per plant and parcel and the mean allelic richness per parcel based on a rarefaction-sample size of six plants per parcel (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data).

Common garden experiment

For this study we considered two plants of each of six to eight genotypes of *P. alpina* of each of the 53 parcels grown in a common garden experiment at 1500 m asl at Davos, Switzerland, totaling 825 plants (A. Weyand, K. Maurer, J. Stöcklin, M. Fischer, unpublished data). These plants had been collected from June to September 2002, and propagated and cut to standard size of 12 cm before planting into the experiment in May 2003. To one of the plants per genotype we applied a clipping-treatment to simulate land use, the other plant served as control.

Over two years, we measured several vegetative and reproductive characters. Of each plant, we harvested reproductive biomass, counted the number of reproductive shoots, and noted the mode of reproduction as seminiferous or pseudoviviparous. From the clipping-treatment plants, we obtained cumulative vegetative biomass sampled at four times during the two years (later on called clipped biomass). At the end of the experiment, we harvested aboveground vegetative biomass of each plant (later on called final vegetative biomass). From these data, we calculated total vegetative biomass by summing up clipped and final biomass, and total overall biomass by summing up total vegetative and reproductive biomasses. Moreover, we calculated the percentage of reproductive biomass relative to total biomass to obtain a measure for allocation to reproduction.

Statistical analysis

We calculated quantitative genetic diversity between genotypes within parcels. For each parcel we calculated broad-sense heritability of the measured quantitative characters separately (final vegetative biomass, total vegetative biomass, reproductive biomass, number of reproductive shoots, total overall biomass, and percentage of reproductive biomass). Moreover, we calculated the mean of the three heritabilites of reproductive characters, of the two heritabilities of vegetative characters, and of all six characters.

For each parcel, to calculate broad-sense heritability, we estimated variance components (VC) for variation among genotypes and for residual variation with expected mean squares (EMS) analyses of variance including the random factor genotype and the fixed factor treatment. The broad-sense heritability, H^2, is given by the quotient of the VC due to differences among genotypes (s^2_G) and the sum of this VC and the one due to residual variance (s^2_{Res}) as $H^2 = \frac{s^2_G}{s^2_G + s^2_{Res}}$ (Falconer & MacKay, 1996).

To analyze whether broad-sense heritabilities overall were significantly different from zero we used both possible tests. First, we tested whether variation among genotypes within grassland parcels was significant (Table 1A). To this end, we analyzed the reproductive and vegetative characters with analysis of variance (ANOVA) using a mixed model including the factors parcel, genotype, treatment, and parcel x treatment interaction. From this analysis, overall H^2 was obtained from the VC for genotypes and the VC residual. Second, we obtained mean and standard error of H^2 from all H^2 estimates across parcels. We used these data for a t-test of the test statistic mean/S.E. (Table 1B).

Table 1 Broad-sense heritability H^2 of reproductive and vegetative characters measured in *Poa alpina* plants from 53 grassland parcels of different land use and altitude in the Swiss Alps. A) Broad-sense heritability estimated from within-parcel variance component among genotypes obtained by Analysis of Variance using data of all parcels (see methods). B) Broad-sense heritability estimated as mean across all single-parcel broad-sense heritabilities. Mean reproductive H^2 denotes mean of broad-sense heritability of three reproductive characters, mean vegetative H^2 denotes mean of broad-sense heritability of two vegetative characters, and mean overall H^2 denotes mean of all six broad-sense heritabilities.

	A		B			
	H^2	P (genotype)	Mean H^2	±SE	t=Mean H^2/SE	P (t-test)
Number of reproductive shoots	0.496	0.000	0.414	0.041	10.011	0.000
Reproductive biomass	0.513	0.000	0.425	0.041	10.342	0.000
% of reproductive biomass	0.647	0.000	0.572	0.046	12.485	0.000
Final vegetative biomass	0.426	0.000	0.363	0.035	10.269	0.000
Total vegetative biomass (clipped and final)	0.466	0.000	0.391	0.041	9.471	0.000
Total biomass (vegetative and reproductive)	0.446	0.000	0.394	0.039	10.065	0.000
Mean reproductive H^2			0.470	0.068	6.946	0.003
Mean vegetative H^2			0.377	0.014	26.805	0.001
Mean overall H^2			0.429	0.029	14.711	0.000

Further, we studied the relationship of topographical and soil characteristics of the parcels with broad-sense heritability estimates with pairwise Pearson product-moment correlations. Then, we analyzed effects of land use, altitude, and cultural traditions on broad-sense heritability with an analysis of variance (ANOVA) model with sequential sums of squares using a hierarchical mixed model including the factors culture, village, altitude, fertilization, current land use, and all two-way interactions. Effects of culture were tested against remaining variation among villages and effects of all other factors against variation due to remaining differences among parcels. To test potential relationships between heritabilities and abiotic parameters we included aspect, slope, geographic coordinates, and the soil nutrients K, Ca, Mg and P of the parcels as covariates into the model. As these did not change levels of significance, we used the model without covariates. Finally, we studied the relationships between heritability and the other parcel-measures of biodiversity with pairwise Pearson product-moment correlations.

Table 2 Relationship between broad-sense heritabilities H^2 of reproductive and vegetative characters of *Poa alpina* and topographical parameters for 53 grassland parcels of different land use and altitude in the Swiss Alps. Pearson product moment correlation coefficients are given. X and Y coord denote geographical coordinates according to the Swiss Grid. Bold italic letters indicate marginally significant correlations ($P < 0.1$).

	Altitude	X coord	Y coord	Slope	Aspect
Number of reproductive shoots	0.070	0.076	0.109	0.002	-0.048
Reproductive biomass	0.128	-0.075	-0.070	-0.044	-0.018
% of reproductive biomass	-0.121	-0.166	-0.010	-0.053	0.172
Final vegetative biomass	0.191	0.157	0.076	0.187	0.025
Total vegetative biomass (clipped and final)	0.130	0.093	-0.048	0.160	-0.108
Total biomass (vegetative and reproductive)	***0.242***	-0.119	-0.166	0.100	-0.069
Mean reproductive H^2	0.046	-0.070	0.007	-0.003	0.045
Mean vegetative H^2	0.163	0.127	0.010	0.178	-0.049
Mean overall H^2	0.164	-0.015	-0.030	0.114	-0.012

Results

Broad-sense heritability in Poa alpina

Variation among genotypes within parcels was significant for all vegetative and reproductive characters (Table 1A). Corresponding broad-sense heritabilities H^2 were between 0.426 (percentage of reproductive biomass) and 0.647 (final vegetative biomass; Table 1A). In line with the above, all means of the estimates of the single-parcel broad-sense heritability over all parcels were significantly different from zero and were between 0.363 (percentage of reproductive biomass) and 0.572 (final vegetative biomass; Table 1B).

The three heritability estimates for reproductive characters were non-significantly higher than the estimates of the two vegetative characters (t-test, df = 3; data of Table 1A: t = 1.671, p > 0.19; data of Table 1B: t = 1.402, p > 0.26).

Table 3 Relationship between broad-sense heritabilities H^2 of reproductive and vegetative characters of *Poa alpina* and soil parameters for 53 grassland parcels of different land use and altitude in the Swiss Alps. Pearson product moment correlation coefficients are given. Bold letters indicate significant correlations ($P < 0.05$), bold italics indicate marginally significant correlations ($P < 0.1$).

	K	Mg	Ca	P	pH
Number of reproductive shoots	***0.243***	**0.333**	**0.314**	0.162	***0.264***
Reproductive biomass	**0.350**	0.202	0.137	0.178	0.126
% of reproductive biomass	**0.307**	0.227	0.083	0.095	0.196
Final vegetative biomass	0.209	***0.254***	0.140	0.170	0.029
Total vegetative biomass (clipped and final)	0.180	0.215	0.177	0.184	0.059
Total biomass (vegetative and reproductive)	**0.361**	0.183	0.149	**0.308**	0.075
Mean reproductive H^2	**0.341**	**0.295**	0.200	0.158	0.230
Mean vegetative H^2	0.200	***0.241***	0.165	0.184	0.047
Mean overall H^2	**0.371**	**0.322**	0.220	***0.241***	0.175

Relationships between topographical and soil characteristics of grassland parcels and broad-sense heritability of **Poa alpina**

Geographic coordinates, aspect, and slope were not significantly correlated with the heritability estimates (Table 2). All correlations of soil nutrient concentrations and pH with heritability estimates were positive. Among these, soil concentrations of potassium, magnesium and phosphorous were significantly positively related with heritability estimates, especially of reproductive characters (Table 3). Whereas potassium concentration was significantly positively correlated to nearly all heritability estimates, concentrations of calcium and phosphorous were significantly positively correlated with only single estimates of broad-sense heritability (Ca: heritability of reproductive shoots, N = 49, R = 0.314, p < 0.01; P: heritability of total biomass, N = 49, R = 0.308, p < 0.01,

Table 3). These correlations indicate that parcels with higher potassium soil concentrations are likely to host populations of *P. alpina* with higher broad-sense heritabilities.

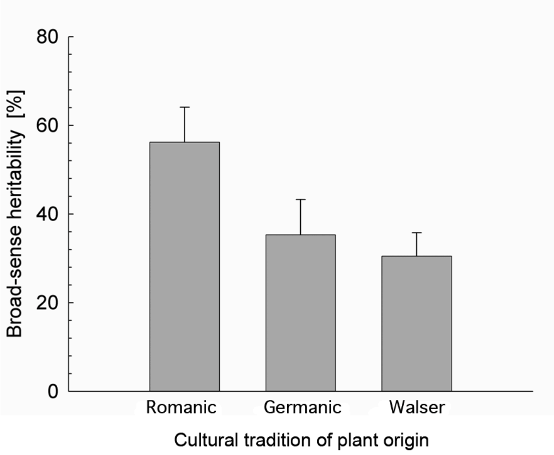

Fig. 1 Relationship between cultural tradition and broad-sense heritability H^2 of total vegetative biomass of *Poa alpina* plants originating from 53 grassland parcels of different cultural traditions, land use and altitude in the Swiss Alps. Error bars denote 1 S.E.

Relationships between cultural traditions, land use, and altitude of grassland parcels and broad-sense heritability of **Poa alpina**

Broad-sense heritability of total vegetative biomass was higher for parcels from Romanic villages than for those from Germanic and Walser villages (N = 53, $F_{2, 9} = 5.1$, $p < 0.05$, Table 4, Fig. 1). Broad-sense heritabilities were higher for grazed parcels than for mown ones. This was especially pronounced for the heritability estimate of percentage of reproductive biomass (N = 53, $F_{1, 10} = 15.0$, $p < 0.01$), and also for overall mean heritability (N = 53, $F_{1, 10} = 6.5$, $p < 0.05$, Table 4, Fig. 2). Heritability estimates were independent of altitude and fertilization of grassland parcels.

Relationships between community biodiversity and broad-sense heritability of **Poa alpina**

Broad-sense heritabilites were independent of plant species richness in the parcels of origin, and they were negatively related to the diversity of biological interactions within parcels (Table 5). Heritability H^2 of total vegetative biomass was negatively related to the diversity of herbivory and fungal pathogen infection. This was significant for the diversity of herbivory per grassland parcel, (N = 49, R = -0.369, $p < 0.01$) and marginally significant for the diversity of herbivory per leaf (N = 49, R = -0.245,

Table 4 Mean broad-sense heritabilities H^2 of different reproductive and vegetative characters of *Poa alpina* plants from 53 grassland parcels of different cultural tradition and land use in the Swiss Alps. Significant differences are indicated by bold numbers. SE denotes standard error. N denotes the number of parcels of plant origin.

		Mean H^2 ± SE							
		Reproductive characters				Vegetative characters			
	N parcels	Number of reproductive shoots	Reproductive biomass	% of reproductive biomass	Mean reproductive H^2	Final vegetative biomass	Total vegetative biomass (clipped and final)	Total biomass (vegetative and reproductive)	Mean overall H^2
Romanic	15	0.405 ± 0.081	0.492 ± 0.059	**0.742 ± 0.058**	0.546 ± 0.056	**0.495 ± 0.066**	0.562 ± 0.079	0.448 ± 0.083	**0.524 ± 0.059**
Germanic	15	0.442 ± 0.075	0.429 ± 0.085	**0.651 ± 0.089**	0.507 ± 0.072	**0.322 ± 0.057**	0.353 ± 0.080	0.376 ± 0.077	**0.429 ± 0.050**
Walser	23	0.400 ± 0.065	0.376 ± 0.067	**0.408 ± 0.067**	0.401 ± 0.056	**0.304 ± 0.054**	0.305 ± 0.053	0.371 ± 0.054	**0.365 ± 0.041**
Mown	13	0.358 ± 0.094	0.287 ± 0.091	**0.297 ± 0.098**	**0.323 ± 0.088**	**0.227 ± 0.071**	0.273 ± 0.073	0.273 ± 0.063	**0.289 ± 0.059**
Grazed	40	0.43 ± 0.046	0.466 ± 0.044	**0.661 ± 0.044**	**0.519 ± 0.036**	**0.407 ± 0.039**	0.430 ± 0.048	0.434 ± 0.046	**0.471 ± 0.031**

p = 0.09) and for the diversity of fungal pathogen infection per leaf (N = 49, R = -0.271, p = 0.06, Table 5).

Relationship between microsatellite diversity and broad-sense heritability of Poa alpina

Heritability estimates of total overall biomass and of final vegetative biomass were marginally significantly positively related with mean allelic richness of microsatellite markers per parcel (Table 5), suggesting positive relationships between selectively neutral and quantitative genetic variation.

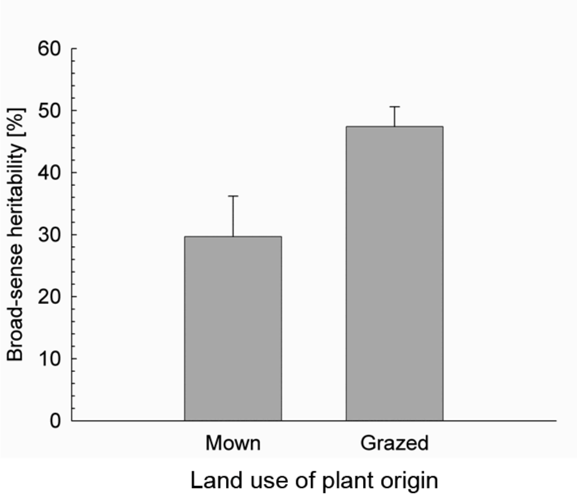

Fig. 2 Relationship between land use and the mean overall broad-sense heritability H^2 of plants of *Poa alpina* originating from 53 grassland parcels of different cultural traditions, land use and altitude in the Swiss Alps. Error bars denote 1 S.E.

Discussion

Broad-sense heritability

Compared with other species, the observed estimates of broad-sense heritability of vegetative and reproductive characters ranging from 0.363 to 0.572 (Table 1) appear intermediate to high. Significant broad-sense heritability of 0.198 was reported for fitness-relevant spatial spread in the clonal plant *Ranunculus reptans* (Fischer et al., 2004). In the grass *Eragrostis tef* a wide range of broad-sense heritabilities between 0.17 for shoot phytomass per plant and 0.74 for panicle length were reported (Assefa et al., 2001). The observed broad-sense heritabilities suggest that a high potential for further adaptation and evolution has been maintained in *P. alpina* even after hundreds of years of selection by agricultural land use.

Table 5 Relationship between broad-sense heritabilities H^2 of reproductive and vegetative characters of *Poa alpina* and landscape diversity of 12 villages and community diversity, and microsatellite diversity of *Poa alpina* for 53 grassland parcels of different land use and altitude in the Swiss Alps. Pearson product moment correlation coefficients are given. Bold letters indicate significant correlations ($P < 0.05$), bold italics indicate marginally significant correlations ($P < 0.1$).

	Number of land use types	Number of plant species	Diversity of herbivory per leaf	Diversity of herbivory per parcel	Diversity of fungal pathogens per leaf	Diversity of fungal pathogens per parcel	Mean number of alleles per plant	Mean allelic richness of six plants per parcel
N	12	49	49	49	49	49	53	53
Number of reproductive shoots	0.298	0.048	-0.107	-0.088	-0.038	0.046	-0.134	-0.103
Reproductive biomass	-0.021	-0.013	-0.156	-0.041	-0.06	0.07	-0.207	0.173
% of reproductive biomass	-0.137	-0.117	-0.079	-0.085	-0.025	-0.023	-0.09	0.108
Final vegetative biomass	0.176	0.039	-0.146	-0.168	***-0.269***	-0.13	0.017	***0.234***
Total vegetative biomass (clipped and final)	0.231	0.156	***-0.24***	**-0.369**	***-0.271***	-0.053	-0.093	0.179
Total biomass (vegetative and reproductive)	-0.018	0.016	-0.149	-0.206	-0.072	0.072	-0.183	***0.269***
Mean reproductive H^2	0.004	-0.045	-0.169	-0.095	-0.068	0.012	-0.189	0.081
Mean vegetative H^2	0.149	0.106	-0.206	**-0.286**	***-0.27***	-0.091	-0.044	0.212
Mean overall H^2	0.114	0.021	-0.239	-0.231	-0.185	-0.023	-0.183	0.204

The relatively high estimates of broad-sense heritability in *P. alpina* may possibly be related to the polyploidy and highly variable chromosome numbers of the species (Müntzing, 1980). For combinatory reasons, there are more possibilities for genetic polymorphisms among polyploid plants than among diploid plants. Moreover, if gene flow among plants of different ploidy levels is restricted (Meirmans et al., 2003), high genotypic variation and broad-sense heritability may be maintained. However, such relationships between quantitative genetic variation and within-species variation in polyploidy have not been explored.

Determinants of heritability

Topography and soil

Our results suggest that the variation in broad-sense heritability between grasslands with *P. alpina* is rarely affected by topography (Table 2). In particular, the higher frequency of sexual reproduction in plants from lower altitudes was not associated with higher broad-sense heritability in parcels at lower altitudes. In contrast, all relationships between broad-sense heritabilities and altitude were non-significantly positive. Possibly, a positive effect of higher frequency of sexual reproducing plants at lower altitudes on broad-sense heritability was superimposed by a negative one of increased fragmentation of grasslands at lower altitudes. The latter was brought forward to explain the higher molecular genetic variation within populations of *Primula farinosa* from higher altitudes (Reisch et al., 2005).

Heritable genetic variation was higher for *P. alpina* from parcels with higher soil nutrient concentrations, especially potassium (Table 3). This may be due to higher spatial or temporal variation in nutrient concentrations in such parcels, which may have led to more heterogeneous selection and thus may have maintained higher heritable genetic variation (Stearns, 1992). Nutrient-rich mountain grassland and hence typical sites for *P. alpina* are usually adequately supplied with potassium according to the nearly closed biological cycle of potassium due to complete recirculation by dung (Bohner & Sobotik, 2000). Accordingly, the higher abundance of the nutrient indicator *P. alpina* in more nutrient rich parcels may have reduced negative effects of genetic drift on heritability (Willi et al., 2005), which may explain the positive relationship between soil potassium concentration and heritability of *P. alpina*.

Current land use and cultural traditions

The broad-sense heritability estimates of several reproductive and vegetative characters in *P. alpina* were on average twice as high for grazed than for mown parcels. These results are even more pronounced than the ones of our microsatellite study of *P. alpina* where the mean number of alleles was also higher in grazed parcels than in mown ones (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). The higher heritable genetic variation in grazed parcels is in line with the hypothesis that increased allocation to reproduction of plants in grazed parcels increases establishment of new genotypes, and thus genetic variation. Moreover, higher allocation to reproduction may also increase gene flow. Because for topographical reasons grazed parcels are somewhat more likely to be situated next to other grazed parcels than to mown parcels, this can also contribute to higher genetic variation within grazed parcels. Higher gene flow among grazed parcels with *P. alpina* was indeed indicated by the lower microsatellite differentiation between grazed than between mown parcels with *P. alpina* (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). An alternative explanation of reduced heritable genetic variation in mown sites could be more spatially homogeneous selection by mowing than by grazing animals. Accordingly, intensive mowing reduced molecular genetic diversity in *Festuca pratensis* compared to natural populations (Kölliker et al., 1998).

The observed higher heritable genetic variation of *P. alpina* for grassland parcels from Romanic regions indicates persisting effects of old cultural traditions on current biodiversity, in this case intraspecific diversity. Most likely this is due to the higher land use diversity especially in the valleys of Romanic villages (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data). We had shown earlier that *P. alpina* is genetically differentiated between parcels of different land use (A. Weyand, K. Maurer, J. Stöcklin, M. Fischer, unpublished data). Therefore, gene flow between parcels with *P. alpina* of different land use is likely to increase heritable genetic variation within parcels. The occurrence of such gene flow is the more likely the more different types of land use occur around a village. Most likely, the higher heritable genetic variation for parcels in Romanic villages is at least partly due to historical rather than to current gene flow, as it was more pronounced in the past, and will decrease in the near future, as far as ongoing land use changes are reducing land use differences between villages of different cultural traditions (Bätzing, 1991).

Land use turned out to be the most important driver of heritable genetic variation in *P. alpina*. This implies that the ongoing rapid land use change in the Alps, where many meadows are converted to pastures (Bätzing, 2003), will lead to increased heritable genetic variation within grassland parcels, at least of *P. alpina*. However, because higher land use diversity also increases quantitative genetic variation between sites, the conservation of quantitative genetic variation requires the conservation of different types of land use, and cannot be achieved by solely maintaining grazed parcels.

Other levels of biodiversity

Our results revealed weak relationships between heritable genetic variation and plant community diversity (Table 5). Thus, our study provides little support for the hypothesis, that plant community diversity could be positively related to heritable genetic variation, because more diverse niches are available in communities that are more diverse. Plant species diversity was weakly positively related with molecular genetic diversity in the forest herb *Trillium grandiflorum* (Vellend, 2004). In our study heritable genetic variation was marginally significantly positively related to molecular genetic diversity (Table 5). If this holds true also in *T. grandiflorum*, it may suggest that plant species diversity is also positively correlated with heritable genetic variation in *T. grandiflorum*. However, positive relationships between plant species diversity and molecular genetic variation were absent in *Ranunculus acris* (Odat et al., 2004) and present, but explained by confounding habitat characteristics, in *Plantago lanceolata* (N. Odat, Helwig, Jeschke, and M. Fischer, unpublished data). We suggest to study the relationship between plant species diversity and genetic diversity for more plant species and not only for molecular, but also for heritable, genetic variation.

The generally non-significant and negative relationships between the diversity of herbivores and pathogens and heritable variation in *P. alpina* (Table 5) appear to not support the hypothesis that a more diverse community of parasites may impose more heterogeneous selection than a less diverse one, and may thus maintain higher heritable variation in plants. However, such negative relationships were in general very weak, and the relationship between the diversity of fungal parasitism per parcel and heritable variation of half of the measured traits in *P. alpina* was non-significantly positive (Table 5). Therefore, the hypothesis should not be dismissed completely. We suggest clarifying these relationships more comprehensively by studying heritable genetic variation of not just one, but several plant species in relation to the diversity of the plant community, and the diversity of plant parasitism. For the latter, it will be interesting to compare the relationships between heritable genetic variation of plants and the diversity of parasites for two cases, first for all parasites in a grassland, and second, only for the parasites actually affecting the target species.

Conclusions

The observed broad-sense heritabilities suggest that a high potential for further adaptation and evolution has been maintained in *P. alpina* even after hundreds of years of selection by agricultural land use. Among the potential drivers of heritable genetic variation in *P. alpina*, land use was most important with heritable genetic variation halved in meadows compared with pastures. Because unfertilized meadows harbor the most species-rich plant communities (K. Maurer, A. Weyand, M. Fischer, J. Stöcklin, unpublished data), and because the higher land use diversity in Romanic villages was associated with higher species diversity and higher heritable genetic variation, we suggest maintaining diverse land use regimes to conserve biodiversity not only at the landscape and plant species level, but also at the level of heritable variation within species, at least of the widespread important fodder grass *Poa alpina*.

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Chapter 9

Summary and general discussion

This thesis studies natural and anthropogenic determinants of grassland biodiversity in the Swiss Alps at the levels of plant communities within a landscape (Chapter 2), species diversity within plant communities (Chapter 2 and 3), biological interactions between plants and herbivores and pathogens (Chapter 4), and diversity within a species (Chapter 5-8). Within-species diversity is studied in the widespread and agriculturally important grass species *Poa alpina* L.

Biodiversity includes several levels of biological integration (landscape, species within community, biological interactions, within species, Primack 2002). High biodiversity is of value from the ethical, esthetical, ecological and economic point of view. Accordingly, its importance has been acknowledged in the Convention on Biological Diversity of the United Nations (United Nations 1992).

The heterogeneous landscape of the Alps is characterized by natural, especially altitudinal, gradients (Theurillat et al. 2003). However, human land use also has been shaping these landscapes for hundreds of years including the clearing of forests which created grasslands of high biological value (Bätzing 2003). Nowadays, species richness of grasslands is three times higher than in the original forests (Zoller & Bischof 1980). Different land use types created a mosaic of diverse meadows and pastures contributing to the Alpine biodiversity. In the Swiss Alps three different cultural traditions (Romanic, Germanic/Alemannic, Walser) can be distinguished (Bätzing 2003). They differed in their agricultural practices and therefore are likely to have affected landscape diversity.

In the Swiss Alps ongoing socio-economically motivated land use changes have been taking place during the last decades. Easily accessible grasslands are used more intensively and those only returning a small yield are abandoned. These changes are likely to affect grassland biodiversity negatively. However, comprehensive studies investigating the effects of agricultural land use on different levels of grassland biodiversity are missing.

To assess the effects of natural and anthropogenic determinants of grassland biodiversity at different levels, we selected 216 grassland parcels at three altitudinal levels in 12 villages in the Swiss Alps. To account for potential regional differences due to settlement history, each of the three cultural traditions was represented by four villages. Each parcel was characterized by a combination of traditional land use (mowing or grazing), current land use (mowing, grazing, or abandonment), and fertilization (unfertilized or fertilized).

Landscape diversity

To assess landscape diversity we analyzed the occurrence of different land use combinations in the study villages and the effects of the diversity of combinations of land use and altitudinal levels on plant species richness occurring in all investigated parcels per village (Chapter 2).

At the valley bottom of Romanic villages, more different parcel types tended to occur than in those of Germanic and Walser villages, suggesting that socio-economic differences among cultural traditions still play a role in shaping landscape diversity. An accompanying socio-economic analysis showed that socio-economic differences among villages are still remarkably pronounced (Pfister 2004). Observed changes in land use comprised either grazing of formerly mown parcels or abandonment of formerly managed grasslands, which reduced the farmers' workload. At the village level, higher man-made landscape diversity, i.e. a higher number of combinations of land use and altitudinal level, was strongly correlated with higher species richness. This result was in line with the mosaic concept, which proposes higher species numbers in landscapes with higher habitat diversity (Duelli 1997).

Plant species diversity

To study the effects of altitude and agricultural land use on plant species richness (Chapter 2) and composition (Chapter 3), we performed two vegetation records in each of the 216 selected grassland parcels and calculated mean values of species numbers and cover per parcel. Additionally, we calculated mean indicator values (Landolt 1977) and percentage cover of the functional groups graminoids, forbs, and legumes per parcel.

Independent of land use and fertilization, plant species richness was highest at intermediate altitudes, probably due to overlapping species pools of lower and higher altitudes. Fertilization and abandonment both reduced species richness. Grazing slightly reduced species richness compared with mowing in unfertilized parcels, while in fertilized parcels it had a positive influence. The highest species richness was observed in mown unfertilized grasslands. Species richness was higher in parcels with low standing crop revealing a conflict between productivity and biodiversity.

Plant species composition changed with increasing altitude when montane species were replaced by subalpine, and subalpine species were replaced by alpine species. In Germanic villages, species composition of parcels indicated moister conditions than in Romanic and Walser villages. Among the land use variables, fertilization and abandonment had the strongest effects on species composition and affected percentages cover of functional groups as well as indicator values. Grazing or abandonment of formerly unfertilized meadows reduced the frequency of their characteristic plant species with a stronger effect of abandonment. However, abandoned parcels also could serve as refuges for certain meadow species. Each land use combination had a different set of dominant species and therefore each of them contributed to landscape diversity.

Diversity of biological interactions

For the analysis of biological interactions between plants and herbivores and pathogens, we sampled 20 leaves of each of the functional groups graminoids, forbs, and legumes in each of 215 parcels (altogether 12’054 leaves) and analyzed the damaged area and diversity of types of herbivory and fungal pathogens (Chapter 4).

Herbivory and fungal pathogen infection were ubiquitous and present on 83 % of all leaves. However, the damaged leaf area was < 4 % indicating that plant-herbivore and plant-pathogen interactions contribute largely to community diversity without causing substantial yield reduction for farmers. Damage by herbivory was highest on legumes, and damage by fungal pathogens was highest on graminoids. More leaf damage by herbivory occurred in traditionally mown sites and at lower altitudes, while damage by fungal pathogen infection was independent of land use and altitude. Most types of herbivory were found on legumes and on leaves from fertilized sites, whereas the diversity of fungal pathogen types was highest on graminoids and in unfertilized sites. As our results did not reveal conservation conflicts between plants, herbivores, and fungal pathogens, we suggest conserving and maintaining a great diversity of plant-herbivore and plant-pathogen interactions by maintaining diverse low-intensity land use.

Diversity within *Poa alpina* L.

We studied within-species diversity in the Alpine meadow grass *Poa alpina* L. (Poaceae). The species is widespread in the Swiss Alps and occurs over a large altitudinal range. Due to its frequent occurrence in pastures and nutrient rich meadows and its high contents of fats and proteins it is among the agriculturally most important fodder grasses (Bachmann 1980; Conert 1998). The species can reproduce sexually or vegetatively producing bulbils on the mother plant. Genetically it constitutes a polyploid complex (Müntzing 1980) with highly variable chromosome numbers (Steiner & Heidenreich 1997). We collected plants in part of the 216 agriculturally used grasslands and additionally in natural sites. Based on a sub-sample of these plants we developed five polymorphic microsatellite markers (Maurer et al. 2005, Chapter 5). In plants from 54 agriculturally used parcels and 20 natural sites we assessed molecular genetic diversity using these microsatellite markers and correlated the number of alleles per plant with chromosome counts in root tips. As measures of genetic diversity we calculated the mean number of alleles per plant and allelic richness per parcel (Chapter 6). Furthermore, we analyzed the occurrence of *P. alpina* in the field among all investigated 216 parcels of grassland and conducted a common garden field experiment including two plants of each of 615 genotypes from 57 agriculturally used parcels and 21 natural sites (Chapter 7). We harvested reproductive and vegetative biomass over two years. One plant per genotype was clipped to simulate land use and one served as control. In 825 plants from 53 agriculturally used parcels we studied the determinants of broad-sense heritability of several reproductive and vegetative traits measured in the common garden experiment (Chapter 8).

Poa alpina most frequently occurred in fertilized and grazed parcels and at higher altitudes, in accordance with its preferences described in the literature (Conert 1998). Within villages, genetic diversity of *P. alpina* was enhanced by a higher diversity of land use types, corresponding with the result of a higher species richness in villages with higher land use diversity. Broad-sense heritability was higher among plants from parcels from Romanic villages than for those from Germanic and Walser villages. Possibly this was due to the higher land use diversity in valleys of Romanic villages.

In the common garden experiment, vegetative reproduction was higher in plants from higher altitudes, in line with the hypothesis of an adaptive advantage of vegetative reproduction in the harsher conditions at higher altitudes (Bauert 1993; Pluess & Stöcklin 2005; Weppler & Stöcklin 2005). At the same time, allelic richness per parcel increased at higher altitudes, indicating addition of alleles typical for vegetatively reproducing plants to the allelic richness of sexually reproducing populations. Allocation to reproduction decreased in plants from higher altitudes reflecting another adaptation to the conditions at high altitudes.

Fertilization neither affected molecular diversity among parcels, as only 0.02 % of the variation of presence and absence of alleles resided between fertilized and unfertilized grassland parcels, nor within-parcel molecular diversity or broad-sense heritability. However, the enhanced common garden yield of plants from fertilized parcels indicates that at least weak adaptation of *P. alpina* to fertilization has occurred.

In the common garden we could observe an increased allocation to reproductive biomass in plants from grazed parcels compared with those from natural sites and a decreased allocation to reproduction in plants from mown parcels. This result was in line with the hypothesis that recruitment is of higher adaptive value in pastures due to the higher availability of microsites for seedling establishment. Moreover, it could be a cause for the observed higher genetic diversity and broad-sense heritability in grazed parcels compared with mown ones. Alternative explanations therefore would be enhanced gene flow through exo- or endozoochorous seed transport by cattle in grazed parcels or stronger selection in mown parcels. The differences in allocation to reproduction indicate divergent selection in response to mowing and grazing. These differences were supported by a smaller genetic distance between pairs of parcels with the same land use than between pairs of parcels with different land use. 1.2 % of variation were between mown and grazed parcels.

The reproductive mode of plants was determined genetically to a large extent as 4.2 % of variation resided between parcels with seed-producing and parcels with bulbil-producing plants. Accordingly, almost no changes in reproductive mode between years or between the two plants of each genotype occurred in the common garden.

The hundreds of years of agricultural land use have left their marks in the genome of *P. alpina* as became apparent in the common garden experiment and the microsatellite study. As regards broad-sense heritabilities, land use was even the most important determinant. Our results suggest that maintaining high genetic diversity of *P. alpina* requires promoting high land use diversity.

Correlations between levels of biodiversity

Despite the large interest in biodiversity research, little is known about the relationship between the different levels of biodiversity. In parcels with a higher plant species richness we found a lower number of herbivory types per leaf and a trend towards more different herbivory types at the parcel-level, while there was no correlation with measures of genetic diversity of *Poa alpina* or broad-sense heritabilities. A higher broad-sense heritability was negatively correlated with diversity of biological interactions within parcels and marginally significantly positively correlated with allelic richness. These results suggest that – despite its wide use as such - plant species richness per study site does not constitute a comprehensive measure of biodiversity considering all levels of biological integration of plants. Furthermore, requirements of one level of biodiversity are not necessarily the same as for others. Moreover, biodiversity should not only be considered at smaller scales but also within a landscape.

Effects of cultural traditions and land use on levels of biodiversity

Cultural traditions affected the level of landscape diversity via the different occurrence of land use types. More different types of abandoned parcels occurred at the valley bottom of Romanic villages, which probably was also reflected by the higher broad-sense heritability. These differences are likely to be caused by differences in hereditary partitioning between the Romanic and Germanic culture with partitioning of parcels among all children of a family in Romanic villages and inheritance of the farm as a whole by a son in Germanic villages. In Walser villages, which are situated at higher altitudes, a higher pressure to use all area that could be used agriculturally probably reduced abandonment of land. However, Swiss agricultural policy rather promotes agricultural uniformity than taking cultural or regional differences into account and therefore remaining differences are likely to disappear within the next decades.

Fertilization strongly reduced species richness and caused changes in species composition. It promoted the diversity of herbivory types while it affected the diversity of fungal pathogens negatively and had no effect on diversity within *P. alpina*. Thus, from the conservational point of view, fertilization had mostly negative effects if there were any detectable. However, fertilized meadows are important in agriculture due to their higher yield compared with unfertilized parcels and therefore will be maintained. In contrast, the area of fertilized pastures with low species richness combined with lower yield should be kept as small as possible.

Abandonment also severely reduced plant species richness and changed species composition towards more common species and an increased percentage cover of graminoid species. However, abandoned grasslands also served as a refuge for some species. We recorded more types of fungal pathogen infection on graminoids in abandoned sites than in managed ones. In the common garden experiment plants from abandoned parcels produced more reproductive shoots than those from managed parcels indicating an adaptation to escape the competitive conditions in abandoned parcels (Fischer & van Kleunen 2001; van Kleunen *et al.* 2001). In the molecular study no plants from abandoned parcels were included. Observed effects of abandonment rather were negative for biodiversity at different levels. Nevertheless, abandoned parcels hosted plants from unfertilized meadows which otherwise were endangered, and they contributed to a high landscape diversity.

Mowing and grazing both contributed to a high species richness and diversity when parcels were unfertilized. As their species composition differed, the occurrence of both types enhanced landscape diversity although grazing of formerly unfertilized meadows reduced species richness of parcels and the frequency of species characteristic for unfertilized meadows. Traditional mowing promoted plant-herbivore interactions, while grazing enhanced genetic diversity of *P. alpina* and broad-sense heritability. *P. alpina* has undergone divergent selection in response to mowing and grazing, and thus plants from mown and grazed parcels are genetically different.

These results suggest that a high diversity of land use types in the landscape would promote biodiversity at the different levels most. Considering ongoing socio-economically motivated changes in land use, moderate grazing can be a valuable alternative to abandonment.

Swiss agricultural policy mostly works at the parcel level. However, to promote a high biodiversity, a high diversity of land use types within a landscape and also a more or less even distribution of land use types across all altitudinal levels are required. Therefore, financial incentives should rather be based on the farm or even village level. Otherwise, abandonment of grasslands at higher altitudes and intensified use at lower altitudes will go on with severe consequences for biodiversity. However, a diverse landscape should also comprise some abandoned parcels. Depending on local conditions abandoned grasslands would need to be kept free of bushes and trees because otherwise within a few years grassland would be replaced by forest.

Conclusion

With our comprehensive study across 12 villages we could demonstrate that human land use affects biodiversity at all levels and has largely shaped present biodiversity during the hundreds of years of agricultural activity. Additionally, we showed that cultural traditions still affect man-made landscape diversity. Still persisting socio-economic differences among cultural traditions are likely to be responsible for this cultural effect.

The reduction of labor for farmers by grazing of formerly unfertilized meadows or abandonment of pastures and meadows will reduce land use diversity, and the number of parcels of high biological value used at low intensity will decrease. Thus, biodiversity will decrease both between and within grassland parcels. Therefore, financial incentives are needed to stop the ongoing changes in agriculture in order not to risk losses in biodiversity. Financial incentives should promote high biodiversity within parcels of land, but as not all levels of biodiversity react in the same way a high landscape diversity at the village level is also necessary.

Unfertilized meadows are the most laborious type of grassland, and therefore they are most likely to get abandoned, especially when they are steep and at high altitudes and thereby not easily accessible. Due to their significance for plant species richness and composition, financial incentives for the conservation of these unfertilized meadows are especially important.

We showed that, in contrast to widespread reservations against grazing, particularly low-intensity grazing has several positive aspects at all levels of biodiversity, and from a biodiversity point of view, grazing is clearly preferable to abandonment of grasslands.

In summary, to protect biodiversity at all levels of biological integration, a highly diverse landscape has to be promoted. Therefore, financial incentives should aim at high land use diversity, which is likely to be most successfully promoted at the village level. A reduced diversity of land use types or even abandonment of whole regions will severely reduce biodiversity. At the same time, landscape attractiveness for tourists will be reduced and the remnants of cultural heritage in the Swiss Alps will be endangered. The conservation of all levels of biodiversity and of their cultural, esthetical, ecological, and economic value requires the persistence of the diverse landscapes of the Alps.

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