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Organic seed propagation of alpine species and their use in ecological restoration of ski runs in mountain regions

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Abbreviations

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BAL	Bundesanstalt für alpenländische Landwirtschaft
CAL	Calcium Acetate Lactate
CEC	Cation Exchange Capacity
C_{mic}	Microbial biomass C
C/N	Soil organic C-to-total-N
d.a.e.	Days after emergence
df	Degree of freedom
DMRT	Duncan's Multiple Range Test
EDTA	Ethylenediaminetetracetic acid
exchang.	exchangeable
extr.	extractable
F	Fischer's Variance ratio
FiBL	Forschungsinstitut für biologischen Landbau
GA_3	Gibberellic acid
GIS	Geographical Information System
hr	Hour
HSD	Honest Significant Difference
HLRL	Hessisches Landesamt für Regionalentwicklung und Landwirtschaft
HPLC	High Pressure Liquid Chromatography
ISTA	International Seed Testing Association
K_{EC}	Extractable part of microbial biomass C
K_{EN}	Extractable part of microbial biomass N
min	Minute
N_{mic}	Microbial biomass N
ÖNORM	Austrian Standard Institute
PCA	Principal Component Analysis

PSD	Pure Seed Definition
rel.	relevé
R_{max}	Maximum potential relative growth-rate
rpm	Revolutions per minute
SD	Standard Deviation
SE	Standard Error
Sig.	Significance
TSW	Thousand Seed Weight
VAM	Vesicular-arbuscular mycorrhiza
V	Volt
WHC	Water Holding Capacity

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Foreword

The whole work presented here deals with a possible approach to prevent and control soil erosion in mountain regions after the vegetation has been damaged or destroyed by natural or man-caused events.

Soil erosion is a natural process, defined as the detachment of individual particles from the soil mass and their transport by erosive agents such as running water and wind (Morgan, 1986). In mountain regions it acquires a particular relevancy. Slope steepness is there a common feature, and it increases the risk of gully erosion and landslides because mass movements are made easier by gravity.

Erosion has also anthropogenic concauses: man influences it in various ways, mainly by favouring the action of the erosive agents and increasing the consequent devastation (Zachar, 1982). This occurs through destruction or deterioration of natural vegetation, increasing and concentrating surface runoff or changing the quality of the soil. In particular, the construction of infrastructures for the mass tourism (i.e. roads, ski lifts, ski runs) has caused in the last four decades increasing erosion problems due to denuded or insufficiently revegetated areas. According to the estimates of Krautzer (1996c) about 5,000 ha of ski runs must be yearly restored. In parallel, the depopulation of mountain areas that is typical of some marginal regions of the Alps (Bätzing, 1991) indirectly promoted an increase of the erosion risk. Cessation of management of pastures and meadows is known to promote appearance of erosion niches. Non-mowed, large tussocks and small shrubs transmit the shear stress of the gliding snow to the turf, thus leading to disruptions of the turf cover (Newesely et al., 2000). Such fissures act as preferential ways for the erosive action of water (Schauer, 1975). Besides, it is a normal practice for farmers to amend small-sized erosion phenomena as soon as they appear. Where land care has gone out, interventions take place only when the erosion problem is already so urgent, that intensive measures are necessary.

An important measure to prevent or control erosion is the substitution or restoration of the vegetal cover on denuded areas. Above-ground vegetation intercepts raindrops, dissipating their kinetic energy and preventing the consequent splash erosion, increases the surface roughness thus reducing the speed of the surface runoff and favouring sediment deposition, filters soil particles out of runoff. Plants and their residues help to maintain soil porosity thereby increasing the degree of infiltration of water into the soil and, in turn, delaying the onset of surface runoff. Root systems mechanically bind or restrain soil particles (Zachar, 1982; Morgan, 1986; Gray, 1995). Furthermore, plants withdraw water to the soil through evapotranspiration, and therefore help maintaing it cohesive (Florineth, 1999).

The method most frequently used for revegetation of ski runs in mountain regions is seeding commercial seed mixtures containing lowland forage species, but such species, bred for agricultural purposes, repeatedly proved not to be adapted to such difficult conditions (climatic, edaphic). In contrast, encouraging results were obtained by using seed of alpine species for the restoration (see i.e. Florineth, 1988; Holaus, 1997; Krautzer, 1997b).

The present work focuses on two aspects of the use of indigenous species for the revegetation of ski runs in the Alps, each of them presented in a separated section: first, the seed propagation of indigenous, alpine species in an organic farming context, and, second, their use for restoration in mountain regions in combination with environment-friendly revegetation techniques, aiming to promote the spontaneous, natural processes of re-colonization. Some basic research about the growth of *Trifolium alpinum*, whose seed production was found to be extremely difficult, was carried out as well and occupies an own section in this dissertation.

Organic seed propagation of indigenous species

1.1 Introduction

The use of indigenous species for the restoration at high altitude implies the availability of adequate quantities of seed or plant material. Various approaches are possible to achieve this goal (Figure 1). The first important decision is whether to use plants or seed material. In the first case possible sources must be identified and seed or vegetative propagation material must be gained. Relatively small amounts of seeds are sufficient for the production of pot-grown plantlets, which can be later reintroduced at the restoration site. In the case of the vegetative propagation material, suitable techniques, such as the subdivision of grass tussocks in small parts (Markert, 1988; Grabherr and Hohengartner, 1989), or the so-called Single Ramet Cloning were shown to be effective for various grasses (Gasser, 1989; Urbanska et al., 1987; Urbanska, 1989) and for a less large number of forbs (Tschurr, 1988; Tschurr, 1992) and legumes (Hasler, 1992) so that large numbers of plantlets could be produced starting from a reasonable quantity of autochthonous plant material. As both techniques imply large use of manpower for the collection of the base material, for the horticultural cultivation of the plantlets and for their transport to and transplantation at the restoration site, they are very expensive (Fahlselt, 1988; Florineth, 1988) and have not found a large-scale diffusion in the practice, with the exception of non-profit, public institutions, such as universities (see the numerous experiments carried out by the high altitude revegetation group of the Geobotanical Institute of the ETH Zürich in Switzerland) or governmental agencies (Florineth, 1992). Nevertheless, for species with sporadic seed production, which propagate themselves mainly by clonal growth, this represents the only chance for their reintroduction (Urbanska, 1985; Florineth, 1988). As an advantage, cloned plants attain in the field precocious flowering and seed production (Urbanska, 1988; Urbanska, 1990). A further consequence is that cloned material represents a single or only few genotypes, so that the genetic variability of the original population is considerably reduced. Some authors (Urbanska et al., 1987; Urbanska, 1988) do not regard negatively this fact, as the cloned genotypes already proved to be successfully adapted to the environment, while others (Krautzer, 1997a; Spatz, 1997) advocate an as wide as possible genetic variability in the restoration material, in order to ensure that the unpredictable conditions of the high altitude environment are successfully met at least by some of the genotypes.

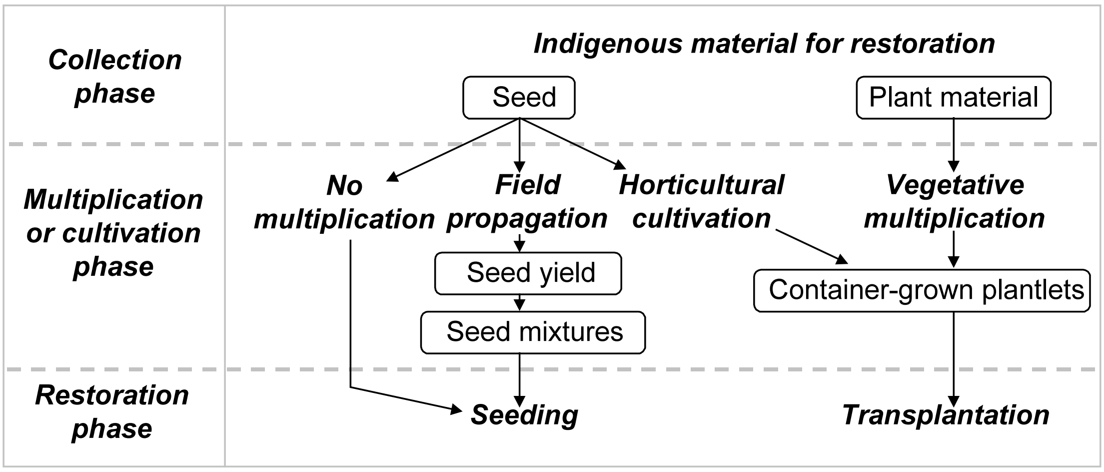

Figure 1: Possible strategies for the obtainment of native restoration material.

An alternative to the strategies described above is the re-introduction of autochthonous species at the restoration site in form of seed. In this case relatively large seed amounts are required, as seeding rates for revegetation vary usually between 100 and 200 kg/ha. Different approaches are possible in order to obtain the desired amount. The manual collection of seed is a quite labour-intensive task, which allows to obtain seed of single species. Vescovo (2000) achieved peaks of maximally 30 g of seed collected in one hour for a group of 15 alpine species, 8 of them having a productivity of less than 10 g/hr. Values up to 150 g/hr and 50 g/hr on average are reported respectively for small-sized grass and forbs seeds collected manually in American national parks (Majerus, 1996). It is intuitively conceivable that factors such as the seed size, the seed production per individual plant, the type of ripening (temporally concentrated or scalar), the plant density of the desired species, the time of the harvest and the terrain morphology of the collection site, play a big role in determining such a wide variation of the collection efficiency. Mechanical collection of seed by stripping (Mahler, 1990; Miller et al., 1996) or vacuum-harvesting (Majerus, 1996; Stevenson et al., 1997) in grassland with botanical composition resembling that which is aimed to has been reported. The advantage is represented by an increased collection productivity (Stevenson et al., 1997). The disadvantage is the obtainment of a seed mixture, whose composition can only to a limited extent be influenced by the collectors (Mahler, 1990; Stevenson et al., 1997). Moreover, the seed production at high altitude is aleatory, depending on the course of the harsh climate. Amount and quality of seed exhibit wide fluctuations from year to year (Marchand and Roach, 1980; Chambers, 1989).

A further approach, represented by the seed propagation of indigenous species in lowlands or in valley locations and their reintroduction at the restoration site in form of seed mixtures, constitutes the subject of the present work, and requires the obtainment of smaller amounts of native seeds. The whole process can be schematically resumed as follows

(Figure 2): in the first step ecotypes of the desired species are separately collected, usually by hand, in locations with ecological features similar to those of the sites to be restored. Then the small amounts obtained in this way are multiplied at lower altitudes in two or more steps. The seed is finally included in seed mixtures with a definite composition for the restoration.

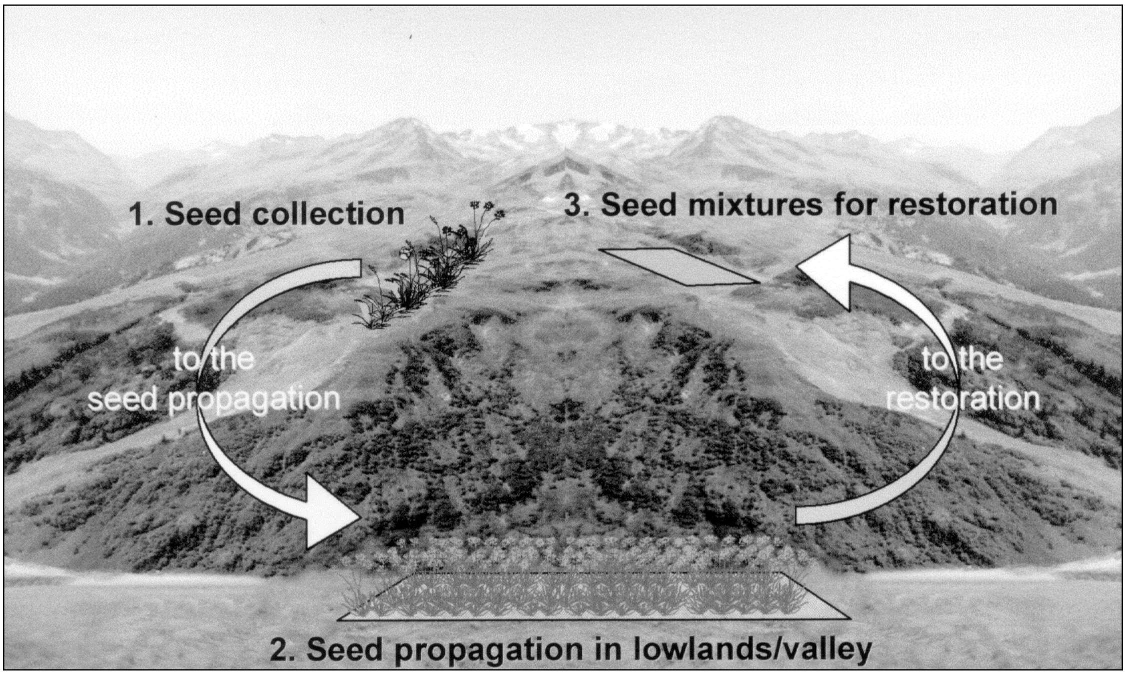

Figure 2: Scheme of the seed propagation of indigenous species for the restoration of eroded areas in mountain regions.

First attempts in Europe to produce seed of alpine plants date back to the beginning of the last century in Austria and were carried on up to the 40's (Krautzer, 1995a). They were resumed since the end of the 80's by Köck et al. (1989), Florineth (1992), Lichtenegger (1994a) and Krautzer (1995a), who made a first screening of a large number of species and found some of them being suitable for the seed propagation and having surprisingly high potentials in terms of seed yield and seed quality. More or less at the same time, a seed production of native species was developed also in North America (Riley et al., 1984; Carlson, 1986; Majerus, 1996).

This approach is based on some considerations about the advantages deriving from the propagation of the alpine species outside their natural areal of distribution and/or under environmental conditions strikingly differing from those of the sites where these species naturally occur.

In first instance, a general increase of the seed germination can often be observed after the seed propagation of alpine ecotypes (Carlson, 1986; Krautzer, 1995a). Seed dormancy is a frequent feature of alpine species, and represents an adaptive mechanism conferring a selective advantage to these species in the natural environment (Amen, 1966). Germination is initiated only under a restricted range of conditions, ensuring that the process of germination and establishment, during which the seedlings are extremely vulnerable to unfavourable climatic events, will take place only under environmental conditions enhancing the chances of a successful establishment (Schütz, 1988). Moreover, seed dormancy represents an efficient strategy for distributing the risks of the first developmental phase over a broad time period; only part of the seeds contribute to the population turnover at a given time, whereas the others remain as seed bank in the soil (Zuur-Isler, 1982). What can be considered an advantage in established plant communities represents instead a serious obstacle in the case of restoration attempts of surfaces devoid of vegetation. In this case it is very important to establish a vegetal cover as fast as possible following soil disturbance, in order to prevent erosion of the fine soil material by water runoff (Mosimann, 1981). Therefore a rapid seed germination, seedling emergence and establishment is desired. Moreover, rapid germination and high viability are particularly important, as the time period with temperatures suitable for seed germination and seedling establishment becomes shorter with increasing altitude (Chambers et al., 1987a). If dormant seeds are included in the seed mixtures, a higher seed rate is required to achieve the desired plant density and this increases in turn the costs for the restoration. This may reduce the acceptance of the use of autochthonous material in the practice, considering that the indigenous seed is generally very expensive. A further point is the faster development exhibited in general by the alpine species if grown at lower altitudes and if supplied with adequate nutrient levels. The time necessary to the plant to achieve the sexual maturity is in some cases reduced, allowing a seed production earlier than at the original locations. Alpine legumes were found to produce seed at low altitudes already in the second year (Krautzer, 1995a), while alpine legumes sown at high altitude entered the reproductive phase in the fourth growing season (Hasler, 1992). Last but not least, mechanisation and accessibility are generally easier for agricultural fields located in plain areas. Both factors bring about a decrease of the seed price.

A possible problem connected with the seed propagation is represented by the loss of intraspecific variability. At several stages of the seed production the genetic diversity of the base seed can be potentially reduced, partly because plants experience conditions very different from the natural regime in the wild, partly because of the techniques used to mechanise the seed production process. If seed dormancy is not broken, mainly plants obtained by non-dormant seeds may be represented in the multiplication plots, intraspecific competition in the seed multiplication field may select genotypes better adapted to favourable climatic and nutritional conditions disadvantaging those more tolerant of stress conditions, the harvesting process may fail to include in the yield early- and late-maturing individuals, the cleaning process may exclude seeds with unusually large or small size and weight (Majerus, 1996). Unfortunately, very little is known on a genetic basis about the consequences of these undesired selection. The only information available shows that the seed produced by the second generation of *Bromus marginatus* did not exhibit significant genotypic variation if compared to the wild seed used for starting the seed propagation (Mitchell-Olds, 1993, cit. in Majerus, 1996). The variability loss is far more enhanced if cultivars are bred, as in this case traits that are typical of crop plants are intentionally emphasized (Meyer, 1996). Moreover, homogeneity is one of the breeders' aims, as it is required for the registration of varieties (Krautzer, 1995a; Spatz, 1997). In an extreme case for example, a variety of *Poa alpina*, which reproduces often by means of apomixis (Müntzing, 1954; Müntzing and Müntzing, 1971), was developed starting from a single plant from a Canadian population (Darroch and Acharya, 1996). Little information is available about the performance of bred varieties of alpine species in revegetation attempts. Schiechtl (1980) generically reports better performances in the Alps of non-bred ecotypes of lowland species in comparison to registered varieties. On the basis of what exposed above it can be therefore inferred that attention should be paid to the preservation of the natural variability of ecotypes. Seed propagation should be preferred to plant breeding, and after maximally three generations new wild seed should be collected and the propagation started again.

At the present time, seed of about 15 alpine species, single or in seed mixtures, is available on the market at accessible prices (Krautzer et al., 2000).

A further aspect taken into consideration in the present work is the attempt to perform the seed propagation in the context of the organic farming.

Organic farming has considerably increased its market share in the last decade. Most of the alpine countries are among the European countries with the highest amount of organic farms. Lampkin (1998) estimated that an overall area of more than 1,000,000 ha was cultivated 1997 in the alpine countries (Austria, France, Germany, Italy and Switzerland) according to the EU-decrees 2092/91 and 2078/92. This area has further increased in the following years and in 2001 achieved a value of 2,670,664 ha (FiBL, 2002). The seed propagation of indigenous species could represent an integration of the income of organic farmers. Moreover, according to the EU-decree 2092/91, starting from 2004, only the use of organically produced seed will be permitted in organic farms. Seed of species suitable for revegetation, pasture establishment and improvement under relatively harsh climate will be required. It has therefore been considered interesting to verify the possibility to propagate seed of alpine plants in the context of the organic farming.

The slow growth rate of the alpine grasses and forbs, their subsequently low competitive capacity and their susceptibility to fungal diseases (Krautzer, 1995b) are thought to make difficult an organic seed production of such species. Krautzer (1995b; 1997a) already pointed out that application of herbicides and fungicides is indispensable for the seed production of alpine species.

The possibility of a seed propagation of alpine species according to the prescriptions of the organic farming has remained until today unexplored.

In the present work three species were singularly investigated: two slow-growing species (*Trifolium alpinum* and *Sesleria albicans*) and one currently propagated species (*Festuca nigrescens*). Aspects of the seed quality before and after the seed propagation and the suitability of techniques for the mechanical weed control and for the harvest were tested. An evaluation of the potential seed production was performed as well.

1.2 Materials and methods

1.2.1 Location of the experimental site

Small scale seed propagation plots were established at the research station Hebenshausen (Figure 3) of the Faculty of Ecological Agricultural Sciences of the University of Kassel. The field trials were located at the Meierbreite (geographical position 51° 23' 25'' N, 9° 54' 70'' E) at about 220 m a.s.l. in the Leine-Ilme depression, about 6 km from Witzenhausen, in the administrative district Werra-Meißner (Hesse, Germany).

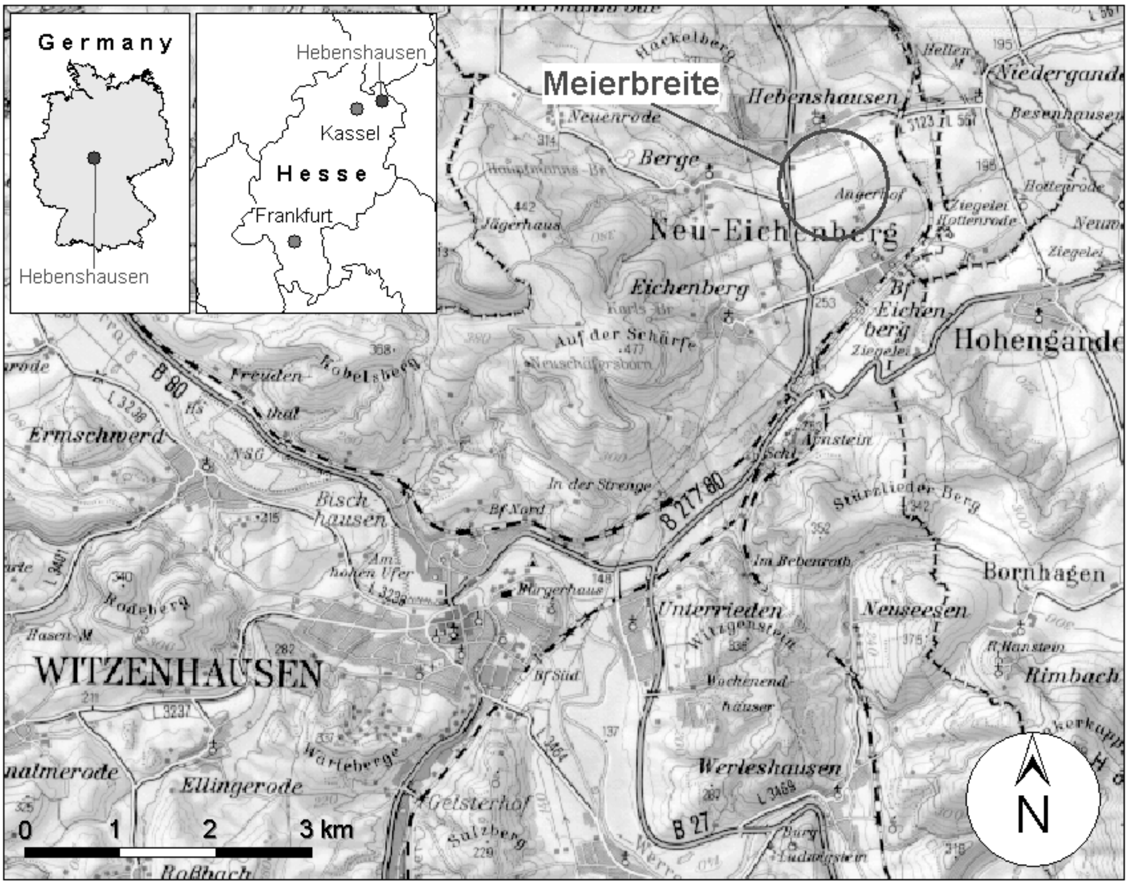

Figure 3: Location of the experimental site of the University of Kassel in Hebenshausen (Hesse, D) at the Meierbreite.

1.2.2 Geology and soil

Loess-loam areas on Trias mother rock sloping down from the margins of the drainage basin to the floodplain are typical of the Leine-Ilme depression. On these substrates luvisols have arisen. Stagnant water appears often in loam-rich subsoils without outflow located in depressions. The soils in the Leine-floodplain have developed into floodplain-loess (Hinze et al., 1979), while in the upper part of the Leine Keuper- and Trias-hills are more frequent (Klink, 1969).

The soil type is a superficially eroded Luvisol. The soil texture is a silt loam with high field capacity (Neuendorff, 1996) and, because of its soil texture, a moderate slaking tendency (Finnern et al., 1996).

Soil analysis carried out prior to the trial set up showed that the soil had a faintly acid to very slight basic pH (6.5 to 7.1 in water). The P_2O_5-content was 19 mg/100 g soil and the K_2O-content was 19 mg/100 g soil.

The area has been agriculturally managed for a long time.

1.2.3 Climate and meteorological survey

The area under investigation belongs to the climatic district "West Mid Germany", which is characterised by the subcontinental climate of Western Europe meeting the continental climate of Eastern Europe. According to the climatic classification of Köppen the investigation area can be assigned to the Cf class (humid-temperate climate with at least 30 mm rainfall in the driest month).

A synthesis of climatic data from the meteorological stations at the experimental site is available for the years 1990-1999. Year data sets are then individually available for the last decade, but they are not complete for all years. Temporary failures of the meteorological station run at the Meierbreite by the Department of Forage Production and Grassland Ecology caused sometimes data losses through time. These were therefore integrated, where possible, with measurements of other meteorological stations run at the same location by the Institute of Crop Science (precipitation data) and by the Department of Ecological Plant Protection (temperature data) of the University of Kassel.

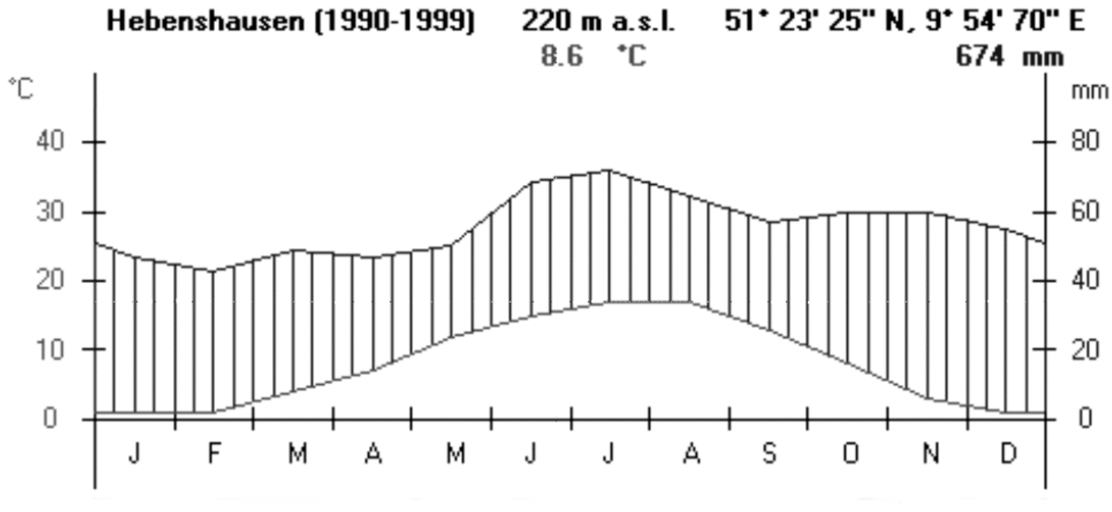

Figure 4: Precipitation and temperature (1990-1999) at the experimental site of the University of Kassel in Hebenshausen at the Meierbreite. Representation according to Walter (1955).

Long-term data (1990-1999) about temperature and precipitation are represented in Figure 4 according to Walter (1955). A yearly temperature of 8.6°C and 674 mm precipitation were assessed on average. The warmest month was July (17.5°C), the coldest January (1°C). The maximum precipitation occurred in July (72 mm), the minimum in February (43 mm). Dry periods are not shown by the climatic diagram

1990-1999, but are not uncommon in April or May (Neuendorff, 1996). The growing season has a duration of about 7 months.

A comparison of the data collected during the experimentation (2000-2002) with the climatic data 1990-1999 shows that the yearly temperatures were 0.6 to 1.1°C higher than the long-term mean and the monthly temperatures were in 21 out of 28 months above the average of the period 1990-1999.

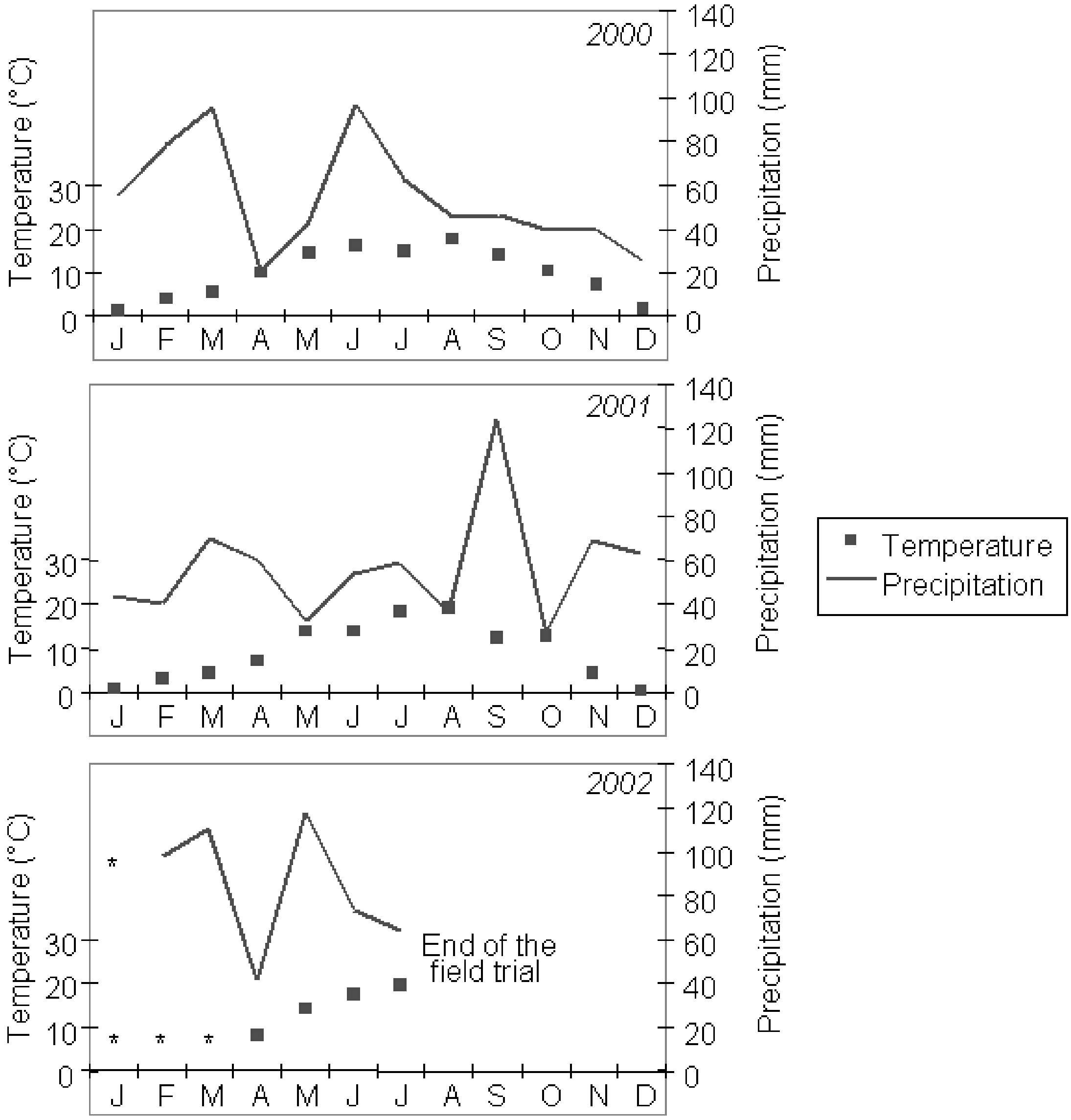

Figure 5: Monthly mean temperatures and precipitation sums at the experimental site in Hebenshausen during the investigation period.

Table 1: Monthly sums of rainy days (daily precipitation>1 mm) and mean relative humidity at the experimental site in Hebenshausen during the investigation period (2000-2002).

Month	Rainy days (No.)			Relative humidity (%)		
	2000	*2001*	*2002*	*2000*	*2001*	*2002*
January	8	12	*	84.8	89.0	*
February	17	6	15	80.9	79.1	*
March	17	13	12	81.9	84.9	*
April	5	12	5	72.4	77.5	74.0
May	8	6	12	70.9	71.0	79.2
June	7	12	8	71.5	76.2	75.4
July	13	8	9	81.7	71.1	77.4
August	6	9	*	73.0	71.4	*
September	8	20	*	83.2	86.8	*
October	10	5	*	84.6	85.2	*
November	6	15	*	*	89.7	*
December	5	13	*	*	89.8	*

* missing data

2000

The mean temperature for the whole year was 9.7°C. The first three months of 2000 were particularly wet, while April was characterised by a relatively warm and dry period. May was also warm and rainfall events were concentrated in the second half of the month. June was characterised by heavy rainfalls concentrated in short and intense precipitation occurring in few rainy days. The period after the trial set up (beginning of May) was therefore quite unfavourable for the germination and establishment of the crop plants, because of the scarce precipitation after the seed germination and because of soil erosion caused in the plots by the intense precipitation events in June. July was on the contrary quite cold and rainy with 13 rainy days. This fact caused difficulties in carrying out the necessary field works in due time, as the soil was too wet to bear the mechanical implements used for the mechanical weed control.

2001

The mean yearly temperature (9.3°C) and the precipitation (645 mm) resembled those of the previous year. Only short frost periods occurred in winter so that none of the months had a mean temperature below zero. March and April were quite wet, while in May was recorded the lowest precipitation amount. Dry periods were quite frequent during the growing season and were recorded in May, August and October. September was instead very rainy (124 mm) with copious precipitation distributed over the whole month.

2002

Because of data losses in the first months, data were available starting from mid February until the end of the field experimentation (July). The mean air temperatures were more or less consistent with those of the previous year, increasing gradually from April to July. It has to be pointed out that May was particularly precipitation-rich, achieving totally more than 100 mm rain throughout the month, with a relatively high frequency of rainy days (12).

1.2.4 Species investigated

Krautzer (1995b) suggests concentrating the propagation efforts on species that either show a wide ecological amplitude or are specialists for extreme climatic- or edaphic situations, in which most species are not able to establish. The present research project focuses on species meeting such requirements. The three species chosen have been studied in the past to different extents. The seed propagation of *Sesleria albicans* has been only partially explored (Krautzer, 1995a, Vescovo, 2000), while the only information source about the seed propagation of *Trifolium alpinum* consisted in the results of preliminary experiments carried out in 1998 and 1999 at the Meierbreite with transplanted, pot-grown plantlets, which indicated that it is a species difficult to propagate because of high mortality and scarce seed production (Rode, verb. comm.). On the contrary, *Festuca nigrescens* is one of the species, together with *Poa alpina*, whose conventionally produced seed is currently available on the market.

1.2.4.1 Trifolium alpinum

Trifolium alpinum is one of the few legumes that are able to establish themselves on acidic soils at high altitude. It is mainly distributed in the Western Alps in the sub-alpine and alpine zone at an altitude between 1,500 and 2,500 m a.s.l. in the Southern Alps (Pignatti, 1982a) and between 1,600 and 3,100 m in the Western and Central Alps (Schauer and Caspari, 1975). It can be found in pastures on acidic soils (pH between 3.5 and 5.5 according to Landolt, 1977) with very low lime content, minutely sandy soil texture, with low or very low nitrogen-content, and with a medium content of humus. *Trifolium alpinum* exhibits nutritional values similar to those of cultivated lowland clovers (Badino et al., 1989) and has a good attitude to soil consolidation thanks to its deep and ligneous taproot, which can grow more than 1 m deep (Flüeler, 1992; Oberdorfer, 1990). It is a characteristic species of the *Nardion*-alliance, but it can also be found in the *Caricion curvulae* (Oberdorfer, 1990). According to the ecological indexes of Ellenberg (1996) and Landolt (1977) it is an heliophilous plant, with little need for warmth, adapted to sub-oceanic to continental climates, with reduced soil moisture-requirements. The seed of this species is not currently available on the market. Pot plants horticulturally grown in plastic containers for at least three months are transplanted in patchy stands of previously restored ski runs in South Tyrol (Gallmetzer and Florineth, 1996).

1.2.4.2 Sesleria albicans

Sesleria albicans is a species showing a large ecological amplitude. It is distributed all over the calcareous Alps in the alpine and pre-alpine region. In the Alps it can be found mainly in the alpine and sub-alpine vegetation belt, at altitudes between 1,500 and 2,600 (3,100) m a.s.l., but can also occur in the colline vegetation belt as dealpine species starting from 100 m a.s.l. (Pignatti, 1982b). *Sesleria albicans* is a pre-glacial hygrophilous element with an adalpine character (Pignatti and Pignatti, 1975), typical of sub-oceanic and relatively continental climate. It can be considered drought resistant because of its xeromorphic features (Dixon, 1986) and it is not able to develop well in static waterlogged conditions, but it seems to tolerate temporarily such conditions, as the plants recover after cessation of the stagnant-water conditions (Dixon, 1996). It is an heliophilous species and can be found on superficial or moderately deep soils. They are often stony, with a moderate humus-content, with neutral to basic reaction. *Sesleria albicans* has a very low requirement for nitrogen and phosphorous (Dixon, 1982). It is a characteristic species of the *Sesleretalia*-order and a constant as well as dominant gregariously living species of the plant communities *Seslerio-Caricetum sempervirentis* and *Ranunculo hybridi-Caricetum sempervirentis* (its vicariant association in the southern part of the Alps, see Feoli Chiapella and Poldini, 1993 and Grabherr et al., 1993), where it plays a fundamental role in building up the characteristic steps-structure (Ellenberg, 1996). In the pre-alpine region it can be found also in dry or semi-dry chalk grasslands (*Festuco-Brometea*) and in xeric pine- (*Erico-Pinion*) and beech-woods *(Cephalantero-Fagenion*), mainly together with other alpine glacial relicts (Oberdorfer, 1990). *Sesleria albicans* is a consolidating plant of the calcareous screes, being able to bind the calcareous debris together and to dam the fine earth with its fine roots (Ellenberg, 1996). The roots are faintly branched in the basal part and can grow 40 cm deep in the soil, with a lateral extension up to 140 cm. Lateral roots are produced starting at a depth of about 10-12 cm (Blaschke, 1991b). The distal root segments are under natural conditions intensively colonised by vescicular-arbuscular mycorrhiza (Blaschke, 1991a). Roots of dead plants can remain in the soil up to 30 years, because the soil activity is strongly reduced (Kutschera and Lichtenegger, 1982). All roots arise from the shoot system as nodal axes and the root system shows a remarkable plasticity (Dixon, 1996). A commercially-produced seed of this species is not available yet on the market. Very small quantities are produced on request and are extremely expensive.

1.2.4.3 Festuca nigrescens

Festuca nigrescens is widespread across the whole Alps in the alpine and pre-alpine region at altitudes between 1,200 and 3,000 m (Pignatti, 1982b; Conert, 1998). It can be mainly found in moderately nutrients-rich and moderately acid pastures and meadows from the montane vegetation belt (*Cynosurion, Polygono-Trisetion)* to the subalpine and alpine belt, mainly in the alliances *Nardion strictae* and *Poion alpinae* (Rothmaler, 2002; Conert, 1998), but also in the calcicole plant community *Seslerio-Caricetum sempervirentis* (Peratoner, 1995; Dietl et al., 1998). It can also be found in light woods and clear cuts (Adler et al., 1994). It is an important forage species of the medium-intensively-managed mountain pastures at high altitudes and can be considered a soil-consolidating plant thanks to the intensive soil colonization achieved with numerous fine roots (Kutschera and Lichtenegger, 1982). *Festuca nigrescens* is hemi-heliophilous (Conert, 1998). The suitability of this species for the seed propagation has already been tested, adequate cultivation techniques have been optimised and a conventional seed production has been already established on the market (Lichtenegger, 1994a; Krautzer, 1995a; Krautzer, 1995b; Werder, 1996). Investigations about the seed propagation according to the prescriptions of the organic farming have not been reported in the literature examined.

1.2.5 Establishment of small-scale seed propagation plots

1.2.5.1 Seed provenance

Trifolium alpinum

About 500 g of seed were obtained in the autumn 1999 from the Sonderbetrieb für Bodenschutz Wildbach- und Lawinenverbauung of the Province of Bozen/Bolzano (South Tyrol, I). The seed was collected at the end of the summer 1999 in a *Carici curvulae-Nardetum* at an altitude of 2,100 m within the ski resort Meran 2000 (Hafling, South Tyrol, I), at the location St. Oswald.

Sesleria albicans

In the summer of 1999 the seed necessary for the establishment of the trial was collected in the Alps at five different locations (Table 2). Further seed of this species (460 g) was obtained from two German companies (Conrad Appel GmbH, Darmstadt, D and Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, D). The provenance was the same in both cases. The wild seed was collected in the Swabian Jura (Baden-Württemberg, D) and was then propagated at the Hohenloher Ebene (490 m a.s.l., Baden-Württenberg, D). The two seed lots differed in the harvest year, that of the company Conrad Appel having been harvested 1996, that obtained from the company Rieger-Hofmann three years later (1999). The seed of the provenance Schwäbische Alb I was therefore almost four years old at the time of the purchase.

About 580 g were totally available for the seed purity analysis and for the trial set up.

Table 2: Provenances of *Sesleria albicans* available for the establishment of the small-scale seed propagation trial.

Provenance	Location	Region, Country	Harvest year	Altitude (m a.s.l.)	Weight (g)
Kleine Traiten	Oberaudorf	Bavaria, D	1999	1,550	10.6
Große Traiten	Oberaudorf	Bavaria, D	1999	1,650	10.7
Monte Baldo	Trento	Trentino - Alto Adige, I	1999	2,000	8.7
Kapal	St. Anton am Arlberg	Tyrol, A	1999	2,300	5.0
Karwendel	Mittelfeld	Bavaria, D	1999	2,350	38.5
Monte Cavallo	Pordenone	Friuli - Venezia Giulia, I	1999	1,650	19.5
Schwäbische Alb I*	Swabian Jura	Baden-Württenberg, D	1996	600	60.0
Schwäbische Alb II*	Swabian Jura	Baden-Württenberg, D	1999	600	400.0

* seed already propagated

Festuca nigrescens

The seed employed for the field trial was gained from a previous conventional seed propagation harvested at the Meierbreite in the summer 1999. The wild seed had been obtained from the Sonderbetrieb für Bodenschutz Wildbach- und Lawinenverbauung of the Province of Bozen/Bolzano (South Tyrol, I) and had been collected in the Winschgau (South Tyrol, I).

1.2.5.2 Harvest, conditioning and storage

The seed of *Trifolium alpinum* was collected and cleaned by hand, dried at room temperature and stored for about four months at 6°C until the trial set up.

The panicles of *Sesleria albicans* were collected by hand, dried first on paper at room temperature for about two weeks and subsequently further oven-dried at 30°C for 36 hrs. The seeds were extracted and separated from the panicles using a laboratory thresher with a fractionating aspirator (Allesdrescher mit Windsichter K21, Baumann Saatzuchbedarf, Waldenburg, D). The seed lots were then sieved and cleaned again with the fractionating aspirator. The provenances that were purchased had already been cleaned by the seed companies prior to selling.

The seed of *Festuca nigrescens* was combine-harvested and dried in cotton bags on an air drier at room temperature for about four days. The seeds lots were then sieved and cleaned with an air-screen cleaner.

All seed lots were stored at 4-6°C for about 4 months until analysis and trial set up.

1.2.5.3 *Purity analysis*

Purity analysis was performed according to the prescriptions of ISTA (1996). The Pure Seed Definition (PSD) No. 10 (genus *Trifolium*) and the PSD No. 33 (genus *Festuca*) were adopted respectively for *Trifolium alpinum* and for *Festuca nigrescens*. The genus *Sesleria* is not included in the list of the pure seed definition numbers by genus. Thus *Sesleria albicans* was analysed in conformity with the pure seed definition of a genus with similar florets anatomy (*Cynosurus*, *Trisetum*) and the PSD No. 28 was used.

Working samples of different size were investigated: 6 g for *Trifolium alpinum*, 1 g for *Sesleria albicans* and 3 g for *Festuca nigrescens*.

Seeds and impurities were individually examined and assigned to one of the three possible fractions (pure seed, other seeds, inert matter). Samples of *Sesleria albicans* and of *Festuca nigrescens* were scrutinized with the aid of reflected light, in order to distinguish filled seeds from empty florets.

For the provenances Große Traiten and Kleine Traiten of *Sesleria albicans* particular rules were adopted because of the high amount of sclerotia of ergot of rye found in the samples: when under reflected light it was not evident, whether caryopsis or sclerotia were included in the floret, palea and lemma were removed. If a caryopsis was found, their were weighed together with the pure seed fraction, in order not to alter the results of the purity analysis. It has to be taken into account that the removal of palea and lemma may lead in *Sesleria albicans* to a germination increase (Fossati 1980; Schütz, 1988), but preliminary tests showed that purity analysis without this kind of supplementary inspection led to substantial errors. Sclerotia wrongly regarded as seeds resulted in estimations of the inert matter fraction which were 50% lower than the values obtained with the additional check.

1.2.5.4 *Seed weight*

Seed weight assessment was performed on the pure seed fraction according to the prescriptions of ISTA (1996). Eight replicates of 100 seeds each were weighed.

1.2.5.5 Seed germination in laboratory

All germination tests were performed on the pure seed fraction. The seeds were placed for each treatment on filter paper circles (9 cm of diameter, mod. 595, Schleicher & Schuell GmbH, Dassel, D) in glass petri dishes (10 cm of diameter). Two ml of distilled water or, if planned, of another medium, were supplied at the beginning of the experiment. Samples were randomly placed in a growth chamber (mod. VEPHL 5/2000, Heraeus-Vötsch, Balingen, D). A temperature regime of 20°C (day, 12 hrs) and 10°C (night, 12 hrs) was used for *Trifolium alpinum* and *Sesleria albicans*, while a constant temperature regime of 20°C (12 hrs light) was adopted for *Festuca nigrescens*. After three weeks, if necessary, the substrate was maintained wet by adding distilled water. Seeds were considered germinated when the radicle pierced the seed coat and became visible. Seedlings were evaluated according to ISTA (1979, 1996). The first count was performed 7 days after beginning of the experiment and later every 7 days. Testing time was 14 to 105 days for *Trifolium alpinum*, 35 days for *Sesleria albicans* and 28 days for *Festuca nigrescens*.

Trifolium alpinum is known to germinate very poorly, if not pre-treated, because of its innate dormancy due to the thick and impermeable seed coat (Weilenmann, 1981; Flüeler, 1992; Gallmetzer and Florineth, 1996). The germinative capacity was therefore tested also on mechanically scarified seeds. Two methods of the mechanical scarification were employed. One sample was gently rubbed between two layers of sandpaper, until small scratches on the seed coats appeared. In a further sample the testa was scarified under a binocular microscope with a razor blade. A control with a non pre-treated sample was also included in the experiment. Contrasting opinions are expressed in the literature about the light requirements for germination. Gallmetzer and Florineth (1996) reported *Trifolium alpinum* to germinate better in the dark, while according to Kinzel (cit. in Hegi, 1964) illumination promotes the seed germination. In a further experiment non-scarified seeds were therefore tested either in a 12-hours-regime or in the dark. All above-described experiment were performed in four replicates of 50 seeds each per treatment.

Vescovo et al. (1998) reported the seed germination of *Sesleria albicans* to be considerably increased by pre-chilling combined with gibberellic acid (GA_3) or with KNO_3, whereas other authors achieved high seed germination with untreated samples (Fossati, 1980; Schütz, 1988). In order to detect eventual dormancy in the seed collected, to choose which seed lots to use for the field experiments and to determine in turn the optimal seed rate, the effects of pre-chilling (storage of the seeds in contact with the moist substrate for 7 days at 4-6°C in the dark) and different mediums [distilled water, 0.2% KNO_3 (P 8291, Sigma-Aldrich Chemie GmbH, Deisenhofen, D), 0.005% gibberellic acid (G 7645, Sigma-Aldrich Chemie GmbH, Deisenhofen, D)] were evaluated in a two-factorial preliminary test. For each provenance, every possible combination of the factors was tested in two replications of 100 seeds each.

The mixtures of more provenances used for the field trials were investigated under standard conditions in four replicates of 50 seeds each.

The germination test of *Festuca nigrescens* was performed in four replicates of 50 seeds each.

1.2.5.6 Field-, seedbed preparation, experimental design and trial set up

Winter wheat was the preceding crop in the area selected in Hebenshausen at the Meierbreite for the experiments. The field was ploughed at the end of November 1999. Because of the satisfactory P- and K-contents detected by the soil analysis, fertilisers were not applied. The secondary tillage took place at the end of April 2000 by means of two consecutive tillages with a rotary harrow (false seeding). Two weeks later, after the emergence of the weeds, the field was harrowed again, in order to get rid of them. The seedbed was prepared immediately before sowing by harrowing and rolling the field.

All trials were established in small plots (Table 3) that were arranged in a two-factorial randomised complete block design (factor 1 = mechanical weed control; factor 2 = harvesting method) with three replicates (Figure 6). A row width of 24 cm was chosen, in order to meet the requirements of the devices to be used for the mechanical weed control. Such a row width had been already been proved to be optimal for *Festuca nigrescens* (Krautzer, 1995a).

Table 3: Details about the establishment of the small-scale seed propagation trials at the Meierbreite.

Trial	*Trifolium alpinum*	*Sesleria albicans I*	*Sesleria albicans II*	*Festuca nigrescens*
Sowing date	4.05.2000	5.05.2000	20.06.2000	9.05.2000
Plot length	3 m	3 m	3 m	5 m
Plot width	1.44 m	1.44 m	1.44 m	1.44 m
Cultivated area	155.52 m²	155.52 m²	155.52 m²	259.20 m²
Sowing technique	Seed stripes	Row drill	Row drill	Row drill
Rows number	6	6	6	6
Sowing depth	1.5 cm	1.5 cm	0.5 cm	1.5 cm
Row spacing	24 cm	24 cm	24 cm	24 cm
Seed rate	4.2 kg/ha	7.2 kg/ha	7.2 kg/ha	5 kg/ha

After the trial set up of *Trifolium alpinum* about 5 mm rainfall occurred in three days between the 8th and the 9th of May. Later on, no precipitation took place for about 10 days. In this period temperatures were relatively high and the soil dried very quickly. As *Trifolium alpinum* had already germinated, the plots of *Trifolium alpinum* and *Sesleria albicans* I were watered on the 10th, 13th, 15th and 18th of May, in order to prevent seedling mortality. Each time about 4 mm of water were supplied.

Trifolium alpinum

Two days before sowing, the seed to be used for the field trial was scarified with sandpaper as described above (see paragraph 1.2.5.5) and then delivered to a seed company (SUET Saat- und Erntetechnik GmbH, Eschwege, D) for its arrangement in seed stripes: the seeds were placed between two very thin paper stripes, whose width was 1 cm. The distance between the seeds in the row was 4 cm (20 seeds/m). Rows were marked out in the field with a seed drill (Amazone D4, Amazonen-Werke H. Dryer GmbH & Co., Hasbergen, D) and the seed stripes were afterwards manually placed in the drills and covered with soil.

In a preliminary propagation test of *Trifolium alpinum* it had been observed that *Rhizobium*-induced nodules were not present on the roots of plants already 1 year old, although successful achievement of the symbiosis had been observed by other authors in pot-grown plants inoculated with autochthonous soil (Weilenmann, 1981; Gallmetzer and Florineth, 1996). Therefore the seed stripes were inoculated with a Radicin® inoculum mix (Jost GmbH, Iserlohn, D) containing three different strains of *Rhizobium leguminosarum*, whose effectiveness had been tested by the producer for *Trifolium repens*, *Trifolium pratense*, *Trifolium hybridum*, *Medicago sativa, Medicago lupulina*, *Melilotus* sp., *Anthyllis vulneraria* and *Lotus corniculatus*. The peat containing the inoculum was rubbed against a sieve (1 mm mesh) and sifted through it. Then it was mixed thoroughly in tap water (9.5 g of peat per litre of water). The suspension was incubated overnight at room temperature. Before covering the seed stripes with soil, 17 ml of the suspension per metre of seed stripe were poured on them with a watering can.

On the 3rd and on the 10th of June two intense rainfall events (30 mm in one hour and 16 mm in 25 min respectively) occurred at the experimental site in Hebenshausen. The resulting water runoff caused soil erosion in hand-rogued plots. Especially in the plots of block 1, which is situated in the lowest part of the field, the water partially bared the roots of quite a large number of seedlings. In the plots strongly affected by erosion plants were earthed up in the day following the heavy rain.

Sesleria albicans

The seed was drilled with an Amazone D4 seed drill (Amazonen-Werke H. Dryer GmbH & Co., Hasbergen, D), that was equipped with special seed hoppers suitable for seeding small amount of seeds. A seeding depth of 1.5 cm, that is unusual for small-sized grass seeds, was adopted in expectation of a dry period in May, in order to reduce the risk of dry up of the seedling. After sowing, the seedbed was rolled, in order to ensure a good seed-soil contact. For this trial the seed of the provenances Schwäbische Alb I and II was used. About 460 g of it were available. After the trial set up, plots were watered as previously described for *Trifolium alpinum*.

Table 4: Weight composition of the seed mixture of different provenances used for the trial *Sesleria albicans* II

Provenance	Weight (g)	Weight (%)
Schwäbische Alb II	400.0	67.1
Schwäbische Alb I	60.0	10.0
Karwendel	38.5	14.6
Monte Cavallo	19.5	6.2
Monte Baldo	8.7	1.6
Kapal	5.0	0.3

As the emergence took a very long time to occur (see paragraph 1.3.1.2) and the density of the seedlings in the rows appeared to be unsatisfactory, a second field trial with a smaller seeding depth (0.5 cm) was started later on the 20th of June. A modified Hege 80 Plot drill machine (Hege Maschinen GmbH, Waldenburg, D) was used for sowing. A seed mixture of different provenances of *Sesleria albicans* was used (Table 4), because the remaining amount of the provenances Schwäbische Alb I and II was not sufficient. The provenances Große Traiten and Kleine Traiten were not used in the seed propagation trials because of the high amount of sclerotia of *Claviceps purpurea* detected in the inert matter fraction during the purity analysis.

Henceforth the two trials will be referred to as "*Sesleria albicans* I" and "*Sesleria albicans* II".

Festuca nigrescens

A seed rate of 5 kg/ha was used. The seed was sown with a seed drill (Amazone D4, Amazonen-Werke H. Dryer GmbH & Co., Hasbergen, D) and then the soil was rolled again, in order to ensure a good seed-soil contact.

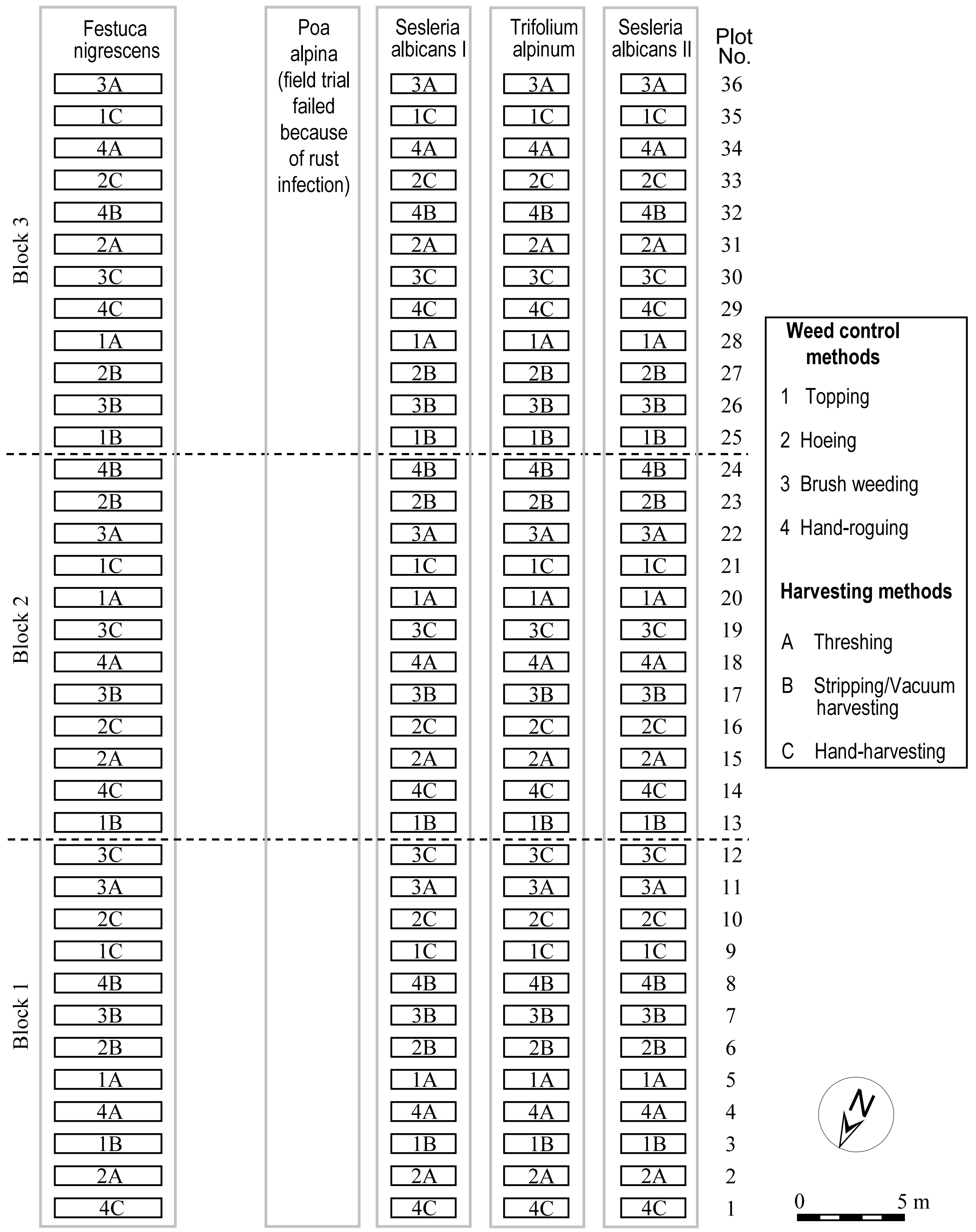

Figure 6: Experimental design of the small scale seed propagation trials of *Festuca nigrescens*, *Sesleria albicans* I *Trifolium alpinum* and *Sesleria albicans* II.

1.2.5.7 Emergence

The plots were examined every week and the crop plant was considered germinated when the rows became visible.

1.2.5.8 Maintenance and fertilisation of the plots

Every year all grasses were mowed near the ground at the end of the summer, in order to stimulate the tillering.

No kind of fertilisation was carried out for *Trifolium alpinum* and *Sesleria albicans*, while at the end of each growing season, 50 kg/ha of nitrogen were supplied to *Festuca nigrescens* in form of liquid manure.

1.2.6 Susceptibility of *Trifolium alpinum* to pathogens

Until mid of July 2000 *Trifolium alpinum* developed well, at least in plots where hand-roguing was performed. Then some plants which had shown a reduced growth rate until that moment became chlorotic. The symptoms appeared first on the oldest leaves, then the whole plants became yellow and died within about two weeks. Dead plants that were extracted from the soil presented necrotic areas at the tip of the roots, but insects could not be found. At the end of August the phenomenon became widespread in the whole trial and also well developed plants died rapidly, showing the above-described symptoms.

Some plants at the margins of the trial were dug up and their roots were examined under the binocular microscope, in order to look for possible damage caused by insects.

As a nutrient deficiency of nitrogen, sulphur, magnesium or potassium may produce similar symptoms, a quick test to exclude this hypothesis was applied. Four groups of 4 plants showing a light or medium symptomatic were marked, in order to make them retrievable, and their leaves were sprayed with one of the following 5%-solutions: urea, $(NH_4)_2SO_4$, $MgSO_4$, K_2SO_4. Pictures of each plant were taken. After two weeks the plants treated were inspected again and their status was compared to the initial one.

In the next step three groups (light symptoms, medium symptoms and heavy symptoms) of three plants were dug up in Hebenshausen together with the soil around the roots. The samples were divided in plants and soil and sent to HLRL (Hessisches Landesamt für Regionalentwicklung und Landwirtschaft - Pflanzenschutzdienst) for investigations on pathogens. Further plant- and soil samples were obtained from the location where the seed of *Trifolium alpinum* had been collected (Meran 2000, South Tyrol, I) and from another small propagation trial located on the mount Meißner (Hesse, D). They were prepared as described above and then sent to HLRL for analysis.

1.2.7 Investigation of mechanical weed control

1.2.7.1 Weed control methods

Four methods of the mechanical weed control were tested in the above-described field trials in combination with three harvesting methods. See details of the statistical design in Figure 6.

The weed control-treatments are defined as follows:

1. Topping: at the time of the first intervention, the biomass of weeds and of the cultivated plant was mowed and removed from the plots with a forage plot harvester (Haldrup, Løgstør, DK). Later on, when the cultivated plants were sufficiently developed, the biomass was mulched with a BVL Schlegel-Häcksler and left in place.
2. Hoeing: hoeing was performed with a mid-mounted Schmotzer hoe with goose foot shares (16 cm width) mounted on individual parallelogram linkages. Rotating discs between the shares protected the crop from being buried by the soil laterally shifted by the shares. Starting from the second weed control intervention, hoeing was preceded by topping, because the rows were otherwise not distinguishable.
3. Brush weeding: this is a method mainly used for vegetables or other high-value crops (Lampkin, 1990; Dierauer and Stöppler-Zimmer, 1994). Flexible brush rolls, consisting of cadmium-free polypropylene bristles, and rotating on a horizontal axis, work to a depth of about 5 cm and remove weeds between the rows (Geier and Vogtmann, 1988b; Kress, 1989). They are brushed out of the soil, complete with roots, and are left lying on the soil surface. Tunnels (8 cm width) protect the crop in the rows. Brush weeding was performed with a mid-mounted multiple brush hoe (model 500 mm, Bärtschi-FOBRO AG, Hüswil, CH). Starting from the second weed control intervention, brush-hoeing was preceded by topping, because the rows were otherwise not distinguishable.
4. Hand-roguing: an inter-row hoeing was coupled with a hand-weeding within the rows.

The mechanical weed control was performed for each treatment when weather and soil conditions made it possible to achieve optimal effectiveness. As different soil conditions are required for hoeing and brush weeding, up to one week elapsed between the different weed control-methods. Nests of *Cirsium arvense* were eradicated by pulling out the plants in all plots on mid July, regardless of the weed control-treatments, because the mechanical weed controls chosen for this experiment are known to be ineffective against this species (Dierauer and Stöppler-Zimmer, 1994).

See Figure 7 for the chronology of the interventions. Deviations from the above-described weed control treatments are described below.

Trifolium alpinum

In the first growing season, the inter-row hoeing in the hand-roguing variant was carried out manually.

Because of the extremely low number of plants still alive at the beginning of the second growing season it was impossible to carry on the investigations of the mechanical weed control. A manual weeding was instead performed around each single plant regardless of the treatment planned for each plot, in order to minimize competition effects of the surrounding weeds and get enough information to measure at least the individual seed production.

Sesleria albicans

In comparison to the small propagation trial of *Trifolium alpinum*, the mechanical weed control in *Sesleria albicans* presented an additional complication in its performance. *Sesleria albicans* required a long time for the emergence and the seedlings are very thin. Therefore it is quite difficult for the machine operator to see the rows from the tractor during the first weed control intervention. In *Sesleria albicans* I no particular countermeasure was taken, whereas in *Sesleria albicans* II the plants were marked with a string stretched along one row. The tractor carrying the hoe or the brush weeder was driven using the string as guide. Starting from the second intervention the rows were visible.

In *Sesleria albicans* I the sole hand-roguing variant could be performed on mid May 2001, while all other mechanical weed control methods could not be employed, in order not to damage the flowering plants. Later on, after the seed harvest in August, they were employed in *Sesleria albicans* II, while it was impossible in *Sesleria albicans* I because of the size achieved at that time by *Lolium perenne* and *Taraxacum officinale*. Their roots had already intensively colonised the soil and attempts of employing the mechanical implements (hoe, brush-weeder) resulted in great damage also to the crop plant, which was eradicated together with the roots of the grass weeds. Thus only the hand-roguing variant could be weeded. In the third year only the hand-roguing variant of *Sesleria albicans* I was manually weeded at the beginning of May.

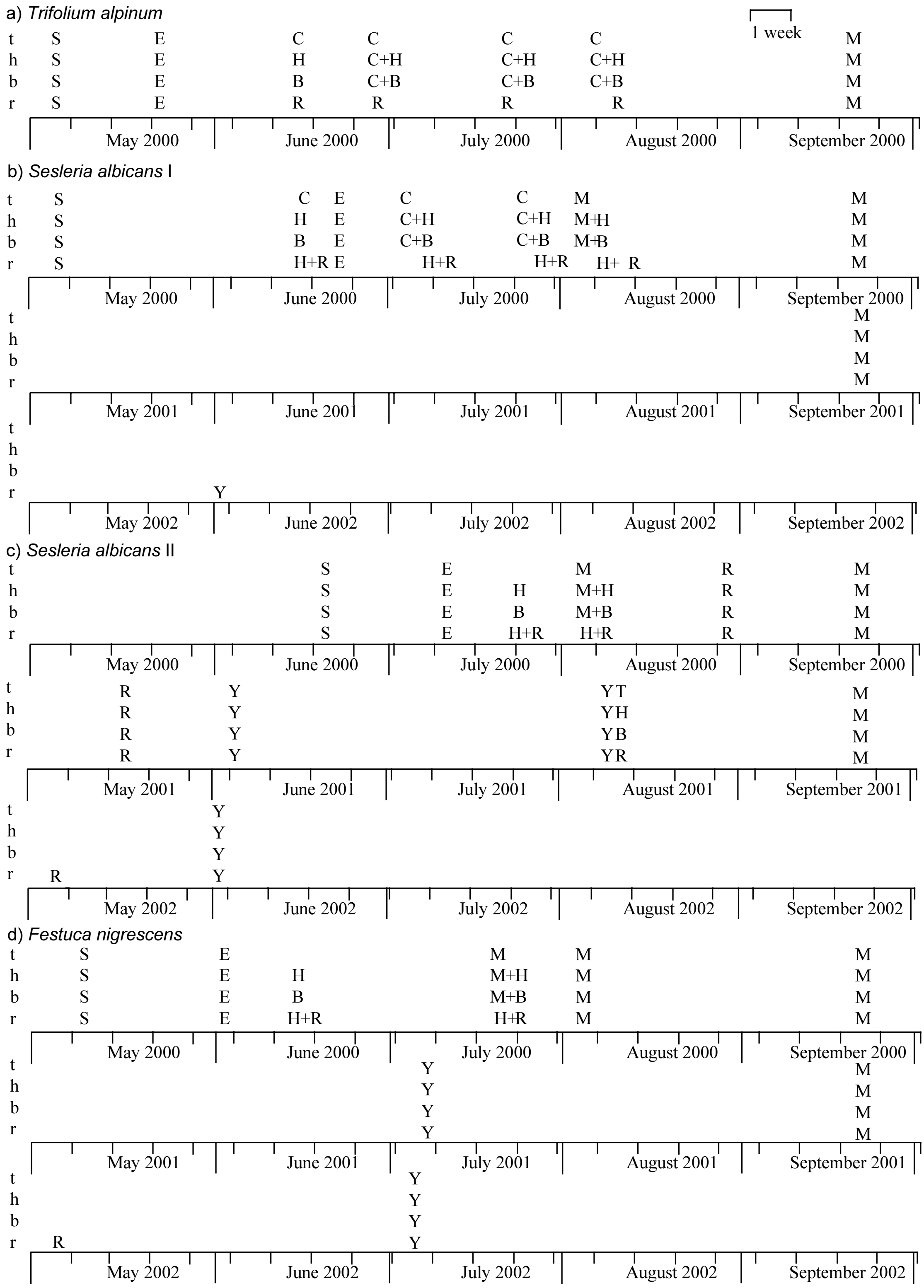

Figure 7: Time table of the field works carried out during the growing seasons 2000-2002 in a) *Trifolium alpinum*, b) *Sesleria albicans* I, c) *Sesleria albicans* II and d) *Festuca nigrescens*. Treatments (t=topping, h=hoeing, b=brush weeding, h=hand-roguing), important date of crop plant establishment (S=sowing, E=emergence) and field works (C=mowing and removal of biomass, M=mulching, B=brush weeding, H=hoeing, R=hand-roguing, Y=harvest) are indicated.

Very early it became evident in *Sesleria albicans* I that the crop plant was not able to withstand the competitive pressure of the weeds in the mechanised variants. Therefore it was decided to perform in *Sesleria albicans* II a manual weeding in all plots each time the weeds overgrown *Sesleria albicans*, in order to keep at least one trial relatively free from weeds and get the possibility to test adequately the harvesting methods. In addition to the planned weed control interventions, all plots in the *Sesleria albicans* II-trial were hand-rogued at the end of August 2000 and on mid May 2001.

1.2.7.2 Assessment of cover and density

Cover and density of the cultivated plant and of weeds were assessed using the so-called Göttinger Rahmen (Bartels et al., 1983), a count frame with an area of 0.1 m² used for the determination of the damage threshold of weeds in cereal cropping. Its original dimensions were modified for this experiment (22 x 45.5 cm), in order to fit along one row (Figure 8a).

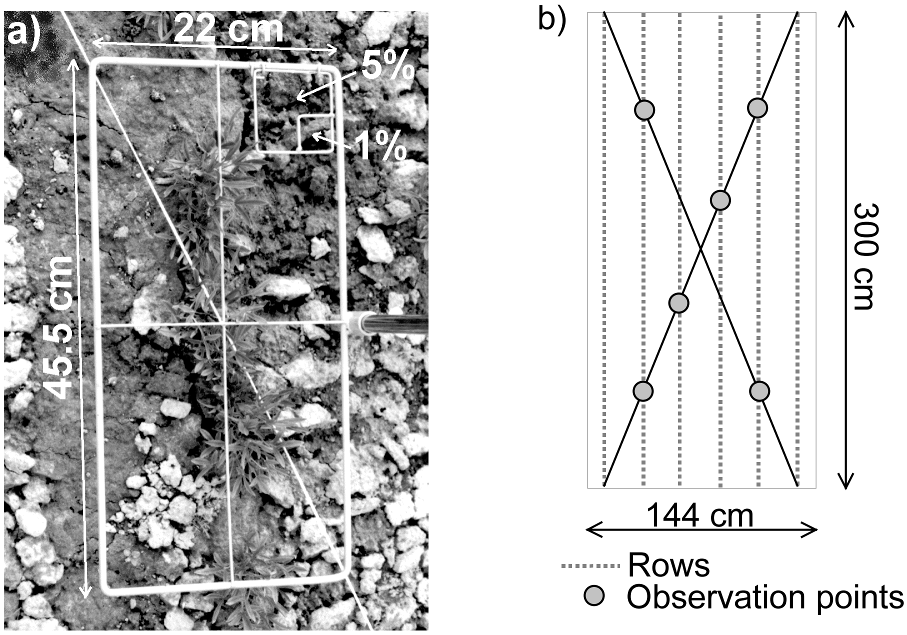

Figure 8: (a) The „Göttinger count frame“ used for the assessment of cover and density in the small propagation trials and (b) the scheme for the positioning of the count frame within the plots.

Cover was estimated by sight with the aid of reference areas of 1% and 5%. Density was defined as the number of plants rooting inside the count frame. In each plot six observation points were established, whose location was retrievable according to the intercepts between plot diagonals and crop rows (Figure 8b). For each plot the average of cover and density values was calculated and referred to the surface unit. The species nomenclature of Rothmaler (2002) was followed. Species with a mean cover ≥3% or with a mean density ≥5 plants/m² at the time of the assessments were defined as problem weeds.

Surveys of cover and density were not performed for the field trial of *Festuca nigrescens*.

Trifolium alpinum

In 2000 cover and density of the crop plant and of the weeds were assessed on the day before the second weed control intervention and again two weeks later.

In 2001 and in 2002 the crop plant density was too low and the plant distribution too patchy to employ the Göttinger count frame. Density counts of the crop plants were therefore carried out within the whole plots before harvesting.

Sesleria albicans

In 2000 in *Sesleria albicans* I the assessments took place on the day before the second weed control and two weeks later. In *Sesleria albicans* II they were performed on the day before the first weed control and two weeks later. Topping could not be carried out at that time, because weeds were still in the initial development stage and a premature mowing could result in copious ramification of some species (i.e. *Matricaria recutita*). Results about this treatment have therefore to be interpreted as a control.

In 2001 only the cover of weeds was assessed, because accurate density counts of most grasses weeds were impeded by strong tillering. For the crop plant both density and cover were surveyed.

In 2002 the cover of *Sesleria albicans* and of the weeds was additionally assessed with the same method.

1.2.8 Investigation of harvesting methods

1.2.8.1 Phenology of the crop plants and choice of the harvest date

In 2000 only few plants of *Trifolium alpinum* flowered at the end of August, but they did not produce any ripe seeds. In 2001 flowering took place quite homogeneously by the middle of May. The seed ripeness was also homogeneously attained at the beginning of July (6^{th} of July) and at this time the plants were manually harvested. In 2002 none of the plants still alive flowered.

A concentrated flowering and seed ripening of *Sesleria albicans* is known to occur under natural condition at high altitude (Vescovo, 2000). In more favourable climatic conditions, as observed in Hebenshausen, a more or less continuous flowering took place throughout the whole growing season. Anthesis occurred starting from the beginning to mid March. Seed ripeness was attained starting from the end of May-beginning of June, in accordance to what observed by Krautzer (1995a). In order to investigate the presence of correlation between the size of the tussocks and the occurrence of floral stems, before the harvest in June 2001 200 plants in each trial were randomly selected and tussock diameter and number of floral stems were assessed. In 2001 two harvests were performed, on the 6th of June and on the 10th of August. In 2002 just one harvest on the 3rd of June was made, because *Claviceps purpurea*-infections caused such a deterioration of the seed quality in the first year that a second harvest seemed not to be advisable.

Festuca nigrescens flowered at the beginning of June in both the second and the third growing season, and attained homogeneously the seed ripeness at the beginning of July.

1.2.8.2 Harvesting methods

Ripe seeds of *Trifolium alpinum* were harvested by hand detaching the pods from the mother plant by gentle sweeping. Due to the extremely low plant density, it was technically impossible to employ other mechanised harvesting methods.

In *Sesleria albicans* the harvesting methods are defined as follows:

A) Threshing: the planned harvest with a combine harvester was not possible because of the small size of the plots and the poor seed production, with a subsequently low biomass to harvest, resulting in high seed losses. The plots were instead mowed with a Haldrup plot harvester, harvesting the seed together with the foliar biomass, in order to include also spikelets with short or prostrated stems. The yield was immediately dried with an drier at room temperature for 4 days. Seeds and panicles were then separated from foliar biomass through shattering and sieving, and then threshed with a laboratory thresher (Allesdrescher mit Windsichter K21, Baumann Saatzuchbedarf, Waldenburg, D).

B) Stripping/vacuum harvesting: the harvest was performed using a prototype (GE-1700) developed at the Department of Forage Production and Grassland Ecology of the University of Kassel (Linsel and Scheidemann, 2000). Briefly, ripe seeds are loosen from the mother plants by flexible plastic brushes rotating on a horizontal axis and are then vacuumed into tubes driving them to containers collecting the seed. An air flux of about 70 l/s is generated by the suction system.

C) Hand-harvesting: the panicles were collected manually using scissors and then threshed with a laboratory thresher with a fractionating aspirator (Allesdrescher mit Windsichter K21, Baumann Saatzuchbedarf, Waldenburg, D).

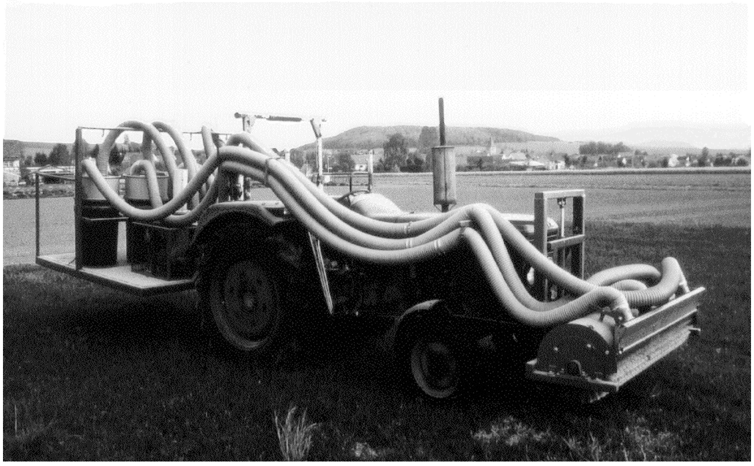

Figure 9: Stripping/vacuum harvesting device (prototype GE-1700). Photo courtesy of T. Fricke.

In *Festuca nigrescens* the harvesting methods were defined as follows:

A) Combine harvesting: the plots were harvested with a plot combine (mod. 125 C, Hege Maschinen GmbH, Waldenburg, D).

B) Stripping/vacuum harvesting: the harvest was performed with the above-described prototype.

C) Hand-harvesting: fertile culms of *Festuca nigrescens* were harvested with electric scissors (model Accu 6, Gardena Kress and Kastner GmbH, Ulm, D) and subsequently fed to the plot combine used for method A.

1.2.8.3 Conditioning and storage

The seed of *Trifolium alpinum* was dried at room temperature for two weeks and then cleaned in two steps. The seeds were first extracted and separated from the pods using a laboratory thresher with a fractionating aspirator (Allesdrescher mit Windsichter K21, Baumann Saatzuchbedarf, Waldenburg, D) and then rubbed against a sieve to get rid of pods residuals.

The seed of *Sesleria albicans* was dried in cotton bags on an air drier at room temperature for about four days. The seed lots were then sieved and cleaned with the fractionating aspirator.

The seed of *Festuca nigrescens* was sieved and cleaned with the fractionating aspirator. The air flux in the aspirator was set to the point that permitted to remove part of the inert matter and of the weeds, but without seed losses of the crop plant, in order to retain the possibility of evaluating the potential pure seed yield. This was achieved running small seed samples (about 30 g) of the presumably least pure seed yield (topped, combine-harvested plots) through the aspirator and examining the resulting fractions. Successive adjustments were performed until a satisfactory result was achieved. The same settings were used in the two harvest years.

The cleaned seed of all species was stored for about 4 months at 4-6°C in the dark until analysis.

In all trials results are affected by margin effects, as whole plots were harvested. For *Sesleria albicans* they can be considered not relevant because of the low plant density within the plots.

1.2.9 Investigation of seed quality

1.2.9.1 Purity analysis

Purity analyses were performed for each species according as described in paragraph 1.2.5.3, with the following modifications.

As extremely low seed amounts were obtained from the single plots of *Trifolium alpinum*, samples for the analysis were obtained pooling the seed yield of several plots.

In *Sesleria albicans* the share of sclerotia of *Claviceps purpurea* within the inert matter fraction were as well determined.

In *Festuca nigrescens* one sample weighing 1 g was investigated for each plot at each harvest.

1.2.9.2 Seed weight

The seed weight was determined as described in paragraph 1.2.5.4.

1.2.9.3 Seed germination in laboratory

The germination tests were performed for each species as described in paragraph 1.2.5.5.

For *Trifolium alpinum,* the germination tests were performed on scarified and untreated seed samples from the alpine collection site and from the propagation site. Scarification was performed by rubbing gently the seeds between two layers of sandpaper, until small scratches appeared on the seed coat. Germination experiments were carried out in four replicates of 50 seeds each per treatment. Besides, seed germination tests performed at the beginning and at the end of the observation period (2000-2002) on 2000-scarified and untreated samples of the alpine accession were compared, in order to assess eventual changes in seed quality through time. A test on a newly scarified sample of the same seed accession was also run at each time point, in order to check the untreated seeds for viability. A third experiment was also carried out, in order to assess the influence of the methods used for seed cleaning (threshing, rubbing the seed against a sieve) on seed germination: thirty-month-old seeds of the alpine provenance, collected and cleaned by hand, were run twice through the laboratory thresher. Part of the seed was then additionally rubbed against a sieve. Samples of these two treatments together with an intact seed sample as control were tested for germination.

Fossati (1980) observed a germination increase of seeds of *Sesleria albicans*, whose glumes had been removed, interpreting this treatment as a sort of scarification. The use of a laboratory thresher for seed cleaning (see paragraphs 1.2.5.2 and 1.2.8.3) was observed to loose quite a large amount of caryopsis from the glumes. Moreover, the purity analysis of the provenance Große Traiten required the removal of the glumes from most of the seeds scrutinized (see paragraph 1.2.5.3). A further experiment was therefore performed, in order to verify, whether the removal of the glumes had influenced the results of the germination tests. A sample of about 1000 seeds obtained in the first harvest year was separated in intact seeds (caryopsis+glumes) and in naked caryopsis. Four replications of 50 seeds per treatment were incubated under standard conditions (water as medium, no pre-chilling).

1.2.10 Statistical analysis

Fulfilment of the assumptions of analysis of variance was preliminarily checked. Normal distribution of residuals was tested with the Kolmogorov-Smirnov's test (Köhler et al., 1996). Variance homogeneity, according to the suggestions of Sachs (2002), was tested with the Cochran's test (Camussi et al., 1990) in case of lightly skewed distribution; in case of normal distribution, the Cochran's test was employed for less than ten samples, otherwise the Bartlett's test (Sokal and Rohlf, 1995) was performed; for distributions flatter or higher than the normal one, the Cochran's test was used up to ten samples, and the Levene's statistic (Sachs, 2002) for ten or more samples. As at least 10 observations per sample are recommended for the Levene's test (Sachs, 1990), where less observations were available the Cochran's test was performed instead. All above-mentioned tests were performed at the 10% level.

Cover percentages and density counts were generally transformed, if necessary, using respectively the arcsine transformation function and the logarithm function, in order to meet variance assumptions before submitting the data to variance analysis (Sokal and Rohlf, 1995). Fulfilment of the assumptions of analysis of variance was checked afterwards as above described. For the presentation of results means were back-transformed and are shown in the original scale.

In case of normal distribution, the comparison of two samples was performed with a two-tailed t-test (Sokal and Rohlf, 1995). For more than two samples, and in case the assumptions of the analysis of variance were met, analysis of variance (ANOVA) was performed. ANOVA and all below-described tests were carried out at $\alpha = 0.05$. If main effects or interactions were significant, multiple comparisons were performed at the highest interaction level with Tukey's Honest Significant Difference (HSD) or with Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. Correlation between variables was investigated with the Pearson's parametric correlation test after positive testing of data for normal distribution with the

Kolmogorov-Smirnov's test (Sokal and Rohlf, 1995).

At the time of the first weed control intervention, in *Sesleria albicans* II cover and density data of the crop plant and of weeds in dependence on the weed control methods were subjected to covariance analysis (ANCOVA). The cover and the density assessed before the weed control intervention were assumed as co-variables. Homogeneity of slopes of data after intervention on data before intervention was tested through ANOVA as preliminary step (Littell et al., 1991).

As measurements of seed yield and seed quality of the same plots of *Festuca nigrescens* were repeated in different years, a repeated measures ANOVA was performed, in which year was included in the analysis as within-subject factor, while weed control, harvesting method and block were regarded as between-subject factors.

If even after transformation data did not meet the requirements for performing an analysis of variance, non-parametric tests were carried out instead. The Mann-Whitney-U test was used to test differences between two groups within a variable, while the Kruskal-Wallis test was employed for more than two groups (Sokal and Rohlf, 1995). *A priori*-multiple comparisons were performed with the Mann-Whitney-U test, *a posteriori*-multiple comparisons with the Nemenyi-test (Köhler et al., 1996).

In case of a suspected non-linear, monotonic relationship between variables, correlation was tested using Spearman's correlation coefficient (Camussi et al., 1990).

ANCOVA was performed with the software SAS (release 6.12, SAS Institute Inc., 1996), all other analyses with the software SPSS (release 7.0, SPSS Inc., 1995).

1.3 Results

1.3.1 Trial establishment

1.3.1.1 Seed quality

Trifolium alpinum

The purity analysis of the base seed showed that the pure seed fraction was 100% of the working sample. Other seeds and inert matter were not found. The thousand seed weight (TSW) of the examined provenance of *Trifolium alpinum* was 4.97 ± 0.04 g. In a controlled environment the scarified seeds germinated very quickly, and 14 days after the beginning of the experiment their whole germination was achieved. The non-scarified control, on the contrary, germinated poorly (12% on average). No significant difference was found between the germination capacities of samples scarified with different methods (Figure 10a). Percentages of abnormal seedlings were also found not to be significantly different depending on the scarification methods (Figure 10b). Most of the abnormal seedlings had the cotyledons trapped inside the seed coat.

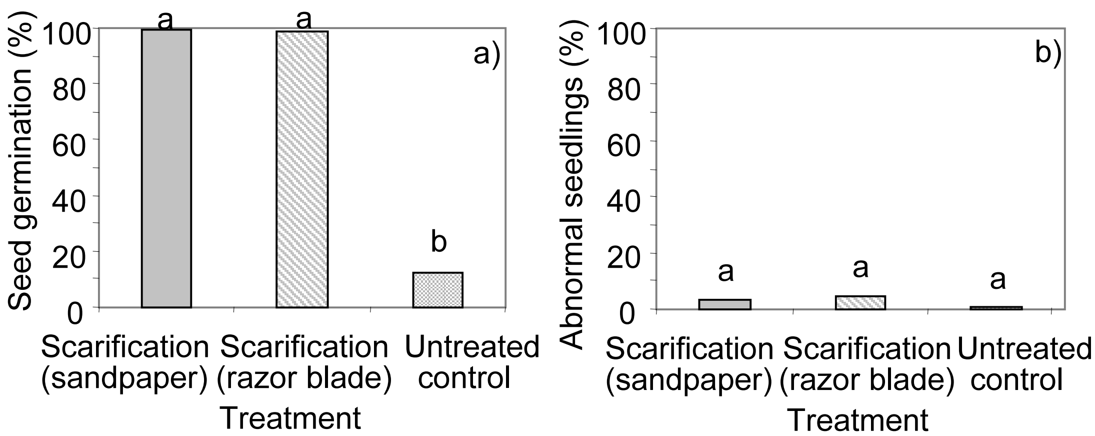

Figure 10: Influence of two different kinds of scarification a) on the seed germination of *Trifolium alpinum* and b) on the amount of abnormal seedlings. Average of four replicates per treatment. Analysis of variance was performed on arcsine-transformed data. Mean separation by Tukey´s HSD at α=0.05. Bars with the same letter are not significantly different.

The investigations about the light requirements for the optimal germination of *Trifolium alpinum* indicated that the seed germination was not significantly different in light or dark conditions at each observation date, although slightly higher value were achieved on average in the dark (Figure 11). Analysis of variance on arcsine-transformed data did not detect significant differences at any of the observation dates.

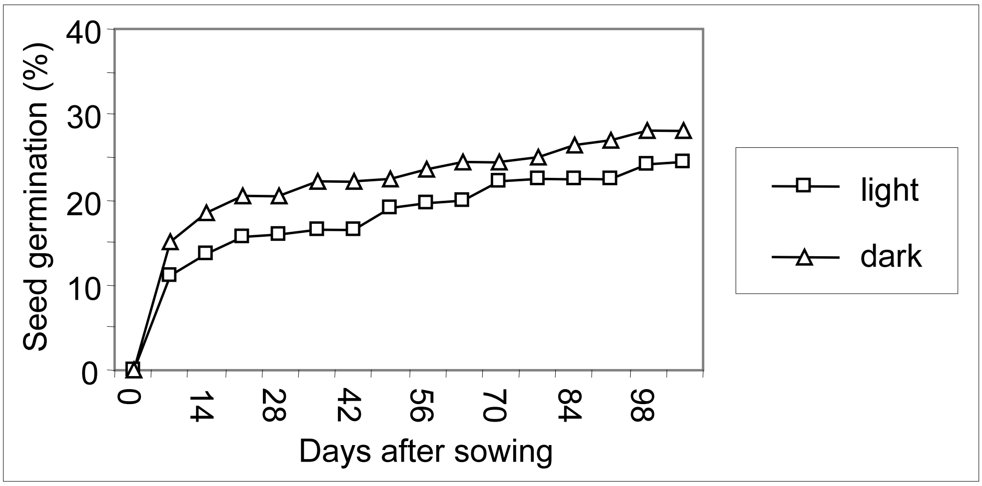

Figure 11: Germination of untreated seeds of *Trifolium alpinum* incubated either in the light or in the dark. Average of four replications of 50 seeds.

Sesleria albicans

The results of the purity analysis are summarised in Table 5. The pure seed fraction was in most cases about 80% or more. Large amounts of sclerotia of ergot of rye were found in the provenances Kleine Traiten and Große Traiten. Traces of other seeds were found only in the provenance Monte Baldo. A large variation of the seed weight was found in the samples analysed.

Table 5: Results of the purity analysis and seed weight assessment performed on various provenances of *Sesleria albicans*.

Provenance	Pure seed (%)	Inert matter (%)	Other seeds (%)	TSW ± SE (g)
Kleine Traiten*	50.2	49.8	0.0	0.90 ± 0.01
Große Traiten*	78.6	21.4	0.0	1.02 ± 0.02
Monte Baldo	79.5	20.3	traces of *Anthoxanthum alpinum*	0.90 ± 0.03
Kapal	80.6	19.4	0.0	1.07 ± 0.01
Karwendel	89.4	10.6	0.0	0.85 ± 0.01
Monte Cavallo	94.0	6.0	0.0	1.63 ± 0.02
Schwäbische Alb I	94.8	5.2	0.0	1.59 ± 0.02
Schwäbische Alb II	98.9	1.1	0.0	2.03 ± 0.02

* not used in the field trials

The provenance Schwäbische Alb II showed the highest values (2.03 g), more than twice the value obtained for the accession Karwendel (0.85 g). A difference in the TSW of the two seed lots of the provenance Schwäbische Alb was also observed, indicating a possible fluctuation depending on the harvest year. The seed weight was 1.97 g for the mixture used for the trial *Sesleria albicans* I and 1.62 g for that employed for the trial *Sesleria albicans* II. Despite of the different composition of the seed mixtures, the weight difference is relatively small because the provenance Schwäbische Alb was in both cases quantitatively predominant.

Concerning the investigation of the seed dormancy of *Sesleria albicans,* the results of the germination tests showed a relatively wide variation among provenances. Assuming the treatment with distilled water and without pre-chilling as standard germination method, the seed germination ranged from 46% to 87% (Table 6).

Table 6: Influence of pre-chilling and of different mediums on the seed germination of several provenances of *Sesleria albicans* 35 days after sowing. Average of two replicates of 100 seeds per treatment. Analysis of variance was performed on arcsine-transformed data. Mean separation was performed by DMRT at α=0.05 for the sole provenance where significant effects were detected (Monte Baldo). Means without common letters are significantly different.

Provenance	Seed germination (%)					
	Without pre-chilling			After pre-chilling		
	H_2O	KNO_3	GA_3	H_2O	KNO_3	GA_3
Große Traiten	75.0	71.1	75.8	71.9	67.1	67.9
Monte Baldo	46.5 [b]	33.7 [b]	74.2 [a]	39.6 [b]	31.3 [b]	32.1 [b]
Kapal	77.0	73.0	66.0	73.0	85.0	62.0
Karwendel	68.7	62.1	59.5	68.7	54.4	63.8
Monte Cavallo	87.2	88.9	85.4	85.4	86.0	83.5
Schwäbische Alb I	62.9	65.5	58.7	57.8	57.0	66.3
Schwäbische Alb II	74.2	68.7	72.7	73.5	73.5	68.7

Medium: H_2O=distilled water, KNO_3=0.2% potassium nitrate, GA_3=0.005% gibberellic acid

All accessions showed germination values over 60%, with the exception of Monte Baldo. The analysis of variance performed separately for each provenance did not detect any effect neither of the pre-chilling nor of the different mediums on the seed germination of the provenances investigated, with the exception of the accession Monte Baldo. Here a significant interaction of the main factors was found ($F_{2,6}$=9.474, $p<0.05$) and the treatment of the seed with gibberellic acid without pre-chilling increased considerably the seed germination (74.2% *vs.* 46.5% under standard conditions).The germinative capacity of the seed mixtures used in the field trials was 69% for the mixture *Sesleria albicans* I and 70% for the mixture *Sesleria albicans* II under standard conditions.

Festuca nigrescens

The purity analysis detected only very small amounts of other seeds (0.5%, mainly of *Alopecurus pratensis*) and of inert matter (1.2%) in the sample investigated, the pure seed fraction being satisfactory (97.8%). A TSW of 1.123 ± 0.004 g was assessed. A high seed germination (97%) was achieved under standard conditions.

1.3.1.2 Emergence

Large differences in the emergence speed were observed between species.

The first seedlings of *Trifolium alpinum* emerged already on the 11th of May, one week after the trial set up, while on the 22nd of May the rows became visible.

In *Sesleria albicans* I the first seedlings emerged on the 2nd of June, four weeks after the trial set up, but were silted up during an intense precipitation on the 10th of June. The rows became then visible on the 23rd of June. The emergence phase took also seven weeks. The first seedlings of *Sesleria albicans* II were noticed on the 4th of July, while the rows became visible on the 11th of July, three weeks after sowing.

The rows of *Festuca nigrescens* became visible on the 2nd of June. The emergence phase took also three weeks.

1.3.2 Mechanical weed control

1.3.2.1 Trifolium alpinum

About two months after emergence very low cover scores (below 3%) were assessed in all treatments of *Trifolium alpinum*, despite of relatively high plant densities. During the two weeks between the mechanical weed control and the second assessment, *Trifolium alpinum* had quite a small cover increase (from 1.6% to 2.8% in the manually weeded variant). The cover in the hoeing- and brush weeding-treatments decreased slightly and did not significantly differ from each other. An analogous trend was shown by the density data (Figure 12).

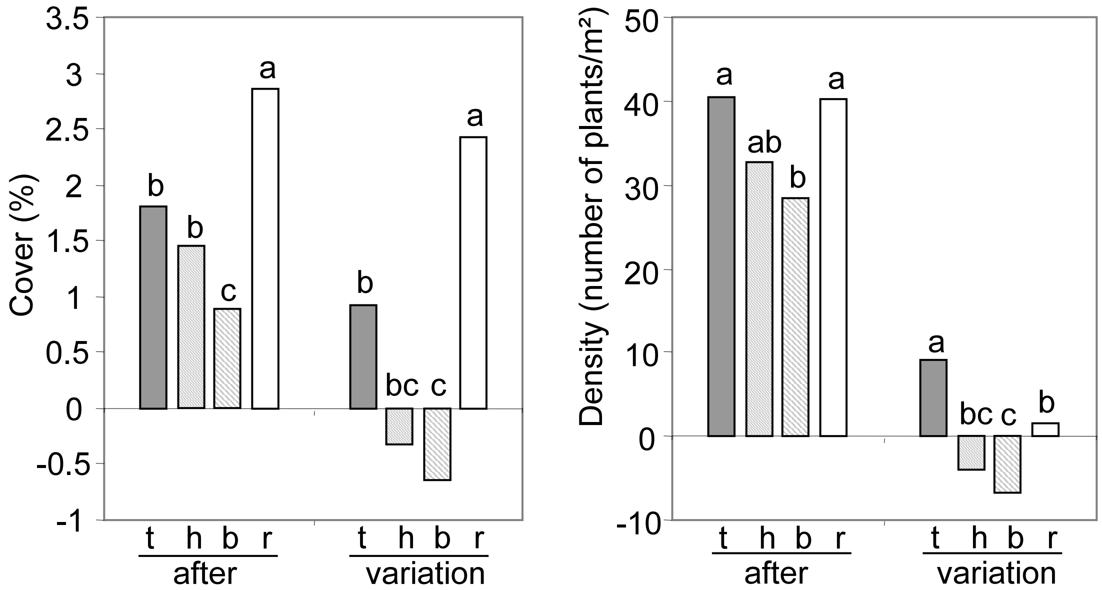

Figure 12: Cover and density of *Trifolium alpinum* two weeks after the second intervention (after) and their variations with respect to the situation immediately before the intervention (variation). Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance of cover and density was performed respectively on arcsine- and logarithm-transformed data. Mean separation by DMRT at α=0.05. Bars with no common letter are significantly different.

Chenopodium album, *Viola arvensis*, *Poa annua* and *Matricaria recutita* were in average the species with the highest cover and density before the weed control was performed and they formed on average 73% of the total cover of weeds (Figure 13a).

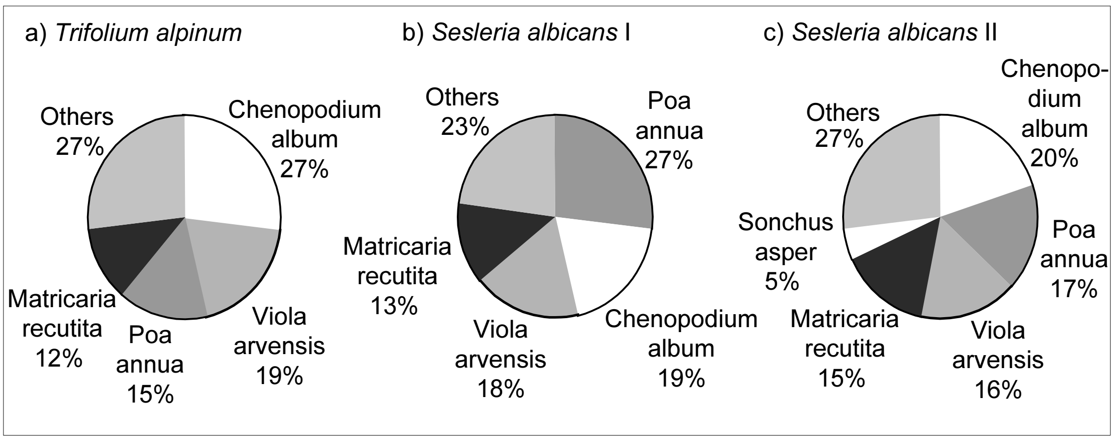

Figure 13: Percentage contribution of weed species to the weed cover before the second mechanical weed control in *Trifolium alpinum and* Sesleria albicans I and before the first mechanical weed control in *Sesleria albicans* II. Average of 36 measurements.

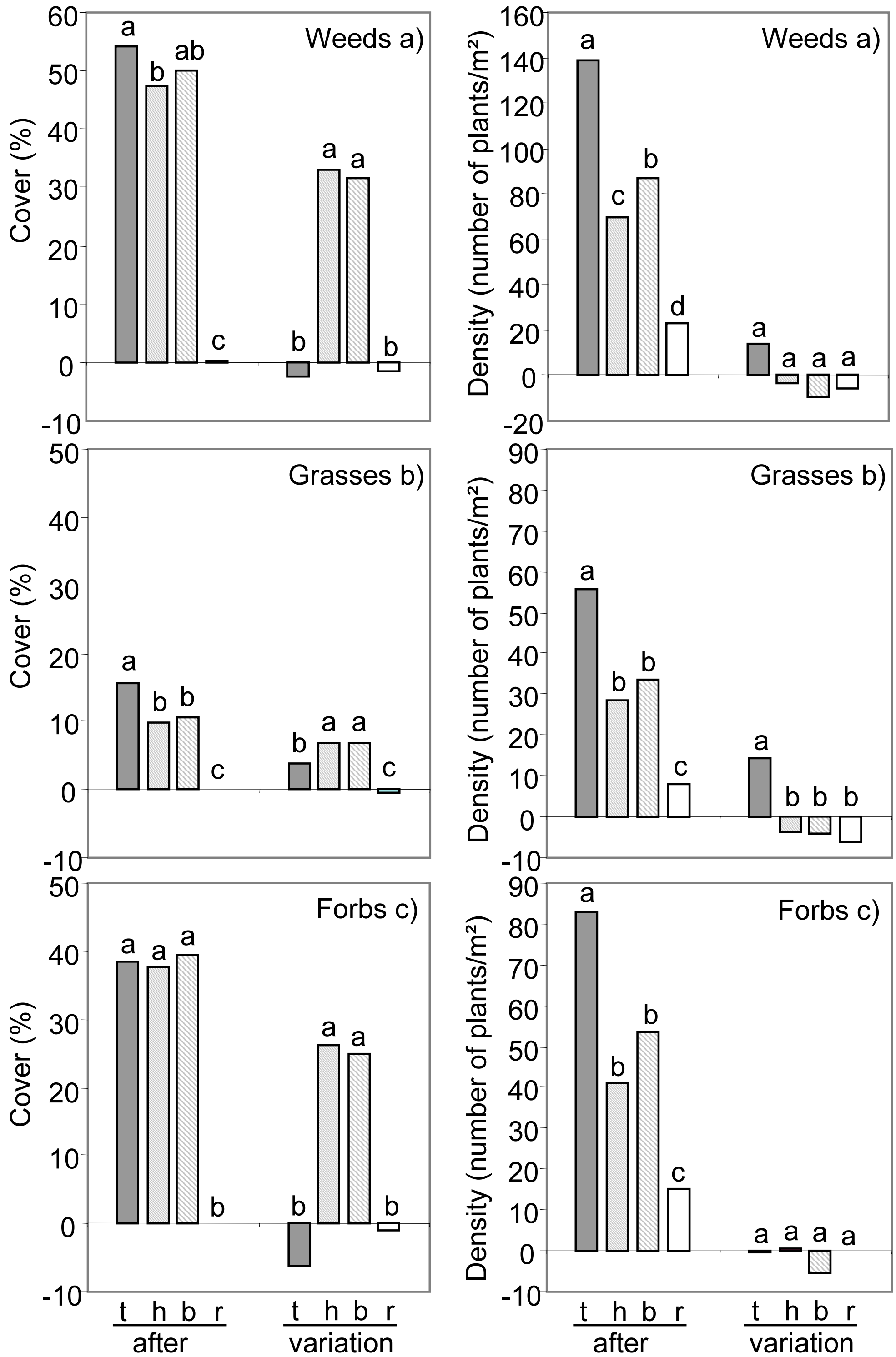

Figure 14: Total cover and density of weeds (a), grasses (b) and forbs (f) two weeks after the second intervention in *Trifolium alpinum* (after) and their variations (variation). Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance of cover was performed on arcsine-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

Only hand-roguing allowed to keep the weeds cover under control. The weed cover was over 45% in all mechanically performed weed control variants. Hoeing and brush weeding did not result in lower weed cover in comparison to the topping variant (Figure 14a) and the weed cover increased consistently in the time between the intervention and the second assessment The weed density was instead lower than in the topping variant. Density changes were very small and there was no significant difference between treatments.

The cover of grasses after the weed control was significantly lower in the hoeing and brush weeding treatments than in the topping variant, but the positive cover variation in these two treatments indicated a tendential cover increase of grasses through time (Figure 14b). Grasses density increased only in the topping treatment, while it remained more or less unchanged in all other variants.

Forbs achieved on average higher values than grasses. They showed a pattern similar to that described for the grasses, with the difference that their cover was not reduced by hoeing and brush weeding. From July 2001 to July 2002 the crop plant density decreased on average from about 35 to less than 1 plants/m², being below 0.5 plants/m² in 2002. In the 36 plots only 134 plants were retrieved in 2001 and their number decreased further on in 2002 (75 plants). The density of *Trifolium alpinum* achieved a value of 3.3 plants/m² in 2001 and 1.9 plants/m² in 2002 in the plots were manual weeding was performed in the first year, while all other treatments were devoid of plants (Table 7). The density was in general extremely low, indicating that only few plants survived to the nematode infection of the summer 2000.

Table 7: Density decrease of *Trifolium alpinum* through time at the Meierbreite. Average of nine replications ± SD.

	Density (plants/m²)			
Mechanical weed control	June 2000 before weed control*	July 2000 after weed control*	July 2001 before harvest**	July 2002**
Topping	31.3 ± 5.7	40.4 ± 7.9	0.0 ± 0.0	0.0 ± 0.0
Hoeing	36.8 ± 7.6	32.8 ± 7,0	0.0 ± 0.0	0.0 ± 0.0
Brush weeding	35.2 ± 6.9	28.3 ± 6.6	0.0 ± 0.0	0.0 ± 0.0
Hand-roguing	38.5 ± 10.1	40.2 ± 10.0	3.4 ± 2.1	1.9 ± 1.8
Total	35.5 ± 7.9	35.4 ± 2.1	0.8 ± 2.1	0.5 ± 1.2

* data assessed using the Göttinger count frame

** data obtained through density counts within whole plots

1.3.2.2 *Sesleria albicans*

In *Sesleria albicans* I, *Poa annua*, *Chenopodium album*, *Viola arvensis* and *Matricaria recutita* had in average the highest cover scores at the time of the second weed control and formed the 77% of the whole weed cover (Figure 13b). In *Sesleria albicans* II the same species with the addition of *Sonchus asper* were considered problem weeds. *Chenopodium album* was the species with the highest cover values, followed by *Poa annua*, *Viola arvensis*, *Matricaria recutita* and *Sonchus asper* (Figure 13c). Other species formed the remaining 27% of the weed cover.

In *Sesleria albicans* I extremely small cover scores of the crop plant were observed in all treatments, cover variations were positive only in the hand-roguing variant. The seedling density decreased in all treatments, particularly in the hoeing- and brush weeding-variant (Figure 15).

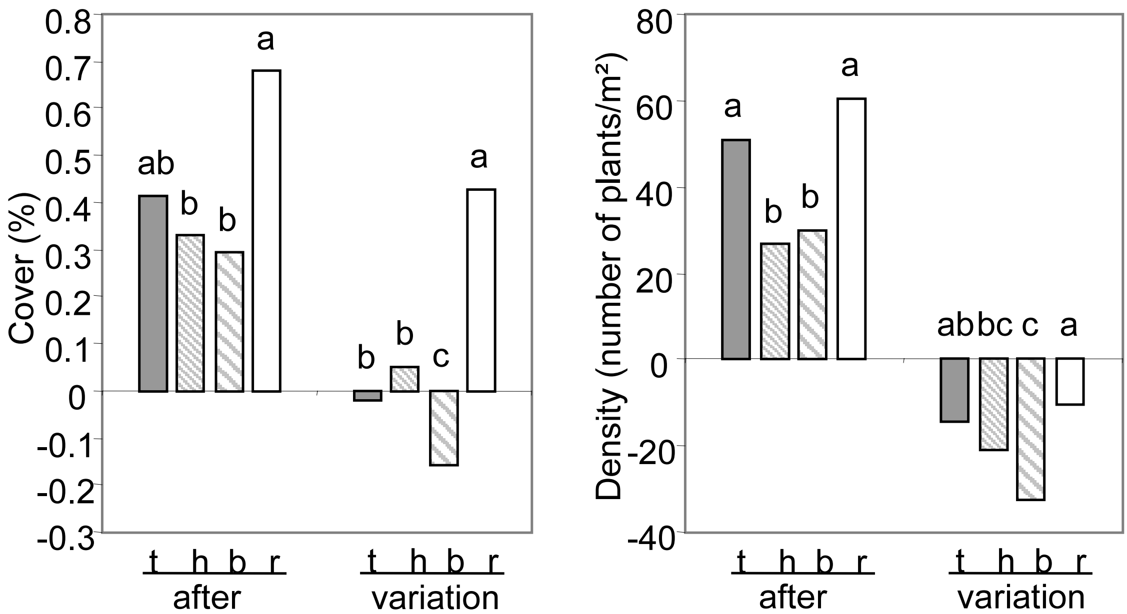

Figure 15: Cover and density of *Sesleria albicans* I two weeks after the second intervention (after) and their variations (variation). Average of nine replications per treatment (t=topping, h=hoeing. b=brush weeding, r=hand-roguing). Analysis of variance of cover and density was performed respectively on arcsine- and logarithm-transformed data. Mean separation by DMRT at $\alpha=0.05$. Bars without common letters are significantly different.

In *Sesleria albicans* II a very small positive cover variation of the crop plant was observed in all treatments after the first weed control intervention, while the seedling density remained similar to the initial situation (Figure 16).

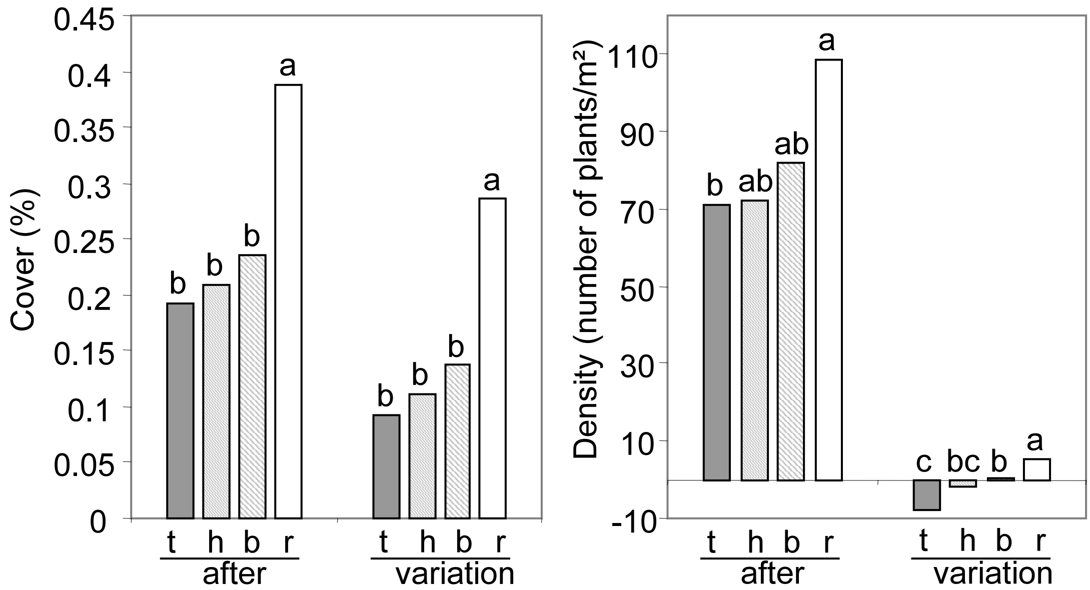

Figure 16: Cover and density of *Sesleria albicans* II two weeks after the first intervention (after) and their variations (variation). Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance of cover and density was performed respectively on arcsine- and logarithm-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

The overall weed cover in the hoeing- and brush weeding- variant in *Sesleria albicans* I after the second treatment presented relatively high scores (about 40%), although it was significantly lower than in the topping variant (Figure 17a). After hoeing and brush weeding, as already observed in *Trifolium alpinum*, the cover increased strongly, whereas it remained almost constant in the other two treatments. The density after the intervention, on the contrary, was about one third of that assessed after topping. Very small density variations were detected in all treatments, with the exception of plots that were manually weeded. The same pattern was recognisable for cover, density and their variations in grasses and forbs (Figure 17b and Figure 17c).

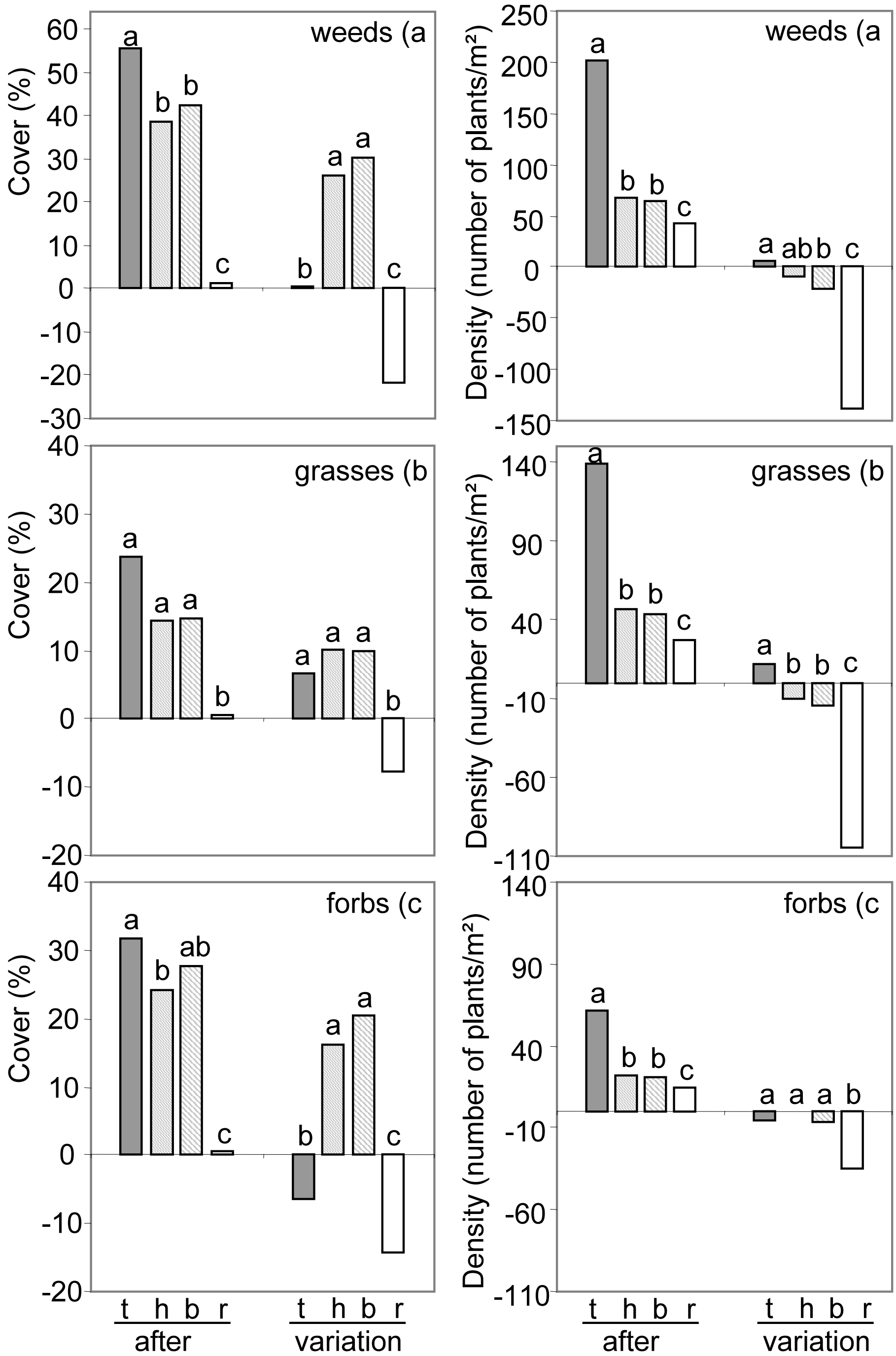

Figure 17: Total cover and density of weeds (a), grasses (b) and forbs (c) two weeks after the second intervention in *Sesleria albicans* I (after) and their variations (variation). Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance of density was performed on logarithm-transformed data. Differences of cover of grasses (after) were tested for significance with the Nemenyi's test at α=0.05 after positive testing of the effect of the weed control with the Kruskal-Wallis test. All other mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

In *Sesleria albicans* II the weed cover after the first intervention was in the control more than four times higher than in the hoeing- and brush weeding-variant (Figure 18). A positive cover variation was nevertheless assessed in all these three treatments. The weed density in the control did not change between the assessment terms, whereas hoeing and brush weeding were less 50% effective than hand-roguing in reducing the weed density.

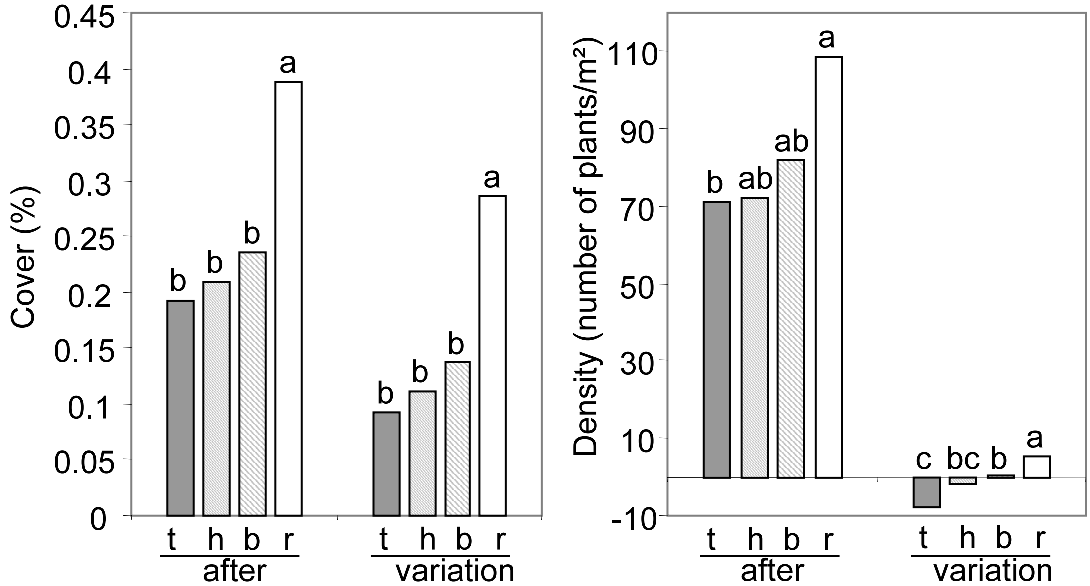

Figure 18: Cover and density of weeds two weeks after the first intervention (after) and their variations (variation) in *Sesleria albicans* II. Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance of cover and density was respectively performed on arcsine- and logarithm-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

Table 8: Efficacy of different mechanical weed control methods on cover and density of weeds, grasses and forbs in *Sesleria albicans* II 14 days after the intervention. Adjusted means are the average of nine replications and have been obtained through ANCOVA. Cover and density scores before the intervention have been used as covariables. Analysis of covariance of cover and density was performed respectively on arcsine- and logarithm-transformed data. Mean separation by DMRT at α=0.05. Values without common letters are significantly different.

Treatment	Weeds		Grasses		Forbs	
	Cover (%)	Density (No./m²)	Cover (%)	Density (No./m²)	Cover (%)	Density (No./m²)
Topping	22.0 [a]	137.4 [a]	6.2 [a]	78.7 [a]	15.0 [a]	52.0 [a]
Hoeing	4.7 [b]	69.0 [b]	2.5 [b]	45.3 [b]	3.1 [b]	21.9 [b]
Brush weeding	6.2 [b]	65.3 [b]	1.5 [b]	39.5 [b]	4.6 [b]	26.0 [b]
Hand-roguing	0.8 [c]	38.7 [c]	0.6 [c]	27.9 [c]	0.2 [c]	9.3 [c]

As shown by the results of the covariance analysis, the efficacy of hoeing and brush weeding in controlling the cover and the density of the weeds was intermediate between the control and the hand-roguing treatment (Table 8). The adjusted cover- and density means obtained through the co-variance analysis were consistently lower for the hoeing and brush weeding variants if compared to the control. Nevertheless they remained significantly higher than in the hand-roguing treatment. The same pattern was observable for grasses and forbs.

In *Sesleria albicans* I the composition of the weed flora changed markedly from year to year: *Lolium perenne*, *Poa annua* and *Taraxacum officinale* achieved in average the highest cover scores at the time of the first seed harvest in 2001, forming together more than 80% of the total weed cover in mechanically weeded plots (Figure 19).

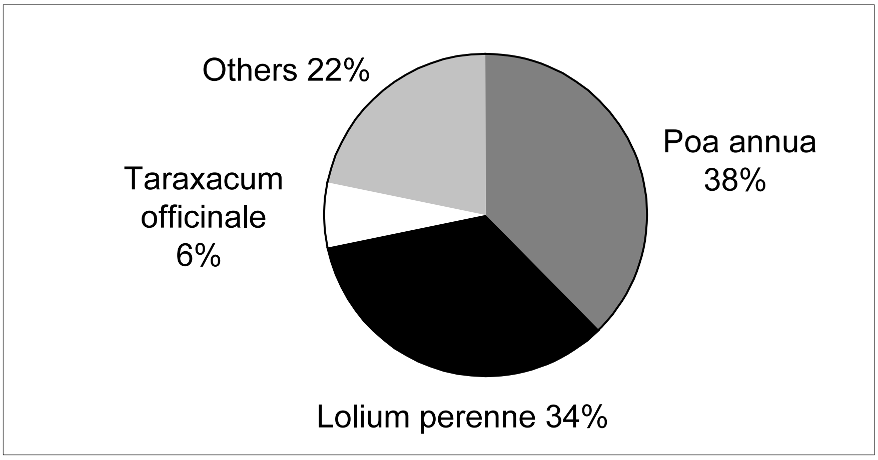

Figure 19: Mean percentage contribution of weed species to the weed cover before the first seed harvest in 2001 in *Sesleria albicans* I. Mean of 27 measurements (only mechanised weed control methods: topping, hoeing and brush weeding).

Among the problem weeds identified in the previous year, only *Poa annua* increased its share. *Lolium perenne*, occurring in 2000 with low density, but with relatively high cover, became in 2001 the main weed species.

The cover of the crop plant in *Sesleria albicans* I was in 2001 quite low, being about 16% in the manually weeded variant and below 8% in all other treatments. Density ranged between 16 and 25 plants/m². The highest values were assessed in manually weeded plots, and the lowest in the mechanically weeded plots, confirming the trend of the first observation year.

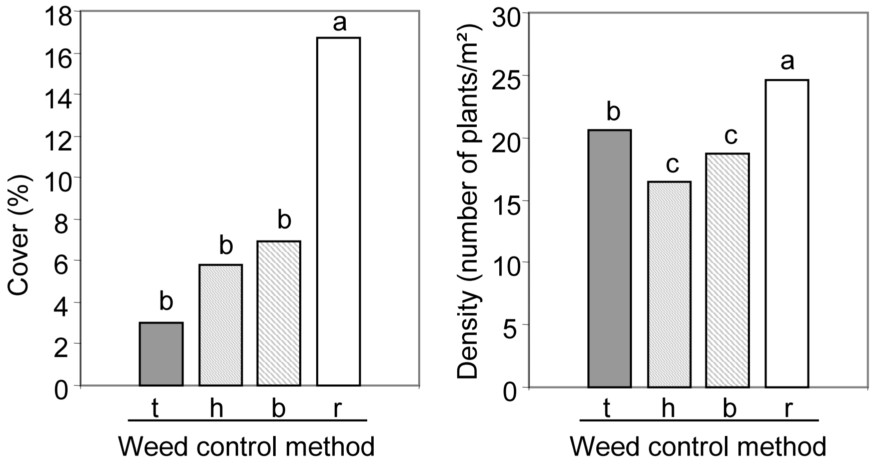

Figure 20: Cover and density of *Sesleria albicans* I before the seed harvest in June 2001. Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance of cover and density was performed respectively on arcsine- and logarithm-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

Topped plots showed intermediate values, and if the cover:density ratio (here called "unitary cover") is considered, it becomes evident that this relatively high density refers to plants that have been overgrown by weeds. *Sesleria albicans*-plants in these plots were still vital, but were small-sized and did not produce, or only to a minimal extent, floral stems. The unitary cover of hand-rogued plots was about 2 to 4 times higher than in the other treatments (Table 9).

Table 9: Mean unitary cover (cover/density) of *Sesleria albicans* I before the seed harvest in June 2001. Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Mean separation by DMRT at α=0.05. Means without common letters are significantly different.

Weed control method	Unitary cover (%)
Topping	0.14 ^{c}
Hoeing	0.36 ^{b}
Brush weeding	0.37 ^{b}
Hand-roguing	0.67 ^{a}

In *Sesleria albicans* II no significant cover differences were observed in 2001 and 2002 between treatments, as in 2001 plots were periodically hand-weeded in addition to the planned weed control methods. Considering the cover variations of *Sesleria albicans* through time in the manually weeded variants of both trials (Figure 21), a very large increase occurred from 2000 to 2002, indicating that the growth of tussocks progresses in a relatively wide time span, and the maximum cover may have not been achieved in the third year yet.

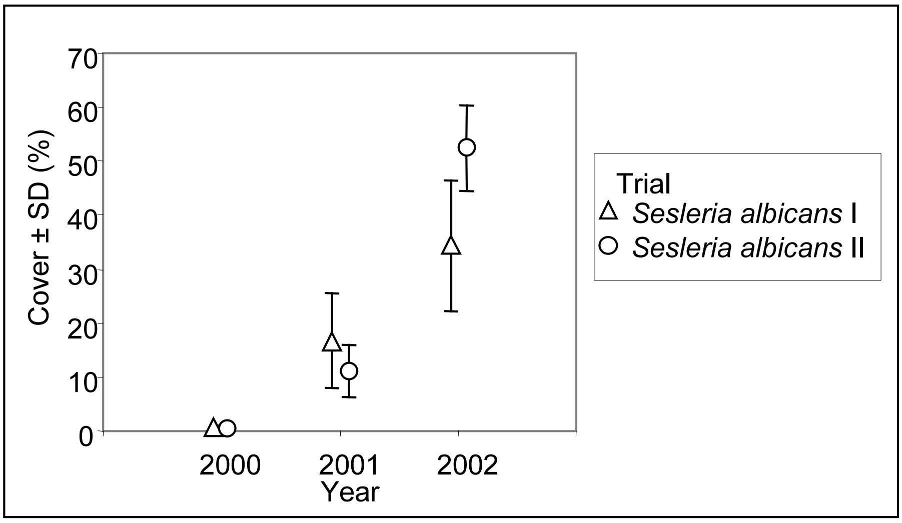

Figure 21: Cover variations through time of the crop plant in *Sesleria albicans* I and in *Sesleria albicans* II (only hand-rogued plots). Average of nine replications ± SD.

Weed cover above 50% was assessed in all treatments in *Sesleria albicans* I in 2001, except in the hand-rogued variant, where it was about 25% (Figure 22). Neither hoeing nor brush weeding proved to be effective in controlling the weeds. Analysing the effect of the weed control method on grasses, topping was the least effective method, hoeing and brush weeding presenting lower values (around 10% lower). Forbs occurred on the contrary in all treatments with similar moderate cover.

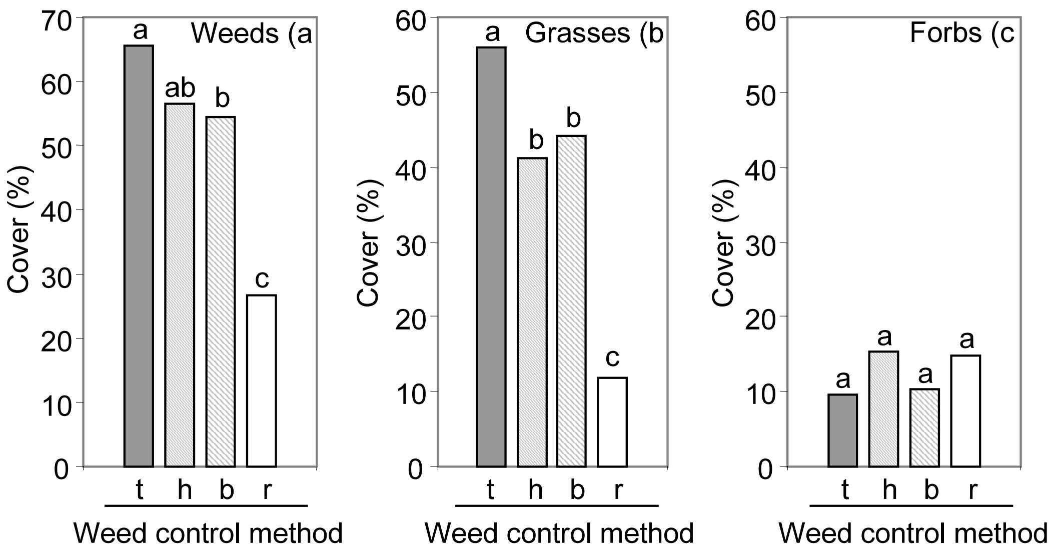

Figure 22: Total cover of weeds (a), grasses (b) and forbs (c) before seed harvest in June 2001 in *Sesleria albicans* I. Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). ANOVA performed on arcsine-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

The small-sized weeds, with the exception of *Poa annua*, were no longer abundant. *Lolium perenne*, whose cover and density were relatively small in the first year, became cover-dominant. It was found to be virtually absent in the hand-weeded plots, while it achieved on average scores between 15% and 35% in the other treatments (Figure 23d). Hoed and brush-weeded plots exhibited significantly lower values in comparison to the topped ones. *Poa annua* was everywhere abundant, and particularly in the brush weeding treatment. Numerous seedling were usually located between the rows, while few plants were found within the rows. Its cover was similar in each treatment, and it was particularly high in the brush weeding-variant. Among the forbs only *Taraxacum officinale* appeared everywhere with cover above 2%, regardless of the weed control method.

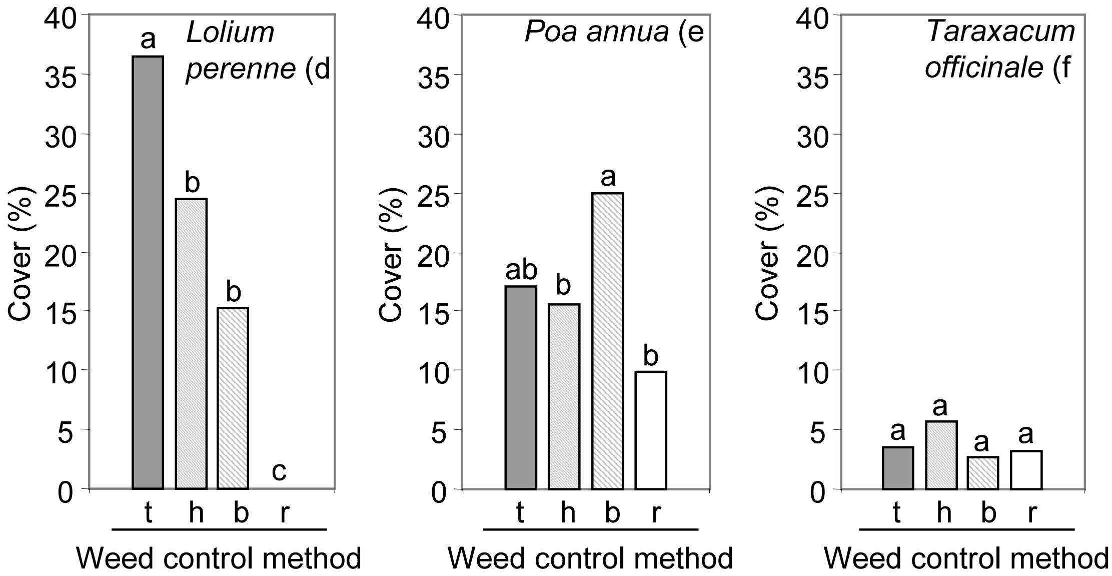

Figure 23: Cover of *Lolium perenne* (d), *Poa annua* (e), *Taraxacum officinale* (f) before seed harvest in June 2001 in *Sesleria albicans* I. Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance was performed on arcsine-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

In *Sesleria albicans* II weeds had in 2001 low cover in all treatments, as periodical hand-roguing was performed. Higher values on average assessed in the topped variant were found to be not significant. As soon as the periodical hand weeding ceased, in 2002, effects of the mechanical weed controls became instead evident; in the topped variant weeds achieved about 30% cover, while hoeing and brush weeding resulted in a reduction of weed cover of about the half (Figure 24).

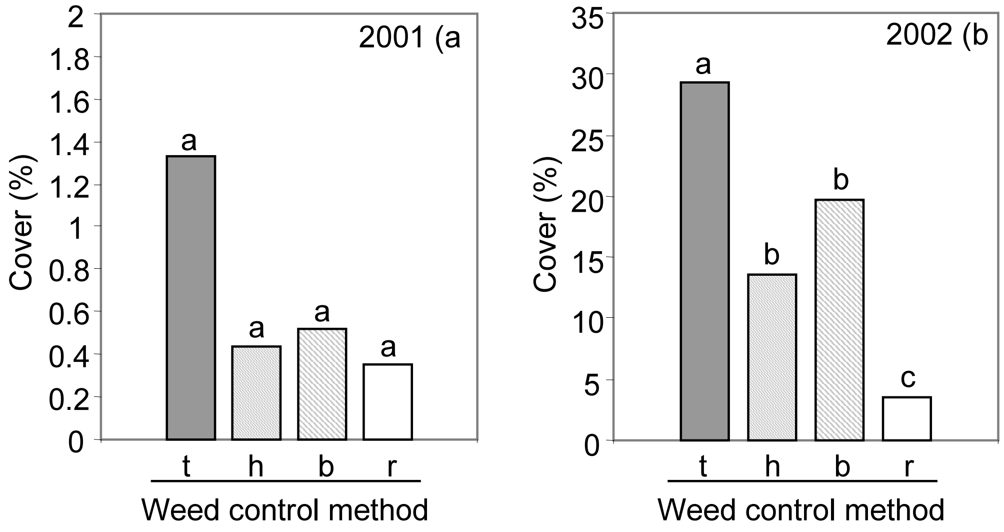

Figure 24: Total cover of weeds in *Sesleria albicans* II before the seed harvest in June 2001 (a) and in June 2002 (b). Average of nine replications per treatment (t=topping, h=hoeing, b=brush weeding, r=hand-roguing). Analysis of variance was performed on arcsine-transformed data. Mean separation by DMRT at α=0.05. Bars without common letters are significantly different.

1.3.3 Susceptibility of *Trifolium alpinum* to pathogens

Rhizobium-induced root nodules were not found on the roots of the plants examined.

No improvement was detected after two weeks in the status of the plants that were supplied with urea, $(NH_4)_2SO_4$, $MgSO_4$ and K_2SO_4. On the contrary, the conditions of some plants worsened. A nutrient deficiency was therefore excluded.

The roots of the sample from Hebenshausen were found to be heavily colonised by phytophagous nematodes of the genus *Pratylenchus*, that had a density up to 10 animals/g of root. The number of *Pratylenchus*-individuals in the soil was also very high, varying from 5 animals/250 g soil of the plants with medium symptoms to 85 animals/250 g soil of the plants with heavy symptoms. Such nematodes were not found in the samples from Meran 2000 and from Meißner. Relatively high numbers of nematodes of the genus *Helicotylenchus* were also found in the sample from Hebenshausen. Other nematodes (*Aphelenchoides* sp.) were observed in the samples from Meißner and Meran 2000. A large number of other saprophyte nematodes was present in the samples of the first location, while the soil of Meran 2000 seemed to be poorly colonised.

The fungus *Thielaviopsis basicola* was found in all root samples from Hebenshausen.

1.3.4 Yield and seed quality

1.3.4.1 Trifolium alpinum

Seed yield

Only an assessment based on the few plants still alive in the second growth period was possible. A mean seed yield of 2.5 kg/ha was assessed in the manually weeded variant, corresponding to a seed production of 0.11 g/flowering plant was assessed. If calculated for the whole number of plants, the seed production was 0.07 g/plant, as only 68% of the plants still alive flowered.

Purity analysis

The pure seed fraction was 99% of the working sample (6 g). Inert matter was also found in small percentage (1%), being mainly constituted by broken or unfilled seeds.

Seed weight

The thousand seed weight (TSW) of the seed harvested at the Meierbreite was 3.84 ± 0.11 g. A considerable TSW decrease of more than 20% was observed in comparison to the wild seed collected in the Alps (Figure 25). The difference in TSW was highly significant (t-test; df=14; t=19.904; p<0.001).

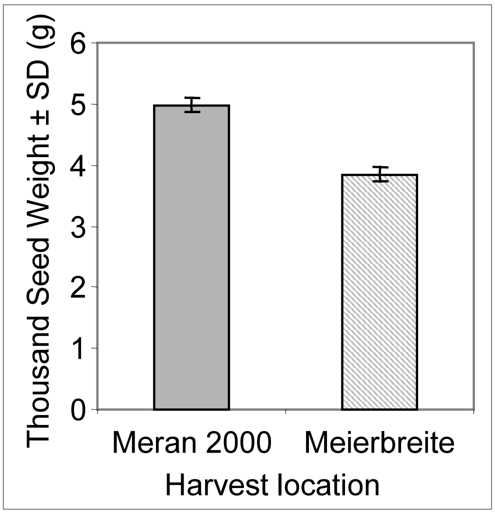

Figure 25: Seed weight of *Trifolium alpinum* from the original alpine location (Meran 2000) and from the propagation site at the Meierbreite. Average of 8 replicates.

Seed germination in laboratory

Scarified seeds of both upland- and lowland-harvest had almost completely germinated already 14 days after sowing, indicating that the seed viability of the sample harvested at the Meierbreite did not differ from that of the alpine provenance (Figure 26).

After an initially rapid increase of about 10% in the second imbibition week, untreated seeds of the alpine provenance showed from the 14th day a more or less linearly increasing trend, achieving about 25% seed germination at the end of the experiment (105 days). The untreated sample of the seed obtained from the propagation plots exhibited on the contrary a quick increase of the cumulative germination in the first three weeks, with scores on average above 50%. Then no further germination increase was observed. On the 35th day the seed germination was about a threefold of that achieved by the untreated alpine provenance. Also the germination speed exhibited a substantial improvement. Starting from the 7th day it was significantly higher than in the untreated Meran 2000-sample.

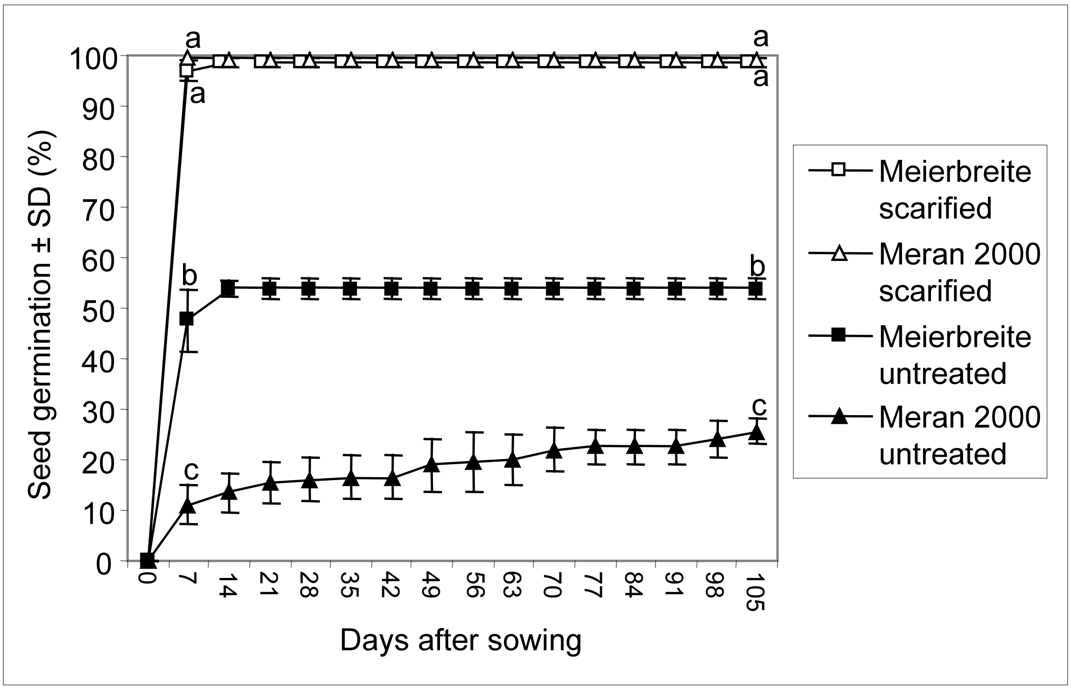

Figure 26: Cumulative germination of scarified and untreated seeds of *Trifolium alpinum* harvested at the original location in the Alps (Meran 2000) and at the lowland-propagation site at the Meierbreite. Different letters indicate significant differences among means (n=4, DMRT at a=0.05). Only changes in significance of means between treatments are marked.

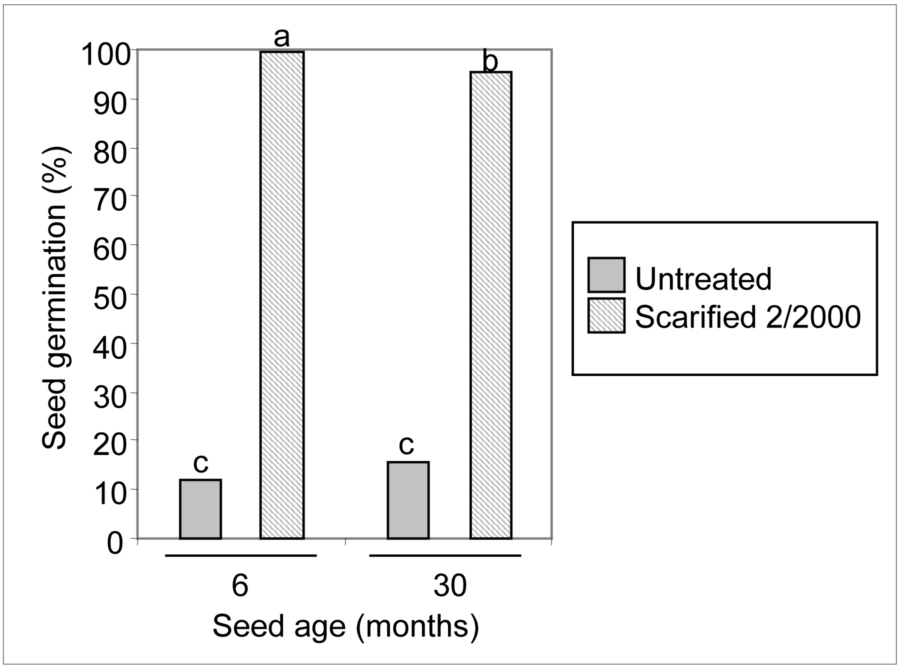

Figure 27: Seed germination achieved 21 days after sowing by 6- and 30-month-old scarified and untreated seeds of *Trifolium alpinum*. Different letters indicate significant differences among means (n=4, DMRT at α=0.05).

The comparison of the cumulative germination of 6- and 30-month-old seed of *Trifolium alpinum* (Figure 27) showed that no significant change occurred for the untreated seeds, while a small, but significant, decrease was detected in the scarified variant 25 months after the scarification was carried out. The seed germination remained nevertheless satisfactory (about 95%).

The use of a laboratory thresher for cleaning the seed resulted in a greatly increased seed germination in comparison to the manually cleaned seeds (Table 10). No further germination increase was caused by rubbing the seed against a sieve.

Table 10: Cumulative seed germination of *Trifolium alpinum* 35 days after sowing depending on the cleaning procedure. Mean separation by DMRT at α=0.05 (n=4). Values without common letters are significantly different.

Cleaning method	Seed germination (%)
Manual cleaning	16.5 [b]
Threshing	87.0 [a]
Threshing + rubbing	85.0 [a]

1.3.4.2 Sesleria albicans

Seed yield

The production of floral culms was found to be significantly correlated to the size of the tussock (Spearman's ranks correlation, $R=0.80$, $p<0.01$). Under a tussock diameter of 10 cm virtually no flowering was observed (Figure 28). A relatively wide variation of the number of flowering stems was observed nevertheless for tussock diameters above 10 cm. For this reason, on the basis of the interpolated curve, only 50% of the variation of the number of flowering stems can be explained by the tussock diameter. A production of about 10 flowering stems appears by tussock diameter above 25 cm.

In the first harvest year the size of the tussocks was significantly higher in *Sesleria albicans* I than in *Sesleria albicans* II (mean values 15.9 cm vs. 12.2 cm respectively, t-test, equal variances not assumed, df=376.37, t=7.148, $p<0.001$).

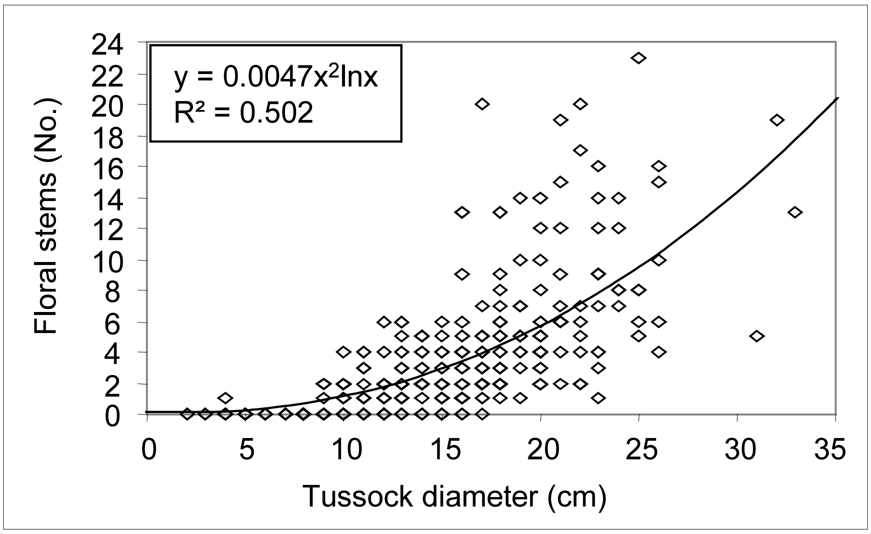

Figure 28: Relationship between tussock diameter and number of floral stems in *Sesleria albicans* I and in *Sesleria albicans* II in June 2001.

In *Sesleria albicans* I the seed production was very low in 2001 (Figure 29a), the hand-harvested variant yielding around 5 kg/ha on average. The seed yield obtained with the other harvesting technique did not differ significantly from the hand-harvesting treatment, despite of a lower mean yield in the stripping/vacuum harvest.

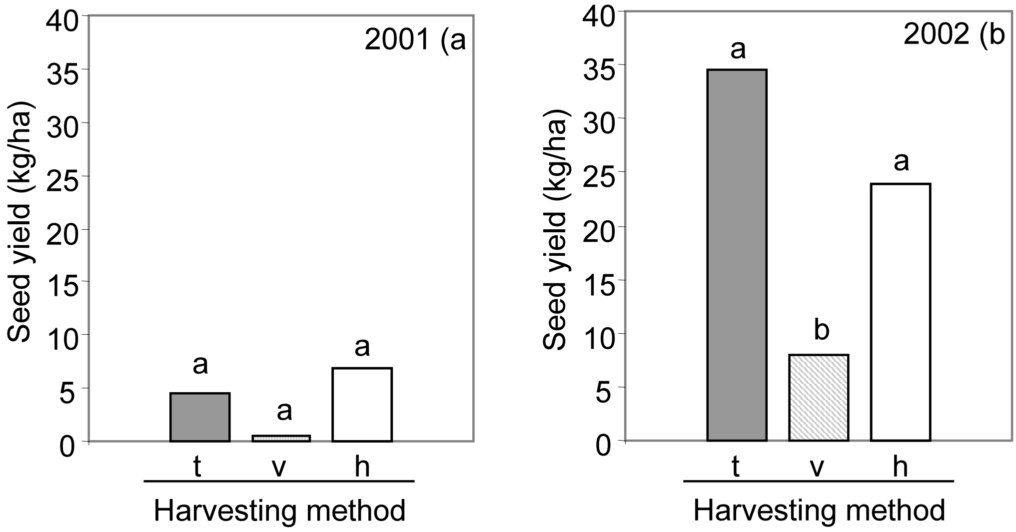

Figure 29: Effect of the harvesting method on the seed yield of *Sesleria albicans* I at the seed harvest in June 2001 (a) and in June 2002 (b) for the manually weeded treatment. Average of three replications per treatment (t=threshing, v= stripping/vacuum harvesting, r=hand-harvesting). Mean separation by Tukey HSD at α=0.05. Bars without common letters are significantly different.

In 2002 the seed yield increased considerably, and values up to 35 kg/ha on average were assessed in the threshed plots (Figure 29b). Hand-harvesting and threshing did not differ significantly, indicating that threshing (as here defined) is a suitable method for harvesting *Sesleria albicans*, while stripping/vacuum harvesting showed very large seed losses (about 60% of the expected yield represented by the hand harvest).

In *Sesleria albicans* II the mechanical weed control methods had no effect on the seed yield in both observation years (Table 11). The seed production was extremely low in the first year (1.5 kg/ha on average) and increased in the second year, achieving yield of 22.6 kg/ha on average in the hand-harvested variant. Again, threshing did not result in a significantly different seed yield, while seed amounts obtained by stripping/vacuum harvesting were about 50% lower than the others.

Table 11: Effect of the weed control method in June 2001 (a) and of both weed control method and harvesting method at the seed harvest in June 2002 (b) on the seed yield of *Sesleria albicans* II. Average of nine replications per treatment. Mean separation by Tukey HSD at α=0.05. Values without common letters are significantly different.

a) 2001

Weed control method	Seed yield (kg/ha)
Topping	1.6 [a]
Hoeing	1.2 [a]
Brush hoeing	2.1 [a]
Hand-roguing	1.2 [a]

b) 2002

	Seed yield (kg/ha)
Weed control method	
Topping	21.7 [a]
Hoeing	14.5 [a]
Brush hoeing	13.5 [a]
Hand-roguing	16.1 [a]
Harvesting method	
Threshing	17.4 [ab]
Stripping/vacuum harvesting	9.4 [b]
Hand-harvesting	22.6 [a]

Purity analysis

In *Sesleria albicans* I (only hand-weeded plots), purity was above 90% in all treatments, with other seeds in percentages below 1%, while inert matter ranged between 3.1% (manual harvest) and 5.9% (other treatments).

Table 12: Results of the purity analysis of seed of *Sesleria albicans* I at the harvesting date in June (average of data of 2001 and 2002).

Harvesting method	Fraction		
	Pure seed (%)	Inert matter (%)	Other seeds (%)
Threshing	92.1	5.8	0.6
Stripping/vacuum harvesting	93.5	5.9	0.6
Hand-harvesting	96.7	3.1	0.1

In *Sesleria albicans* II, analogous purity values above 90% were assessed in all treatments. Regarding the effect of the weed control methods, plots with mechanical weed control exhibited higher content of other seeds (up to 2.4% in the topping variant). Concerning the harvesting methods, the same pattern observed in *Sesleria albicans* I was found.

Table 13: Results of the purity analysis of seed of *Sesleria albicans* II at the harvesting date in June (2002 data).

	Fraction		
	Pure seed (%)	Inert matter (%)	Other seeds (%)
Weed control			
Topping	93.4	4.2	2.4
Hoeing	94.0	4.6	1.3
Brush weeding	93.6	3.9	2.4
Hand-roguing	91.5	7.9	0.6
Harvesting method			
Threshing	92.1	5.8	0.6
Stripping/vacuum harvesting	93.5	5.9	0.6
Hand-harvesting	96.7	3.1	0.1

Extremely high amounts of sclerotia of *Claviceps purpurea* were found in the seed collected at the second harvest date (August) in 2001. They made up to 44% of the samples investigated.

Table 14: Purity analysis and content of sclerotia of *Claviceps purpurea* in seed of *Sesleria albicans* I and *Sesleria albicans* II at the second harvesting date in August (2001 data). Sclerotia of *Claviceps purpurea* are regarded as a sub-fraction of the inert matter.

	Fraction			
	Pure seed (%)	Inert matter		Other seeds (%)
Trial		Sclerotia of *Claviceps purpurea* (%)	Other inert matter (%)	
Sesleria albicans I	55.0	43.7	1.3	0.0
Sesleria albicans II	58.7	39.3	1.6	0.4

Seed weight

In both *Sesleria albicans* I and *Sesleria albicans* II the weight of the seed used for the propagation was relatively high, being slightly lower (TSW = 1.62 g) for *Sesleria albicans* II, a mixture of an already propagated colline provenance (Schwäbische Alb) and various lighter alpine provenance.

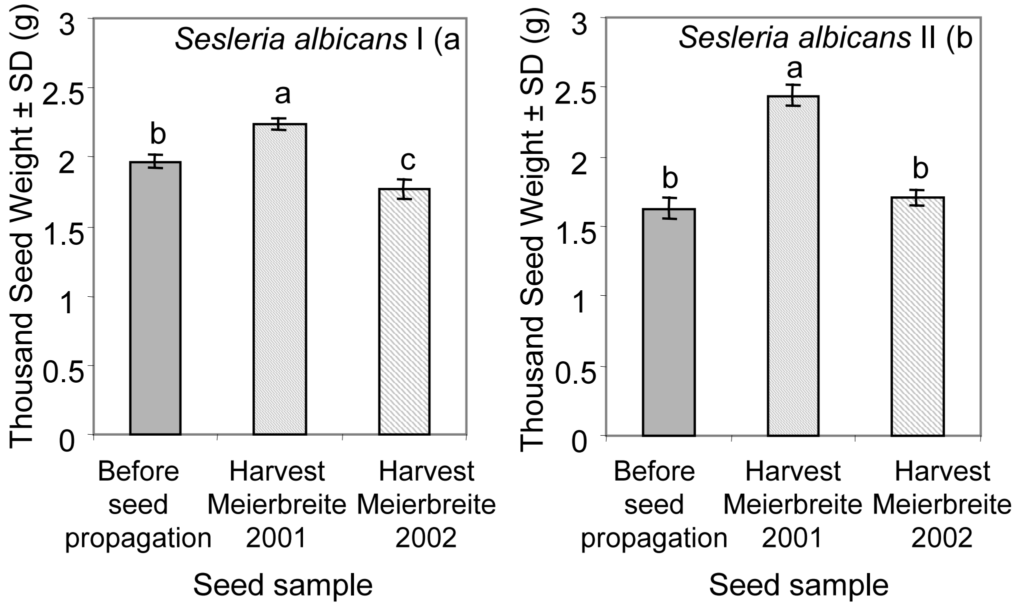

Figure 30: Seed weight of *Sesleria albicans* I (a) and of *Sesleria albicans* II (b) before and after the seed propagation at the Meierbreite (harvest 2001 and 2002). Average of 8 replicates. Mean separation by Tukey HSD at α=0.05. Values without common letters are significantly different.

Sesleria albicans I was instead formed by the sole provenances Schwäbische Alb I and Schwäbische Alb II and its TSW was around 2 g. In both trials an increase of seed weight up to 2.4 g was assessed in the first harvest year, while in 2002 the seed weights were similar to those of the basis seed.

Considering the seed weight variation of single alpine provenances cultivated in small plots at the Meierbreite, one with relatively low seed weight (Monte Baldo, TSW of the wild seed = 0.9 g) and the other with high seed weight (Monte Cavallo, TSW of the wild seed = 1.6 g), the comparison with the seed weights achieved at the harvest 2002 shows that more or less the same seed weight was assessed, regardless of the different TSW of the wild seed. As shown by Figure 31, the provenance Monte Baldo increased considerably its TSW (t-test, equal variances not assumed, df=8,748, t=-16.076, p<0.001), while for the other it remained unchanged (t-test, df=14, t=0.052, p>0.05).

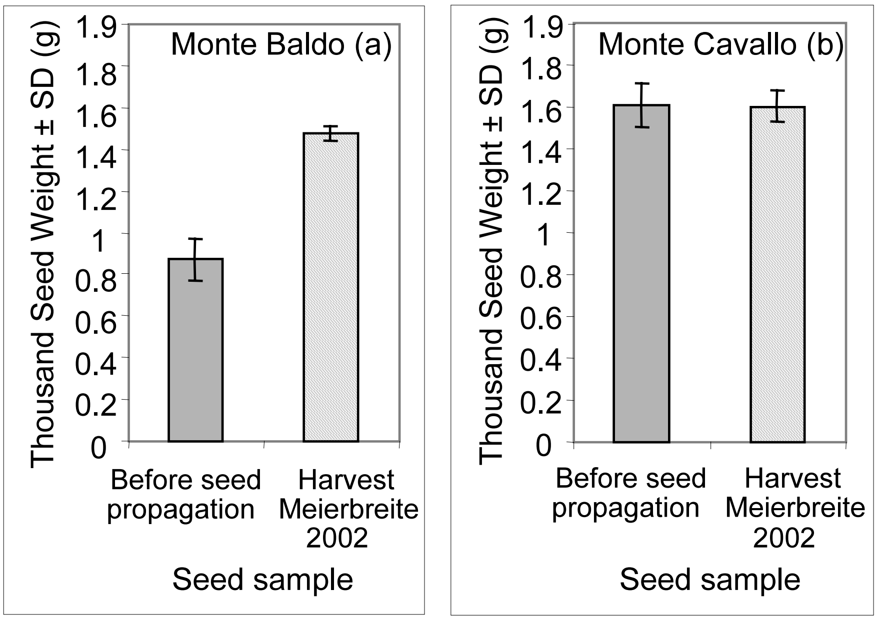

Figure 31: Seed weight of two alpine provenances of *Sesleria albicans* before and after the seed propagation at the Meierbreite (harvest 2002): Monte Baldo, 2,000 m a.s.l. (a) and Monte Cavallo, 1,650 m a.s.l. (b). Average of 8 replicates.

A significantly negative correlation (Pearson's correlation coefficient, R=-0.806, p<0.01) was found between the altitude of the harvest location and the TSW of various provenances collected in the Alps and of seed harvested at the Meierbreite (Figure 32).

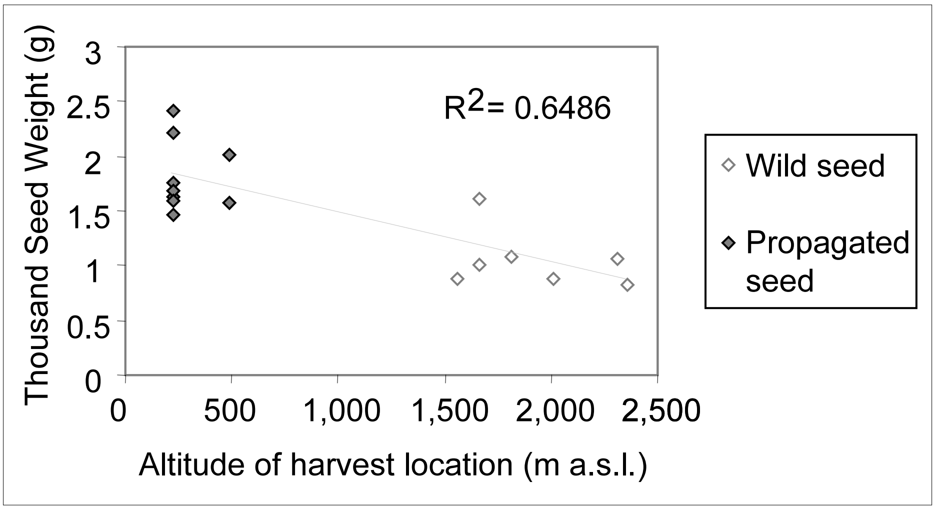

Figure 32: Relationship between altitude of the location where the seed of *Sesleria albicans* was collected or harvested and the seed weight.

Seed germination in laboratory

The germinative capacity of seed of both *Sesleria albicans* I and in *Sesleria albicans* II increased after the seed propagation (Table 15). Fluctuations resembled those observed for the seed weight in 2001 and 2002 (compare with Figure 30) with the difference that, despite of a decrease of seed weight of *Sesleria albicans* I from 2001 to 2002, the seed germination remained unchanged.

Table 15: Germinative capacity of the seed of *Sesleria albicans* I and of *Sesleria albicans* II before and after the seed propagation at the Meierbreite (2001 and 2002 data). Results refer to scores assessed respectively 35 and 42 days after starting the experiment. Average of 4 replicates. Mean separation by DMRT at α=0.05. Within each column, values without common letters differ significantly.

Seed sample	Seed germination (%)	
	Sesleria albicans I	*Sesleria albicans* II
Basis seed (2000)	71.0 [b]	68.5 [c]
After propagation (2001)	90.5 [a]	92.5 [a]
After propagation (2002)	92.5 [a]	76.5 [b]

A significantly positive correlation (Pearson's correlation coefficient, R=0.789, p<0.01) was found between TSW and seed germination of various alpine provenances and of seed harvested at the Meierbreite (Figure 33). In order to exclude from this analysis undesirable effects due to storage time and storage modalities, only recently harvested seed provenances were considered in this calculation, as the germination capacity of *Sesleria albicans* was reported to fluctuate through time (Fossati, 1980) and the seed germination of alpine grasses tends in general to decrease drastically with increasing age if storage temperatures are not adequately low (Vescovo and Scotton, 2002).

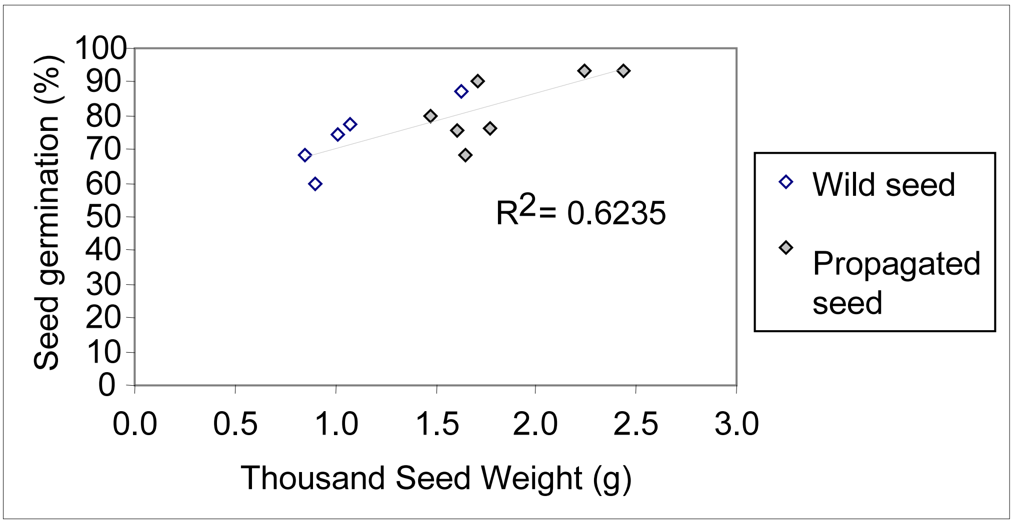

Figure 33: Relationship between seed weight and seed germination in *Sesleria albicans*. Data displayed here refer to tests of recently-harvested seed samples performed under homogeneous controlled conditions. Seed lots were not considered, which storage conditions and storage duration were unknown, or if seeds had been stored for more than 2 years.

1.3.4.3 *Festuca nigrescens*

Seed yield

In contrast to what observed for the other species investigated, a seed yield could be achieved in all treatments investigated.

The analysis of variance performed on the seed yield detected a very highly significant effect of all main factors and significant interactions of year with weed control method and harvesting method (Table 16).

Table 16: Effect of main factors (weed control method, harvesting method and year) and of their interactions on seed yield and pure seed yield (amount of pure seed in the seed yield) of *Festuca nigrescens* (repeated measures ANOVA: $^{n.s.}$=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001).

		Seed yield		Pure seed yield	
Source	df	F	Sig.	F	Sig.
Between-subjects effects					
Weed control method (W_C)	3	17.43	***	14.13	***
Harvesting method (H_M)	2	68.29	***	36.40	***
W_C x H_M	6	0.13	n.s.	0.24	n.s.
Block	2	0.09	n.s.	4.15	*
Error	22				
Within-subjects effects					
Year (Y)	1	55.98	***	27.77	***
Y x H_M	2	3.91	*	1.59	n.s.
Y x W_C	3	8.42	**	5.94	**
Y x H_M x W_C	6	1.56	n.s.	1.40	n.s.
Y x Block	2	2.00	n.s.	3.22	n.s.
Error	22				

In the first harvest year a seed production between 642 and 1,217 kg/ha was achieved depending on the mechanical weed control. Hoeing and brush weeding resulted in mean seed yields of about 800 kg/ha and did not differ significantly from each other. They did not differ from the topping treatment, while yielding lower value than the hand roguing treatment. In the second harvest year they were intermediate between the hand roguing and topping treatments, representing respectively the upper and the lower extreme and presenting the sole significant yield differences. The seed yield decreased considerably, but remained above the limit of 500 kg/ha. Considering the effect of the harvesting methods, the highest seed yield was obtained with the combine harvesting. Stripping/vacuum harvesting resulted in the contrary in high seed losses and in a seed yield being roughly half of that, while hand-harvesting resulted in intermediate values very similar to those of the combine-harvested plots. The mean seed yield, calculated across weed control methods and harvesting methods, decreased on average of about 27% from 2001 to 2002.

Table 17: Seed yield of *Festuca nigrescens* depending on mechanical weed control method and harvesting method. Post-hoc comparisons were performed separately for each year where an interaction W_C x Y or H_M x Y was detected by ANOVA, otherwise for the mean calculated across the other factors. Mean separation by Tukey's HSD at α=0.05. Means without common letters within a factor and the same variable are significantly different.

	Seed yield (kg/ha)			Pure seed yield (kg/ha)		
	2001	2002	Average	2001	2002	Average
Weed control						
Hand roguing	1,217 a	722 a	969	1,070 a	688 a	879
Hoeing	856 b	669 ab	762	737 b	602 ab	670
Brush weeding	765 b	610 ab	688	664 b	560 ab	612
Topping	642 b	552 b	597	523 b	489 b	506
Harvesting method						
Hand-harvesting	916 b	720 a	818	850	703	777 a
Combine harvesting	1,164 a	813 a	988	928	689	808 a
Stripping/vacuum harv.	529 c	382 b	456	469	362	415 b

Similar results of the analysis of variance (Table 16) and the same pattern between treatments and from the first to the second year was detected for the pure seed yield (Table 17). This variable was obtained calculating the amount of pure seed contained in the seed yield on the basis of the results of the purity analysis as follows:

$$PSY_i = SY_i \times PS_i / 100$$

PSY_i Pure seed yield (kg/ha) of the $_i$-th observation
SY_i Seed yield (kg/ha) of the $_i$-th observation
PS_i Pure seed (%) of the $_i$-th observation

Concerning the methods of the mechanical weed control, also for the pure seed yield significant differences between hand roguing and topping were consistently detected through time, while the pure seed yield of hoed and brush hoed plots did not longer differ in the second year from the hand-rogued variant. Even in the topped variant, the seed production remained in the second year near the 500-kg/ha-threshold. Considering the effect of the harvesting methods, hand-harvesting and combine harvesting showed equal efficiency, while high seed losses were evident for the stripped/vacuum-harvested variant.

Purity analysis

The proportion of pure seed in the yield was influenced by harvesting method and year, while the effect of all main factors was detected by the analysis of variance of other seeds. All interactions were not significant for both variables.

Table 18: Effect of main factors (weed control method, harvesting method and year) and their interactions on seed quality of *Festuca nigrescens* (repeated measures ANOVA: [n.s.]=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001).

		Pure seed [a]		Other seeds [b]	
Source	df	F	Sig.	F	Sig.
Between-subjects effects					
Weed control method (W_C)	3	2.47	n.s.	5.59	**
Harvesting method (H_M)	2	35.96	***	101.42	***
W_C x H_M	6	0.91	n.s.	0.78	n.s.
Block	2	3.73	n.s.	2.97	n.s.
Error	22				
Within-subjects effects					
Year (Y)	1	74.65	***	4.42	*
Y x H_M	2	1.36	n.s.	0.03	n.s.
Y x W_C	3	0.89	n.s.	0.29	n.s.
Y x H_M x W_C	6	0.72	n.s.	1.09	n.s.
Y x Block	2	1.67	n.s.	0.52	n.s.
Error	22				

[a] arcsine-transformed data
[b] logarithm-transformed data

Percent of pure seed ranged between 92.6% and 87.8% depending on the weed control method, although differences were not significant. Greater differences were instead caused by the harvesting methods. A satisfactory purity was achieved in hand-harvested plots (95.4%), that of the stripped/vacuum-harvested yield being only 2% lower. The purity of combine-harvested yields was instead relatively low (82.2%). In the second year the seed purity increased consistently in all treatments: a 6%-increase was observed on average.

The proportion of other seeds remained instead unchanged in both years. Hand roguing resulted in a mean seed content around 3%, while the yield of topped plots had almost a double content of seed of weeds. Hoed- and brush-weeded plots exhibited intermediate values around 4%, not differing from the extremes. Larger differences were caused instead by the harvesting methods. The manual harvest limited the content of other seeds to a minimum (1.5%), indicating that despite of the selective harvest performed, some culms of other species were nevertheless included in the yield material to be threshed. The yield obtained through stripping/vacuum harvesting, that was quantitatively far lower than that of the other variants, showed also relatively low content of other seeds (around 4%), thus denoting a relatively good seed quality, if it is considered that the cleaning process was limited to a minimum in order to avoid seed losses of the crop plant. In the combine-harvested treatment the proportion of other seeds was instead a threefold of it (13.3%). *Lolium perenne* and *Alopecurus pratensis* were in both years the main weed species occurring in the seed yield and their share was around 6% each in the combine-harvested variant.

Table 19: Seed quality (percent of pure seed and other seed) of *Festuca nigrescens* depending on weed control method and on harvesting method. Mean separation by Tukey's HSD at α=0.05. Means without common letters in a column are significantly different.

	Pure seed [1] (%)	Other seeds [2] (%)
Weed control method		
Hand roguing	92.6 [a]	3.0 [b]
Hoeing	90.5 [a]	4.3 [ab]
Brush weeding	91.0 [a]	4.5 [ab]
Topping	87.8 [b]	6.4 [a]
Harvesting method		
Hand-harvesting	95.4 [a]	1.5 [c]
Combine harvesting	82.2 [c]	13.3 [a]
Stripping/vacuum harvesting	92.1 [b]	4.2 [b]

[1] post-hoc tests performed on arcsine-transformed data, back-transformed means are shown
[2] post-hoc tests performed on logarithm-transformed data, back-transformed means are shown

Seed weight

The TSW of *Festuca nigrescens* did not change significantly from one year to the other if the base seed was compared to the seed manually harvested in 2001 and in 2002 (ANOVA, $F_{2,21}=1.392$, $p>0.05$). In 2001 the mean TSW was 1.14 ± 0.04 g, in 2002 1.13 ± 0.05g.

Table 20: Mean seed weight of *Festuca nigrescens* harvested at the Meierbreite in 2001 and in 2002 depending on the harvesting method. Average of 16 replicates. Mean separation by Tukey's HSD at α=0.05. Means without common letters are significantly different.

Harvesting method	TSW ± SD (g)
Hand harvesting	1.13 ± 0.04 [a]
Combine harvesting	1.10 ± 0.06 [ab]
Stripping/vacuum harvesting	1.08 ± 0.04 [b]

Analysing the seed weights depending on the harvesting method and on the harvest year, no change was observed through time, while a significant effect of the harvesting methods was detected by a two-way ANOVA ($F_{2,42}=9.375$, $p<0.05$). The seed obtained through stripping/vacuum harvesting resulted significantly different from the manually harvested seed.

Seed germination in laboratory

Analogously to what observed for the seed weight, differences in seed germination were not significant between base seed and the seed yield of the two following harvest years (manually collected seed; ANOVA, n=4, $F_{2,12}$=3.706 $^{n.s.}$). The seed germination was in all three years around 95% (97% in 2000, 94% in 2001 and 95% in 2002). Considering the effect of the harvesting methods on the seed germination, no significant difference was found between seed lots harvested with different methods (ANOVA, n=4, $F_{2,12}$=1.140 $^{n.s.}$). Stripped/vacuum-harvested seeds germinated as good as manually or combine-harvested seeds. The seed germination on average was 94 %.

1.4 Discussion

1.4.1 *Trifolium alpinum*

The results of the investigations about the susceptibility against plant pathogens strongly suggest that the cultivation of *Trifolium alpinum* can be seriously endangered by nematodes. The phytophagous nematodes of the genus *Pratylenchus,* found in all samples of the Meierbreite, are able to penetrate in the root tissues and to proliferate there. The tissues affected necrose. With the penetration of the nematodes are also provided new infection gates for other pathogens (Dropkin, 1980). The high values registered for *Pratylenchus* sp. in both soil- and root samples from the Meierbreite were over the damage threshold and a causal relationship between their occurrence and the symptomatic observed is very likely. This hypothesis is also corroborated by the fact that these nematodes were not found in healthy plants from other locations (Meran 2000, Meißner) and in the corresponding soils. The other nematodes (*Aphelenchoides* sp.) found in these samples are saprophytic and can be found in soils with a large amount of organic matter. *Thielaviopsis basicola*, found as well in all examined plants from the Meierbreite, is known to be the primary agent of the root rot disease in various crop plants (Smith et al., 1988). In the case examined it is impossible to determine, whether this fungus caused the symptoms described and to which extent, but the penetration of *Pratylenchus* sp. in the roots has surely made easier the infection by *Thielaviopsis basicola*. Further investigations are required to elucidate the causal effect between the pathogens and the symptoms (see chapter 2 about this subject). The whole results presented here are likely to be strongly affected by the nematode infection occurred at the end of the summer 2000. The drastic density decrease of *Trifolium alpinum* following the nematode infection suggests that this clover species is extremely susceptible against *Pratylenchus* sp. and that in case of infection an almost complete loss of the crop plant has to be expected.

A further crucial point for the cultivation of *Trifolium alpinum* in an organic farming context is the very slow growth of its epigeal biomass. This has been observed by other authors (Weilenmann 1981; Flüeler 1992) in greenhouse trials, the growth strategy of this species consisting mainly in the production of hypogeal organs (Gallmetzer and Florineth 1996). The low cover scores (2.85%) achieved in the most favourable variant in the field trials at the Meierbreite about three months after the trial set up clearly denote its scarce competitive capacity.

Concerning the mechanical weed control methods tested in the present experiments, a premise about the variants with an inter-row tillage is necessary. The decreases of density of *Trifolium alpinum* observed in the hoeing- and brush weeding-variants after the performance of the mechanical weed control indicate that such methods damaged the plants in the rows. This may be explained by the fact that in the manual positioning of the seed stripes the necessary accuracy in the inter-row distance was not achieved. Similar damages are not noticeable in the hand-roguing variant, where inter-row hoeing was manually performed, and in the topping variant, where no kind of inter-row tillage took place. Adequate mechanised techniques allowing to maintain precise and constant row width would help to minimize damages of this kind. It is remarkable that the density of *Trifolium alpinum* of the topping- and hand-roguing-variants did not differ significantly two weeks after the second intervention, although cover clearly did and the weed cover in the topped plots was on average higher than 50%. It seems that at this stage the crop plant was still able to remain vital, even under high competitive pressure. A similar behaviour of *Trifolium alpinum* was observed by Lamesso (verb. comm.) in field trials carried out by the Forage Crops Department of the Institute of Genetics and Agriculture "N. Strampelli" (Vicenza, I).

In the present experiment hoeing proved to be slightly more effective than brush weeding in controlling the density of weeds, but not their cover. Nevertheless, both seem to be unsuitable weed control methods, because after the second intervention the weed cover was very high in both treatments and it does not differ from that observed in the topped plots. Moreover, in hoed and brush weeded plots the weed cover strongly increased after the weed control, although at the same time their density did not change. This suggests that the plants already located within the rows and those too deeply rooted to be eradicated by the shares or by the brushes, quickly expanded in the space that was still available, until a cover of about 50% was achieved, and competition effects between weeds occurred. Very small changes of weed cover were indeed observed after topping, where scores of 50% on average had been already achieved in the plots before the weed control. Control of any of the problem weeds by hoeing or by brush weeding was unsuccessful, as relatively strong cover increases occurred after the intervention, and cover scores similar to those of the topping variant were achieved. Among the methods investigated, hand-roguing was the only one showing satisfactory results in controlling the weeds, but the high manpower-costs connected with its employment make it unsuitable for a commercially production of *Trifolium alpinum*.

The crop plant occurred in the second growing season only in the plots where manual weeding had been performed in the year before. Its absence in all other treatments may be due to the fact that the plants already weakened by the nematodes were easily overgrown by the weeds and could not stand the competition for light and nutrients. The density decrease observed from 2001 to 2002 regarded plants that were kept free from competition by weeds by manual weeding. It is therefore likely that the mortality observed was related to effects of the nematode infection, rather than to competition effects.

The cultivation of *Trifolium alpinum* in lowlands resulted in a precocious achievement of the reproductive phase by the plants. This may be related to an increased growth and development rate under favourable climatic and nutritional conditions. A minimum plant size must be generally achieved by plants before reproduction takes place (Samson and Werk, 1986). Hasler (1992) indicates a minimum stage of six ramets as threshold for the beginning of flowering in *Trifolium alpinum*. At the Meierbreite seed was consistently produced already in the second year, while at high altitude flowering was still rare in the four-year-old plants, which had achieved the minimum size (Hasler, 1992). The effects of the nematode infection may contribute to explain why plants flowered only in the second year and no flowering took place in the third year. They may have been debilitated to a such extent in the third year, that they were unable to produce the necessary amount of photosynthetic active structures to sustain the flowering processes.

The TSW of the wild population used as source for the propagation experiments at the Meierbreite (5 g) closely resembled those measured by Hegi (1964) and Flüeler (1992) in the Swiss Alps, and differed slightly from that (4.6 g) assessed by Urbanska and Schütz (1986). The seed weight seems therefore to be a relatively constant feature in the natural environment.

A seed weight reduction for seed of alpine legumes propagated in lowlands was generally observed by Krautzer (1995a) and by Flüeler (1992), so that the seed weight reduction of *Trifolium alpinum* observed in the present study after the cultivation in lowlands is in accordance with the previously published data. It remains unclear, whether the nematode infection may have played a role in determining the seed weight decrease.

The results of the germination tests confirmed what already observed by various authors (Lüdi, 1932; Weilenmann, 1981; Flüeler, 1992; Gallmetzer and Florineth, 1996). *Trifolium alpinum* germinates at a very low, more or less steady rate if seeds are not scarified, whereas scarification of the seed coat leads to an extremely fast, virtually complete germination. Dormancy in most *Leguminosae* is caused by the occurrence in the seed coat of a thick-walled palisade layer formed by the so-called Malpighian cells (Werker, 1997), which are impregnated with water-repellent substances as cutin, suberin or ligning (Rolston, 1978) and make therefore the seed coat impermeable to water. In hard seeds water exchange with the surrounding environment is regulated by the hilum, which acts as an hygroscopical-activated valve, allowing water loss in dry conditions without permitting water uptake in a moist environment (Hyde, 1954), thus preventing germination. Such kind of dormancy can be defined as innate according to the definition of Urbanska (1992), as the seeds do not germinate after the separation from the mother plant despite of a favourable environment. The statement of Gallmetzer and Florineth

(1996) that intact seeds germinate better in the darkness could not be confirmed here.

The germination test with razor blade- and sandpaper-scarified seeds gave some indications about suitable techniques for seed scarification. Assuming that the razor blade-variant represents the most accurate kind of scarification (there is a direct control of the whole process), the absence of differences in germination between the methods and the lack of significance in the percentage of abnormal seedling prove that sand paper-scarification is just as reliable in breaking seed dormancy in *Trifolium alpinum*, with the advantage of being less time-consuming. A possible alternative, not tested here, for breaking the dormancy of legumes is represented by the short immersion of the seeds in boiling water (Villiers, 1972). This technique was successfully tested also on *Trifolium alpinum* but it seemed to be less effective than the mechanical scarification, because it led to the death of some of the embryos and subsequently to slightly lower germination percentages (Flüeler, 1992).

About the seed germination of lowland-propagated alpine leguminous species literature data are discordant. Krautzer (1995a) observed for most species an increase, while Flüeler (1992) reported a deterioration of the seed germination, despite of the unchanged seed viability. Also in the present case study the large decrease in seed weight of the propagated seed had no influence on the seed viability, which remained about 100%, as shown by the germination curve of the scarified seeds. It is not possible to give a definitive answer about the cause of the seed germination increase of the untreated seeds harvested at the Meierbreite, but it may be due to the methods used for cleaning the seed, as shown by the seed germination increase after the use of the laboratory thresher. More evidence would be required to assert it with certainty. A source of uncertainty is the fact that the germination values achieved are higher than those observed for the seed harvested at the Meierbreite (85% vs. 55%), although it was cleaned in the same way. These differences are possibly due to the different seed size of the two seed lots and to the fact that the seed of the lowland-provenance was still included in the pods. These could have acted as a screen between the seed surface and the angle bars of the threshing unit, reducing lesions of the seed coat.

The slight mid-term viability losses of scarified seeds under proper storage conditions through a lapse of time of 30 months confirms what already observed by Flüeler (1992) for most of the other alpine legumes. If stored at low temperatures around 6°C by low air moisture, the seeds maintain to a large extent their germinative capacity for a relatively long time, even if a seed coat scarification removed the mechanical obstacle to aqueous and gaseous exchange between embryo and external environment. The deterioration of the seed viability proceeds for scarified seeds slightly faster than for intact seed.

The increase of the seed germination and of the germination rate of *Trifolium alpinum* subsequently to seed propagation at lower altitudes is a feature with very important applications for the use of this legume in seed mixtures for restoration at high altitude:

- Seed that has been produced at low altitude and that has been mechanically cleaned with devices that could damage the seed coat has to be considered as scarified seed. The first consequence of it is that particular care must be taken in the seed storage, because the impermeable seed coat ensures high longevity by allowing retention of low moisture content in the seed and thus preventing desiccation of the mature embryo (Werker, 1997). As shown by the experiment with scarified seeds, if the seed is stored at low temperature, ist quality will be mid-term preserved. The second consequence is that scarified seed germinates homogeneously at once in the field, regardless of the meteorological conditions, as observed in the field propagation trials at the Meierbreite and, at high altitude, by Flüeler (1992). The seedlings could therefore desiccate because of lack of water or be exposed to freezing temperatures in a developmental stage, in which they are particularly vulnerable. Flüeler (1992) observed a good seedling emergence followed by an elevated mortality caused probably by soil freezing and consequent ripping of the fine roots, leading to the death of the plantlets. Adverse climate conditions at this time are likely to affect the whole seedling population established by seeding. For these reasons, scarified seed should not be used for autumnal seeding. It has to be kept in mind that, as shown by the germination tests, the mechanical cleaning does not result in a complete scarification of the seed. A certain seed aliquot, depending on the cleaning methods and on the intensity of the cleaning process, would therefore be able to germinate later in the field.
- Seed scarification leads to a cost increase. In the case of seed of *Trifolium alpinum*, which is expected to be very expensive because of the difficulties connected with its propagation, avoidance of costs would be a crucial point to increase acceptance of its employment in seed mixtures by restoration companies. A partial seed scarification connected with the seed cleaning can be therefore positively considered from this point of view, because an additional step for its scarification would be not necessary.

1.4.2 *Sesleria albicans*

Sesleria albicans showed a very slow growth under the favourable climatic conditions of the lowland. In both *Sesleria*-trials the crop plant achieved in the first growing season extremely low covers in the first developmental stage, even in the most favourable conditions, here represented by the hand-roguing variant (0.68% approximately four weeks after the emergence in *Sesleria albicans* I and 0.39% approximately four weeks after the emergence in *Sesleria albicans* II).

Plant size increases were observed at the Meierbreite from year to year through the whole observation period, confirming that the achievement of adequate cover scores, allowing the plant to compete with the weeds, requires at least three years. For most of the other alpine grasses investigated by Krautzer (1995a) in seed propagation trials, the maximum plant size was achieved instead already in the second growing season. *Sesleria albicans* has a low relative growth rate: according to Grime and Hunt (1975) its R_{max} is 0.75 g/g/wk. In natural conditions the root : shoot ratio is >1 for young plants and >5 for adult plants (Blaschke, 1991b), showing that energies are primarily invested in the hypogeal biomass production. Concerning the clonal growth, discordant results are reported in the literature: it was found to be slow by Urbanska et al. (1987) in a single tiller cloning experiment, the production of new tillers being virtually absent for six weeks after transplantation, while Vescovo (2000) reports a triplication of the tiller number in about three weeks. In a comparative experiment in a controlled environment with some lowland- and upland-species of the genus *Poa,* the cause of the inherently slow growth of the alpine taxa was identified by Atkin et al. (1996a) in the lower specific leaf area, which is a consequence of increased leaf thickness. This reduces the leaf area available for carbon gain per unit leaf dry mass. As respiration rates were found to be independent from the relative growth rate, it was also concluded that alpine species use a larger proportion of the fixed carbon for respiration. Increase of leaf thickness and decrease of specific leaf area is a widespread feature of alpine plants (Körner et al., 1989). Woodward (1979) found out that the growth of *Sesleria albicans* is faintly stimulated by temperature increases because leaf-initiation and leaf-expansion are insensitive to it. The cultivation at lower altitudes does not result therefore in a considerably increased growth speed. Moreover, the nitrogen fertilisation allows barely growth speed increases, as upland grasses exhibit reduced nitrogen productivity (amount of biomass accumulation per mole organic nitrogen and time) (Atkin et al., 1996b), suggesting that nitrogen supply may represent an advantage for weeds rather than for the crop plant. As acceptable cover values of the crop plant were first achieved in the third year, a duration of the culture exceeding three years should be plausible.

The growth of *Sesleria albicans* is stimulated by cuts, as plants are able to overcompensate the loss of biomass (Rainer and Erschbamer, 1996) and large tussocks are then produced after the cessation of grazing or clipping (Dixon, 1982). Similar conditions were provided in the trial at the Meierbreite, where the plants were cut close to the ground at the end of the summer. They showed in the following year consistent cover increases.

A general impediment to an optimal performance of the mechanical weed control is the long period required between seeding and emergence, which can amount up to 7 weeks and increases with increasing seeding depth. This makes very difficult a mechanical weed control in due time when the weeds are in an initial stage of development, because the rows are not visible yet. As shown by the results, marking one of the rows with a string prevents the accidental removal of part of the crop seedlings by the hoe or by the brush weeder, although it is connected with a considerable increase of the manpower necessary for this task.

Concerning the efficacy of the weed control methods, similar considerations as for *Trifolium alpinum* can be made. Considering together the results of both trials, at the time of the first weed control it seems possible to control the weeds to a certain extent by hoeing and brush weeding by reducing the density of weeds, but not their cover, which showed a great relative increase after the intervention. At this time weed cover values were still near the 5%-threshold. Before the second weed control intervention the weeds in the topped variant already exhibited cover scores over 50% on average, and competitions effects probably prevented the establishment of new weeds or the cover expansion of those already established in the plots in the time between the assessments. On the contrary, the weed cover in the hoeing- and brush weeding-treatments increased strongly without large variations of their density, indicating that the weeds, prevalently located within the rows, expanded their cover. It seems that at this stage a control of the weed cover becomes impossible by the mechanical practices investigated. Hoeing and brush weeding do not show at any stage differences in their effectiveness against weeds, the differences observed between them being of little interest. The divergence from the effectiveness of these implements observed by other authors in the weed control in cereals (Koch, 1964a; Koch, 1964b; Schmid and Steiner 1989) or vegetables (Geier and Vogtmann, 1988a) are to ascribe to the different crop plants, which are able to withstand the competitive weed pressure. The crucial point seems to be the competition within the rows, where weeds are not affected by the action of the hoe shares or of the brushes and are able to overgrow *Sesleria albicans*. Hand-roguing is therefore among the investigated weed control methods the only one showing satisfactory results. In this case, its coupling with a preliminary hoeing between the rows reduces considerably the manpower commitment, which remains nevertheless a serious obstacle to the commercial seed propagation of this species. Without manual weeding, no seed production of this species in an organic-farming-context seems to be possible.

Differences from year to year in the botanical composition of the weed flora and in the abundance of single weeds may be caused by seasonal fluctuations of single species and to succession processes taking place from year to year. The faint competition exerted by the crop plant and the partial effectiveness of the mechanical weed control methods investigated made possible a naturally-like succession within the plots. Van Elsen (1994) found analogous strong increase through time of *Lolium perenne* and *Taraxacum officinale* and decreases of *Viola arvensis*, *Matricaria recutita* and *Chenopodium album* in fallow land. The strong occurrence of

Lolium perenne is due to a seed propagation of local ecotypes, which was carried out there at the beginning of the 90´s. Since then, the occurrence of this species was observed as weed in the crops preceeding *Sesleria albicans*.

The production of fertile culms in the present experiments was related to the size of the mother plants. This took place only if the plants had achieved a minimum size (around 10 cm tussock diameter). A relationship between tussock diameter and panicle numbers similar to that observed at the Meierbreite was observed for a British ecotype grown in a controlled environment under different water regimes (Dixon, 1996), suggesting that this threshold is a common feature of this species and it was not bound to growth conditions or to the seed provenance. In *Sesleria albicans* II, which was sown six weeks after *Sesleria albicans* I, the main tussock size was in the second year about 4 cm lower than in *Sesleria albicans* I, and this resulted in a mean seed production that was more than 75% lower. As a consequence for the practice, seeding in the first year should not take place later than at the beginning of May, in order to allow the achievement of the minimum size, if at least a small seed production in the second year is desired.

In contrast to seed yield amounts of about 120 kg/ha obtained by Krautzer (1995a) with pot-grown plantlets transplanted in the field, a very low seed production was achieved in the present experiment. Even the amounts obtained in the third cultivation year are about 1/4 of it, indicating that the advantage gained by pot-grown plantlets during the greenhouse-phase in absence of weeds competition and with optimal water supply is an important factor for achieving lower seedling mortality in the field and in turn more dense plant stands and satisfactory seed yield. On the contrary, as observed in the course of the experimentation at the Meierbreite, the achievement of optimal seedling densities is a difficult task by seed drill.

No definitive answer was gained in the present experiment about adequate large-scale harvesting techniques. Threshing, as defined in the present experiments, seems to be a suitable harvesting technique, but the necessity of doing it in several steps (mowing, drying, sieving and threshing) increases considerably the costs. The use of a combine harvester would require an increase of the cutting height, in order to avoid too high amounts of foliar biomass to be processed, and therefore accepting higher seed losses caused by short or prostrated culms, which would not be clipped by the mowing bar. Further tests in bigger plots would be required to get a definitive answer about it. The indeterminate flowering of *Sesleria albicans* in lowlands would suggest a suitability of the stripping/vacuum harvesting for a repeated harvest of the plant stand, in order to increase the amount of seed and to include in the yield seeds with a as large as possible range of phenotypes. The results show that the implement used in the present experiments has still a wide improvement need, seed losses being too high for achieving acceptable seed yields. Moreover, seed ripening was observed to be more or less concentrated at the time of the first harvest (end of May) and to become more markedly indeterminate during the summer. As the seed quality of the late harvests was spoiled by the sclerotia of *Claviceps purpurea* and it seems therefore advisable to carry out a single harvest, the advantages of a repeated stripping/vacuum harvesting may be of limited importance, even if this harvesting technique would be refined and optimised.

The satisfactory seed germination obtained for untreated samples of the wild provenances used for the experiments at the Meierbreite are in accordance with the results of a number of tests performed by various authors (Lüdi, 1932; Fossati, 1980; Schütz, 1988; Krautzer, 1995a). An exception are the very low values reported by Vescovo et al. (1998) for an ecotype of the dolomitic area. They are considerably lower than those obtained for the provenance Monte Baldo, but in both cases a treatment with gibberellic acid resulted in an at least doubled germination percentage. Gibberellic acid is included in the class of hormones of the gibberellins, which are directly involved in the control and promotion of seed germination (Ziegler, 1991). They are found at relatively high concentration in developing seeds but usually drop to a lower level in mature dormant seeds; if applied to mature seeds, they are known to relieve seed dormancy (Hartmann et al., 1990). It can be concluded that the occurrence of physiological dormancy in *Sesleria albicans* is not a common feature, although there are indications for its occasional occurrence.

The seed propagation in lowlands proved to result in an increased seed quality. The maternal plant environment is reckoned to be in general partly responsible for variations of seed weight and seed germination (Werker, 1997). At high altitudes the life cycle of the plants is strongly influenced by the extreme climatic conditions. Factors as the duration of the growing season or the occurrence of extreme temperature events can be crucial for a plant to accomplish the seed ripening process. Great variations of the amount of filled seeds, of the seed viability and of the seed germination are possible at the same site from year to year depending on the severe nature of the environment (Chambers 1989). Hermesh and Acharya (1992) demonstrated that favourable temperatures at the time of the seed ripening result for *Poa alpina* in higher seed weight and seed germination. Such seed quality improvements under climatic conditions more favourable for the plant growth, were observed for a number of alpine grasses after a seed propagation at lower altitudes (Krautzer 1995a) or after the cultivation in botanical garden (Urbanska and Schütz, 1986). The present experiments provide evidence that also the seed weight of *Sesleria albicans* is largely influenced by the climatic conditions in which the mother plant produced the seed. Similar patterns in the seed weight fluctuations were observed in *Sesleria albicans* I and *Sesleria albicans* II, which were exposed to the same environment during the experimentation. The theory is also corroborated by the fact that the seed weights achieved in 2002 under the same climatic conditions are more or less the same for all four provenances investigated (*Sesleria albicans* I, *Sesleria albicans* II, Monte Baldo and Monte Cavallo), regardless of the seed weight assessed before the propagation. The negative correlation between TSW and altitude is explainable with the fact that the altitude gives indeed a rough estimate of the climatic conditions of the harvest location, as temperature is known to decrease of about 0.55°C for each 100 m-increase of altitude (Landolt, 1992). The climate is nevertheless strongly influenced also by other factors, such as latitude or macro- and microtopography (the latter is crucial at high altitudes). Particularly important appears to be the temperature and the rainfall in the period, in which seed ripening occurs. In May 2002, which was quite rainy and exhibited higher mean air humidity in comparison to the values of 2001 for the same month, significantly lower seed weights were achieved in comparison to the previous year. High rainfall in the latter stages of seed maturation results often in increased seed respiration and, in turn, in reduced seed weight (McDonald and Copeland, 1997). Also the date of harvest may possibly be important, because a delay would allow the seed to gain further weight, or ripe seed could be shed before being harvested. As the seed germination is in turn positively correlated with the seed weight, it appears evident that the seed propagation at lower altitudes results in an advantage if the seed is used in seed mixtures for the restoration at high altitudes. In such situations an as large as possible seed germination and establishment is required, in order to rapidly achieve protection against soil erosion.

The problems arising during the seed propagation of *Sesleria albicans* in an organic farming context and the conclusions for the practice can be summarised as follows:

- All mechanical weed control methods tested are not effective in controlling the weeds and do not represent an alternative to the manual weeding. A commercial seed propagation of this species according to the guidelines of the organic farming seems not to be advisable because of the manpower costs connected with it.
- *Sesleria albicans* must be sown early enough in the spring, because it grows very slow. If the plants in the second year have not achieved a minimal size (a tussocks diameter of at least 10 cm), they do not produce floral stems at the beginning of the growing season. Beginning of May proved in my experiment to be an adequate sowing date.
- This species starts to flower already at the end of the winter and requires a long time for attaining the seed ripeness (from the beginning of March until end of May).
- Due to the slow growth and consequently faint competitive ability against weeds, weed control interventions would be necessary also in

the second year, but early flowering makes impossible the use of any mechanical implement, in order not to damage the floral stems. During this time weeds are able to overgrow the crop plant.

- Ripening occurs relatively homogeneously within the spikelets, but new floral stems are produced throughout the whole growing season, the seed ripening being relatively concentrated at the end of spring and less concentrated later on. Ripe seeds fall easily from the mother plants. In the last part of the ripening process seed losses caused by intense precipitation or hail can be considerable. Reckoning the optimal time point for harvesting is therefore problematical and compromises between seed quality and seed amount are necessary.
- High cover of the crop plant was achieved only in the third year. The row spacing adopted in this study (24 cm), which is the minimum required for the use of hoe and brush-hoe, proved to be too large for *Sesleria albicans*. Weed control interventions would have been required even in the second year. In a conventional farming smaller row spacing could be tested.
- Phytosanitary problems are caused by *Claviceps purpurea* and rusts. The latter seem to depend on seed provenance (high altitude provenances proved to be more susceptible than ecotypes collected at lower altitude) and can cause high mortality of the crop plant.
- *Claviceps purpurea* infections spoil considerably the seed quality of the late harvests, so that only one seed harvest towards the end of the spring is advisable, despite a continuous flowering throughout the whole growing season.
- Combine harvesting seems to be a suitable technique for harvesting, but it can become problematic if a small biomass is processed, i.e. in case of small propagation plots or low density of the crop plant. Incorporating the foliar mass, in order to minimize seed losses can lead to higher costs for seed cleaning.
- The seed production of drilled plots is exiguous: in the second year is below 10 kg/ha; in the third year seed yields of 35 kg/ha can be achieved.

1.4.3 Festuca nigrescens

The experimental question about the effectiveness of the different weed control methods on the weed species can not be resolved on the basis of the present experiment, as no specific investigations about cover and density of weeds were performed, but their effect on seed yield and seed quality can be evaluated.

In accordance to what stated by Krautzer (1995a), *Festuca nigrescens* was confirmed to be a very competitive species. Even in the plots that were only topped, a yearly mean yield of 500 kg/ha was achieved, indicating that even a simple limitation of the weed size during the first developmental stage of *Festuca nigrescens* is sufficient for allowing the crop plant to establish and later to compete successfully with the weeds. This is strikingly contrasting with the very poor competitive attitude of *Trifolium alpinum* and *Sesleria albicans*. A further positive feature of this species is that no important phytopathological problem was assessed in the observation period, small rust infections remaining of very little importance. Large difference in the susceptibility to rust were observed for this species by Krautzer (1995a) depending on the provenance.

As a preliminary remark it has to be pointed out that because of the occurrence of margin effects, caution is required in comparing the seed yield obtained at the Meierbreite with those reported by other authors.

The maximum seed production achieved in this experiment is similar to that reported by other authors for conventionally produced seed: *Festuca nigrescens* is considered to have a high potential for seed production (Werder, 1996) and seed yields of more than 1,000 kg/ha are not unusual for the first harvest (Krautzer, 1996b). A difference with the literature data was instead observed in the yield decrease from the first to the second harvest: the decrease assessed at the Meierbreite is clearly inferior to that reported by Krautzer (1995b; 1996b). The second yield at the Meierbreite was on average about 73% of the first one, whereas values of 30% to 60% are reported in the above-mentioned literature. The lower seeding rate used in the present experiment, being respectively about the half of that adopted for the Austrian propagation trials (10-12 kg/ha), may have resulted in a reduced intraspecific competition, thus allowing the mother plants to achieve a greater yield in the second harvest year.

A satisfactory mean seed production of more than 600 kg/ha per year was assessed on average in the hoeing and brush weeding treatments. Lower yields, with respect to the manually weeded variants, were found only in the first year. The absence of differences between treatments in the second year is likely to be mainly due to the cessation of weed control interventions following the expansion of the crop plant and the complete occupation of the space available for growth. Considering the pure seed yield amount, it is therefore concluded that standard mechanical weed control methods are suitable for the organic seed production of *Festuca nigrescens*.

Concerning the harvesting methods, the prototype used for stripping/vacuum harvesting exhibited also in this experiments very high seed losses (around 50% in comparison to the other treatments), whereas combine-harvesting proved to be as efficient as the most accurate method, represented by the manual harvest, if the pure seed yield is considered. As *Festuca nigrescens* attains homogeneously the seed ripeness, the performance of repeated harvests in the same growing season by stripping/vacuum harvesting, in order to maximize amount of and genetic variation within the yield, appears to be superfluous.

The major problem in the organic seed production of *Festuca nigrescens* is represented by the achievement of a sufficient seed quality. Krautzer (1995b) has proposed for this species a minimum seed purity of 90%, with an allowed occurrence of other seeds not exceeding 2%. Considering plausible standard combinations of methods for the praxis, such as hoeing or brush weeding as weed control method and combine harvesting as harvesting method, a satisfactory seed production can be expected, but the proportion of weed seeds observed would not be acceptable. Combine-harvested plots yielded seed lots with percentages of other seeds above 10%. However, it has to be recalled that in the present experiment the cleaning process was intentionally maintained at a minimum, in order to evaluate the potential seed production. Depending on the implements available for seed cleaning, increased seed purity can be achieved with variable loss of seed of the crop plant. In the case of too high seed losses of the crop plant during the cleaning process, stripping/vacuum harvesting may represent an alternative to combine harvesting; as counterpart to high seed losses, a better seed purity can be achieved. The seed weight decrease observed for stripped/vacuum-harvested seed lots does not represent a decrease of the seed quality as it did not result in a decrease of the seed germination. A first possible reason for the reduced occurrence of seeds of weeds in the stripped/vacuum harvested variants seems to be the suction power of the vacuuming device, which proved to be insufficient for collecting the heavy seeds of both the crop plant and the weeds. This is demonstrated by the smaller seed weight of the stripped/vacuum harvested variant in comparison to that of the other treatments. As a general rule, weed seeds with TSW greater than that of the crop plant are not vacuumed and are subsequently lost. A second reason for the reduced weed amount in the seed yield is that weed species with a ripening time differing from that of *Festuca nigrescens* (i.e. *Lolium perenne*) are not harvested, as the stripping device is set in order not to detach unripe seeds from the mother plant. A combine would collect instead the whole culms by mowing, the unripe weed seeds would be processed by the threshing unit, pooled with the other seeds and could possibly complete the ripening process during seed conditioning. Thus an optimization of the stripping/vacuum harvesting device would likely result in an increase of the weed proportion in the seed yield.

The increase of the proportion of pure seed from 2001 to 2002, associated to a very small decrease of other seeds is not explainable with certainty. A possible hypothesis is that fluctuations of the percentage of filled seeds can be expected from one year to the other. Growth temperatures, for example, are reported to influence floret fertility in perennial ryegrass (Bean, 1980), but very similar meteorological data were recorded in 2001 and in 2002 at the Meierbreite at the time of seed ripening. A further possibility is that slightly different settings of the laboratory thresher used for cleaning the seed allowed better results in the second year.

The seed germination observed for both harvest years can be considered satisfactory and meets the suggested acceptability standard of 75% (Krautzer, 1995b). The absence of germination increases after the seed propagation are not surprising, as the base seed used for the experiments had been obtained from a previous seed multiplication. For the wild seed a lower germination is reported by other authors (Wilhalm, 1990; Krautzer, 1995a), the results ranging between 50% and 76%. Large germination increases of 39% on average following seed propagation in valley locations were observed by Krautzer (1995a). The considerations about the influence of the maternal plant environment on seed germination made in the previous paragraph seem therefore to apply also to *Festuca nigrescens.* Further evidence about the influence of the maternal plant environment on the seed germination of this species is provided by the results of the fertility experiments described in paragraph 3.2.16. The lowland-propagated seed used in the field trial at the Sudelfeld had a germination capacity of 88% to 94%, while that produced by the resulting mother plants at the restoration site in a climatically less favourable environment achieved, under the same test conditions, only 68%.

2 Basic investigations about the plant growth of *Trifolium alpinum*

2.1 Introduction

Most legumes growing in the alpine area in the subalpine and alpine vegetation belt can be found on basic to slightly acidic soils. *Trifolium alpinum* represents an exception, being one of the few legumes that can be found at high altitudes in the Alps on acidic soils. In the natural environment, *Trifolium alpinum* contributes effectively to the nitrogen cycle through nitrogen fixation (Arnone, 1999; Jacot et al., 2000a). *Trifolium alpinum* is considered to be an important species for reintroduction on siliceous sites above the timberline by seeding or planting (Flüeler, 1992). Beside the mechanical protection against erosion given by the above-ground biomass and the anchoring-action exerted by the tap root system, legumes may represent a nitrogen source for artificial plant communities resulting from revegetation programmes (Johnson and Rumbaugh, 1986; Holzmann and Haselwandter, 1988; Reeder, 1990). Reducing the nutrient input in restoration sites is particularly important at high altitudes, because the spontaneous re-colonization of native plants from the surrounding vegetation can be impeded by artificial fertilisation favouring more competitive species introduced by seeding (Urbanska, 1989; Tappeiner, 1996).

Hegi (1964) reported repeatedly failed attempts to cultivate this species. The results of the previous chapter confirm that the seed propagation of *Trifolium alpinum* is a task hard to accomplish. As described in paragraph 1.2.6, major difficulties due to infection of phytophagous nematodes and fungi were experienced in the field trial of *Trifolium alpinum*, so that the results obtained seem to be largely affected by the plant damage caused by pathogens. A reliable evaluation of the growth parameters of *Trifolium alpinum* is therefore not possible on the basis of the data collected in the field.

Moreover, the damage caused by the nematodes could have masked other growth disturbances caused by the different chemical composition and biological activity of the soil at the Meierbreite. One of the problems of the propagation of upland plants in lowland sites is the choice of an adequate cultivation site. Abiotic (physical and chemical soil characteristics, climate) and biotic parameters (soil biota) differing from those of the sites where the species naturally occurs may play a decisive role in determining the success or failure of propagation. Autochthonous soil collected at the original location and added to the growth substrate is an effective measure to achieve plant infection by *Rhizobium*. Weilenmann (1981) and Gallmetzer and Florineth (1996) observed in one to three-month-old pot-grown plantlets of *Trifolium alpinum* the formation of rhizobial nodules. Also the extraction of rhizobia from the autochthonous soil in a nitrogen free solution proved to be an effective method for the inoculation of a sterile substrate such as vermiculite (Jacot et al., 2000a).

Beside an adequate nutrient supply and the control of weeds and eventual diseases and pests, also the absence of noxious compounds in the soil is important. In nature, *Trifolium alpinum* is strictly bound to soils with poor lime content (Heß et al., 1970; Landolt, 1992; Adler et al., 1994). On lime-rich geological substrate it can thrive only over deep humus layers (Hegi, 1964). Weilenmann (1981) observed that plants of *Trifolium alpinum* grown in carbonate-rich soil severely suffered and exhibited damage leading to reduced growth and subsequently to death. Similar symptoms were exhibited by *Gnaphalium supinum*, an alpine forb typical of siliceous soil, if grown on calcareous soil in the greenhouse (Fossati, 1980).

The arable soil of the field at the Meierbreite is likely to be quite different from the humus-rich, strongly-acid soil of the original location in the Alps.

A further investigation on these topics seems therefore to be appropriate. For this reason experiments in a controlled environment were planned, in order to investigate some aspects related to the establishment and growth of *Trifolium alpinum* on soils with characteristics (pH, nutrient availability, occurrence of symbionts) differing to various extents from those of the soil at the original location.

The following experimental questions were addressed:

- Is it possible to grow *Trifolium alpinum* in soils with physical and chemical properties differing from those of the soils on which this species naturally occurs? Does this result in any growth advantage?
- Is the growth of *Trifolium alpinum* influenced by the occurrence in soil of symbionts such as *Rhizobium* or mycorrhiza? Does the introduction of these micro-organisms in the soil of the propagation site result in any advantage for the growth of *Trifolium alpinum*?
- Is it possible to introduce these micro-organisms in soils with characteristics different from those of the original soil?

On the basis of the information gained from the experiments devoted to elucidating the experimental questions formulated above, two further questions were addressed:

- Can *Trifolium alpinum* become P-limited in its growth on the autochthonous substrate if growth temperatures are increased?
- Is it possible to exclude a detrimental effect of the chemical composition of the soil of the Meierbreite on the plant growth of *Trifolium alpinum*? Were phytophagous nematodes the main cause of the plant decline in the field?

2.2 Materials and methods

2.2.1 Soil sampling and preparation

Soil around the root system of plants of *Trifolium alpinum* was excavated at the end of the growing season at the ski resort Meran 2000 (2,200 m a.s.l., St. Oswald, Hafling, South Tyrol, Italy), where the seed used for the seed propagation had been collected. The collection area is a former acidophilous scanty pasture (*Carici curvulae-Nardetum* according to Grabherr, 1993) on a steep slope, which has been excluded from grazing since the mid 80´s, in order to prevent erosion problems due to cattle trampling (Spatz, 1984; Löhmannsröben and Cernusca, 1987). Acidophilous grasses and graminoids, such as *Nardus stricta*, *Carex curvula* ssp. *curvula*, *Avenella flexuosa* and *Avenula versicolor* are common species. *Trifolium alpinum* is the only legume found at this site. The grassland vegetation is strongly colonised by dwarf shrubs (*Calluna vulgaris*, *Vaccinium vitis-idaea*, *Vaccinium uliginosum* and *Rhododendron ferrugineum*). Further soil samples were collected in the same season at the propagation site at the Meierbreite and at four locations in the mountain region of Harz (Lower Saxony, D) on acidic mother rock (grey wacke), assuming that they were likely to have soil properties similar to those of the original soil. Of the four samples collected in Harz, the one chosen (550 m a.s.l., Dammhaus, Clausthal-Zellerfeld, Lower Saxony, D) had a pH and water capacity closely resembling those of the original soil. Details about the collection sites are given in Table 21.

The soils were sieved through a 2-mm sieve in order to remove roots and stones and stored in plastic bags at 6°C in the dark until the experiments were started.

Table 21: Collection sites of the soils used as substrates for the experiments with *Trifolium alpinum*.

Location	Soil use	Altitude (m a.s.l.)	Grassland vegetation	Geological substrate
Meran 2000 - St. Oswald	abandoned pasture	2,150	*Carici curvulae-Nardetum* with dwarf shrubs	Ifinger tonalite
Dammhaus	abandoned meadow	550	*Trisetum flavescens*-meadow with *Nardus stricta*	Grey wacke
Meierbreite	arable field	220	-	Loess

2.2.2 Soil analysis

Soil pH was determined in 0.01 M $CaCl_2$ with a 1:2.5 soil-to-solution ratio (Schlichtling et al., 1995). Total C and total N were measured by gas chromatography after combustion at 1,200°C using a Carlo Erba ANA 1400 analyser. Exchangeable Ca was measured according to Mehlich (1942) after elution with an unbuffered 0.1 M $BaCl_2$ solution (Schlichting et al., 1995). Microbial biomass (C_{mic}, N_{mic}) was estimated after extraction-fumigation with $CHCl_3$ (Brookes et al., 1985; Vance et al., 1987). Values of K_{EC} (extractable part of microbial biomass C) K_{EN} (extractable part of microbial biomass N) according respectively to Wu et al. (1990) and to Brookes et al. (1985) were used. Measurement of ergosterol was performed by reversed-phase HPLC analysis after ethanol-extraction according to Djajakirana et al. (1996)[1]. Plant-available P and K were measured through CAL-extraction (Schlichtling et al., 1995).

2.2.3 Seed planting and growth conditions of plants

All seeds employed in the experiments described here (*Trifolium alpinum*, ecotype, harvest year 1999, Meran 2000, 2,200 m a.s.l., South Tyrol) were surface-sterilised by immersion in 70% ethanol for 5 min and in 5% sodium hypochlorite for 5 min. They were then repeatedly washed with distilled water and allowed to dry on filter paper at room temperature.

To ensure rapid and uniform germination, seeds were mechanically scarified with sandpaper and planted at a depth of 3 to 5 mm in the substrate. Until germination the soil was kept adequately moist by daily watering. Where vermiculite was used as a substrate, seeds were germinated on filter paper and three-day-old seedlings of uniform size were transplanted into the pots.

Experiments I and II were conducted in a growth chamber of the experimental glasshouse of the faculty of Ecological Agricultural Sciences in Witzenhausen with the following settings: 18/13°C, 60% relative humidity. Supplementary lightning at 10,000 lux was supplied to ensure a 16-hr day photoperiod. The temperatures set for the experiments have to be considered purely indicatively, as analysis of the data collected during the experiments showed that the temperatures inside the chamber were strongly influenced by external temperatures and by solar radiation.

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[1] Analyses were performed by G. Dormann at the Department of Soil Biology of the University of Kassel.

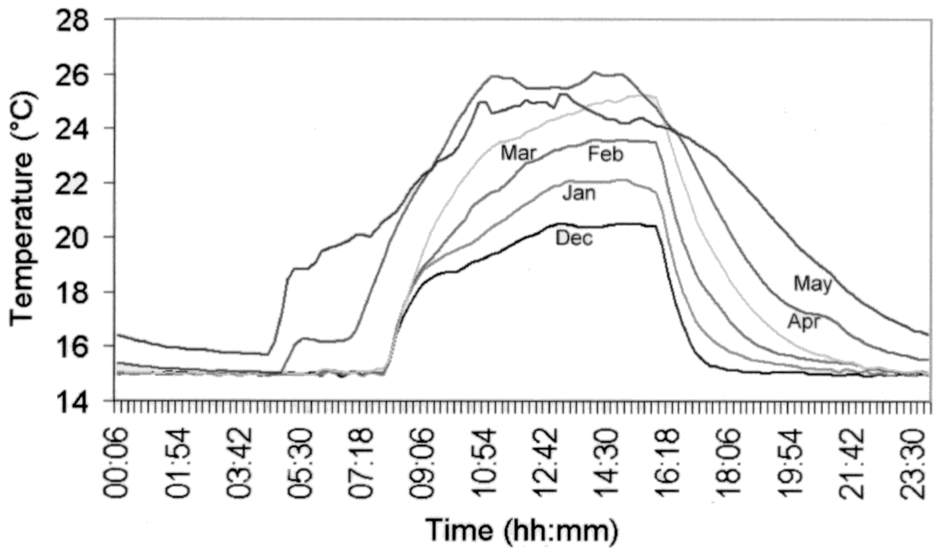

Figure 34: Mean temperature daily patterns in the greenhouse on a monthly basis and duration of the experiments conducted in the greenhouse.

Experiments III and IV were carried out in a growth chamber (mod. VEPHL 5/2000, Heraeus-Vötsch, Balingen, D) at 21/14°C, 16-hr day photoperiod, light intensity of 12,500 lux, 25% humidity. The temperature regime was set to roughly resemble that of the daily pattern in the greenhouse (December and January) and that in the field in August 2000 (data not shown).

Seedlings were grown in transparent plastic pots (5 cm diameter, 10 cm high, 200 cm³ volume) coated with aluminium foil and filled with substrate. All pots were punctured at the bottom to prevent water stagnation inside, except those of the treatments in which nutrient solution was supplied in known amounts. In each experiment, pots were randomly placed on 1.5 x 1 m tables with grids. Every two days rain water (about 10 ml) was supplied to the plants from above, ensuring an adequate substrate moisture throughout the whole experiment duration.

As germination and emergence took place with a slight time delay between different substrates, the day of germination for each treatment was defined as the day in which 80% of the seedlings had emerged (the cotyledons became visible).

For each seedling to be grown for the experiments, three seeds were planted. One week after emergence seedlings were selected for uniform size and vigour and supernumerary seedlings were excised with scissors. Trapped cotyledons were loosened from the seed coat if necessary.

75 ml of nitrogen-free nutrient solution (Jacot et al., 2000a) were supplied to the seedlings grown in vermiculite at the beginning of the experiment. Plants were watered four times in 48 days with a further 110 ml of nutrient solution in total.

2.2.4 Harvesting and analysis

Harvesting was done 48 and 120 days after emergence (d.a.e.), the required number of pots for each treatment being collected randomly. The first harvest date corresponds to the time span needed in all treatments to attain at least the fourth-leaf stage, the second date to the time at which plant decline was observed in the field.

Roots were individually washed with a water jet over a sieve set with decreasing mesh size (from 5 to 1 mm). The sediment production was collected in a container. Sediments accumulated in the sieves and in the container were accurately checked afterwards for eventual root losses. Plants were placed on black paper and copied in original scale with a photocopier. Growth parameters of the harvested plants (number of active and dead leaves, nodulation and number of nodules) were recorded, then epigeal and hypogeal biomass were separated, oven-dried (105°C, 36 hrs), and weighed.

2.2.5 Experiment I: growth of *Trifolium alpinum* on different substrates and its dependence on soil symbionts

The growth of *Trifolium alpinum* was studied in three substrates: autochthonous soil from the Alps (Meran 2000), a similar grassland soil from the Harz (Dammhaus) and the agricultural soil used in the seed propagation trials (Meierbreite), which has very different characteristics from the other two soils. Seeds of *Trifolium alpinum* were planted in the three substrates as described above. In the other two treatments a small quantity (10 g, 1/14 of total substrate weight) of the original soil was added to the surface of the other two soils, so that it formed a thin top layer of about 1 cm depth. The experiment was performed in 26 replications per treatment. Half of the plants were harvested at 48 d.a.e., the others at 120 d.a.e.

As 120-day-old plants grown in soil from Hebenshausen showed symptoms similar to those observed in the field for plants infected by *Pratylenchus* sp., nematode occurrence was checked in these treatments. Fresh root samples of 120-day-old plants were cut from 13 freshly washed plants per treatment, pooled and then sent in sealed plastic envelopes to HLRL (Hessisches Landesamt für Regionalentwicklung und Landwirtschaft - Pflanzenschutzdienst) for analysis. They were checked there for the presence of phytophagous nematodes with the Baermann's funnel method (Janetschek, 1982).

2.2.6 Experiment II: survival of autochthonous *Rhizobium*-strains under different soil conditions and infection occurrence after inoculation using autochthonous soil and soil extract

Samples of the allochthonous substrates (Harz and Hebenshausen) used in the previous experiment were inoculated by mixing them with 10 g of original alpine soil (1/14 of total substrate weight) or by use of a soil extract. The extract of the soil from the Alps was made according to Jacot et al. (2000a). Fresh soil (150 g) was suspended in 690 ml of sterile nitrogen-free solution and agitated at room temperature for 1 hr at 150 rpm. The suspension was then filtered through filter funnels (5 µm mesh, Whatman, Maidstone, England) and the 3-day-old seedlings were watered with 10 ml of it in proximity of the collar. Two controls in fine sterile vermiculite (0.5-2 mm, Goetz, Bischweier, D), one of them inoculated with the soil extract in order to verify the effectiveness of the extraction procedure, were also included in the experiment.

The experiment was performed in 30 replicates. All seedlings were harvested 48 d.a.e., which is known to be an adequate time span for the achievement of nodulation in *Trifolium alpinum* (Jacot et al., 2000a). Roots were examined with a binocular microscope and checked for rhizobial nodulation.

2.2.7 Experiment III: Phosphor requirements of *Trifolium alpinum*

As plants grown exclusively in soil from Meran 2000 showed the lowest values of dry matter production and for this soil the plant-available phosphate was lower than in the other two soils, the growth of seedlings of *Trifolium alpinum* in original alpine soil and in P-enriched original soil was investigated. Phosphor-enriched substrate was prepared by adding 0.81 g of $CaHPO_4 \times 2H_2O$ to 1,500 g (fresh weight) of soil from the Alps, corresponding to an addition of 45 mg P_2O_5 per 100 g of soil dry weight. The pH of the substrate was checked after phosphor enrichment, in order to exclude differences in nutrient availability between treatments due to different pH. The temperature settings of the growth chamber corresponded roughly to the regime experienced in the greenhouse by the plants of experiment I.

Plants were grown in 5 replicates per treatment. The same parameters assessed for experiment I were investigated.

2.2.8 Experiment IV: effect of pathogen infection

In order to exclude the influence of pathogens on the growth of *Trifolium alpinum*, plants grown in untreated and sterilised soil of the Meierbreite were compared. Half of the available soil was autoclaved for 30 min, the other half was not altered. For each treatment, 10 replicates were grown for each harvesting date.

Water stress was indirectly favoured by a low air moisture level (25%) in the growth chamber and was directly induced twice in the investigation period at 83 and 110 d.a.e. by interruption of the water supply for one week, in order to make visible the effects of the expected root damage caused by nematodes. Dead plants were immediately harvested and kept at -20°C until analysis.

Mixed root samples of 120-day-old plants were taken for each treatment as described in experiment I and sent to HLRL. There they were checked for nematode occurrence with the Baermann's funnel method (Janetschek, 1982).

2.2.9 Statistical analysis

Prior to analysis, data were checked for fulfilment of the assumptions of analysis of variance. Normal distribution of errors was checked with the Kolmogorov-Smirnov statistic (Köhler et al., 1996); variance homogeneity was tested with Cochran's test (Camussi et al., 1986) for samples with less than 10 replicates, otherwise the Levene test was used (Sachs, 1990; Sachs, 2002).

In case of failed fulfilment, logarithmic transformation of data was performed. Fulfilment of the assumptions of analysis of variance was checked afterwards as described above.

In case of normal distribution, the comparison of two samples was performed with a two-tailed t-test (Sokal and Rohlf, 1995). For more than two samples, and if all assumptions of the analysis of variance were met, analysis of variance (ANOVA) was performed. ANOVA and all tests described below were carried out at $\alpha = 0.05$.

If, even after transformation, data did not meet the requirements for performing an analysis of variance, non-parametric tests were carried out instead. The Kruskal-Wallis test was used to test the significance of main factors (Sokal and Rohlf, 1995). *A posteriori*- multiple comparisons were performed with the Nemenyi-test (Köhler et al., 1996).

All analyses were performed with the software SPSS (release 7.0, SPSS Inc., 1995).

2.3 Results

2.3.1 Soil properties

The results of the soil analysis are summarised in Table 22. Water holding capacity, pH and CAL-extractable K differed only slightly between the soil samples Meran 2000 and Dammhaus. They were strongly acid, while the arable soil of the Meierbreite had a considerably higher pH and thus a higher content of exchangeable Ca. The highest microbial biomass was detected in the sample from Dammhaus, the lowest was found in the field soil of the Meierbreite, the values of Meran 2000 being intermediate between the other two and exhibiting the highest C_{mic}/N_{mic} ratio. A similar relationship between the three soils was observed also for the C/N ratio.

Table 22: Results of the analysis carried out on the soils used as substrates for the experiments with *Trifolium alpinum*.

Location	Meran 2000 - St. Oswald	Dammhaus	Meierbreite
WHC (%)	129.4	112.1	68.5
pH (CaCl2)	4	3.6	5.9
C_{mic} (µg/g)	790.4	1091.4	392.3
N_{mic} (µg/g)	83.4	175.7	60.4
C_{mic}/N_{mic}	9.5	6.2	6.5
Ergosterol (µg/g)	8.7	1.5	0.7
C%	7.6	5.3	1.8
N%	0.5	0.5	0.2
C/N	15.9	11.1	10.5
Plant-available K_2O (mg/100g)	11.2	12.5	8
Plant-available P_2O_5 (mg/100g)	7.6	11.5	14
CEC Ca	n.m.	3.7	14.3

n.m. not measured

The highest ergosterol content was found instead in the alpine soil sample and it was 6 to 9 times higher than in the other soils investigated. The soil from Meran 2000 exhibited the lowest plant-available P-content, those of Dammhaus and Meierbreite being about 1/3 and two times higher than it respectively.

Altogether, the results show that the soil of the Meierbreite, from a chemical and micro-biological point of view, is very different from the autochthonous soil of Meran 2000.

2.3.2 Experiment I: growth of *Trifolium alpinum* on different substrates and its dependence on soil symbionts

The growth of *Trifolium alpinum* in the greenhouse proceeded very slowly, as already observed in the field trial. After 48 days none of the treatments yielded plant biomass above 50 mg. Four-month-old plants showed a wide range of values between 93 and 670 mg/plant on average. At both harvests the lowest values were found for plants grown in the autochthonous soil of Meran 2000 (Figure 36). A two-way ANOVA including only the treatments employing allochthonous substrates detected highly significant effects of both the substrate and the inoculum at each harvesting date. An effect of the inoculation with a small amount of the autochthonous soil used as the top layer was consistently observed at 48 and 120 d.a.e.

Table 23: Effect of substrate and inoculum on total dry weight of *Trifolium alpinum* 48 and 120 days after emergence (ANOVA, N=13, [n.s.]=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001). Only the substrates Dammhaus and Meierbreite are included in the statistical analysis. Inoculum has to be intended as the addition of a small top layer of the alpine soil Meran 2000 to the substrate.

		48 d.a.e.		120 d.a.e. [a]	
Source	df	F	Sig.	F	Sig.
Substrate (S)	1	19.874	***	56.300	**
Inoculum (I)	1	18.916	***	117.118	***
S x I	1	0.282	n.s.	0.003	n.s.
Error	48				

[a] logarithm-transformed data

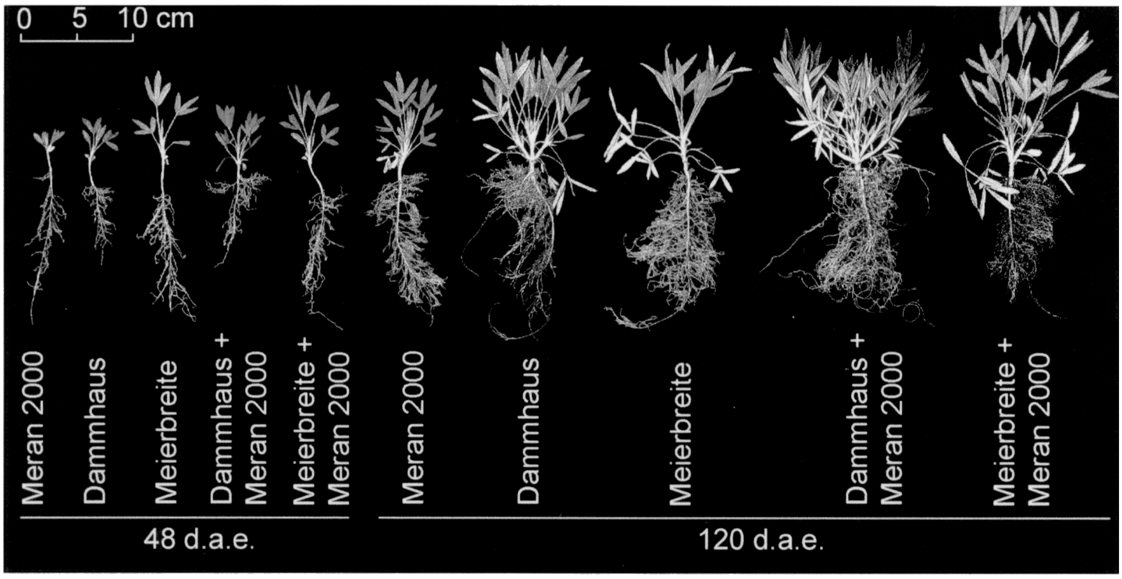

Figure 35: Development of *Trifolium alpinum* grown in different substrates 48 and 120 days after emergence.

Concerning the allochthonous substrates, the soil from the Meierbreite yielded higher dry weights at 48 d.a.e., while that from Dammhaus gave better performances at the second harvest date (Figure 36). At this time, plants in the soil from the Meierbreite had a reduced number of active leaves, particularly if not inoculated with autochthonous soil (Table 24) and showed symptoms similar to those observed in the field following the nematodes infection.

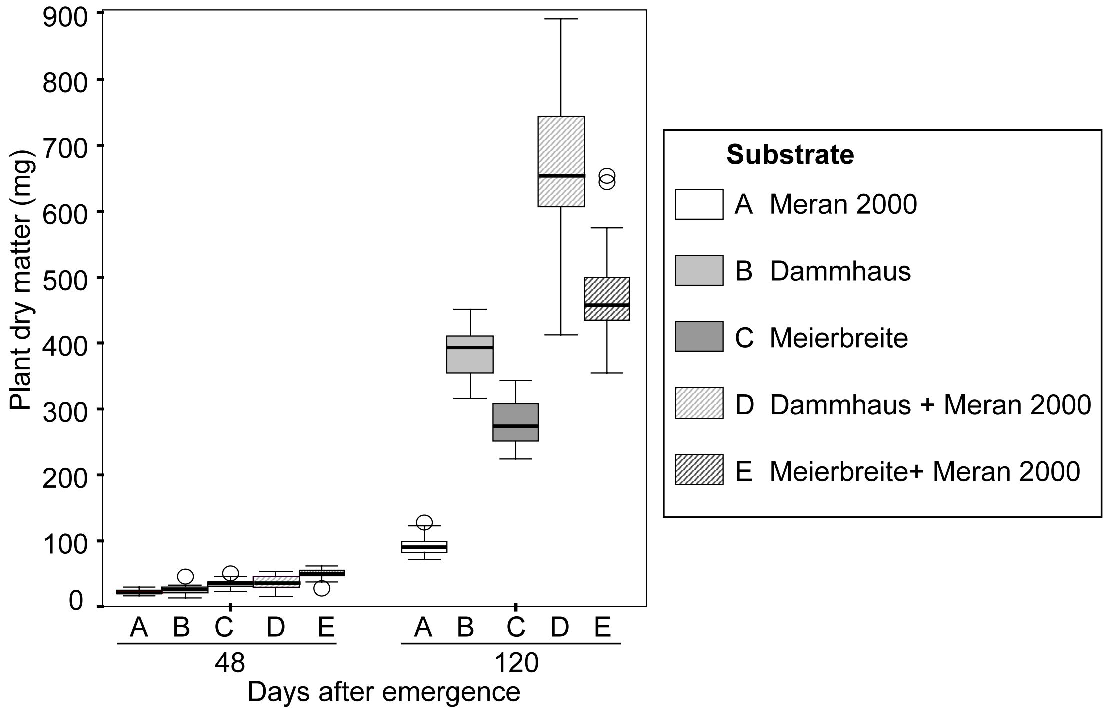

Figure 36: Plant dry matter of *Trifolium alpinum* grown in the greenhouse in different substrates for 48 and 120 days. Average of 13 replicates.

The test for the presence of phytophagous nematodes detected very high colonization densities of *Pratylenchus* sp., with more than 1,000 nematodes/g fresh weight in roots of plants grown in the soil from the Meierbreite.

Table 24: Number of nodules in roots and active leaves in *Trifolium alpinum* grown on different substrates 48 and 120 days after emergence. Mean separation by Nemenyi's test at α=0.05. Average of 13 replicates. Values without common letters in each column are significantly different.

	Nodules (No./plant)		Active leaves (No./plant)	
Substrate	48 d.a.e.	120 d.a.e.	48 d.a.e.	120 d.a.e.
Meran 2000	3.7 a	7.9 a	3.4 b	7.8 b
Dammhaus	0 b	0.1 b	3.6 b	15.1 a
Meierbreite	0 b	0.1 b	4.8 a	7.3 b
Dammhaus + Meran 2000	2.3 a	31.5 a	5.4 a	28.3 a
Meierbreite + Meran 2000	1.5 a	24.2 a	4.9 a	14.8 a

Examination of the roots of all treatments revealed a *Rhizobium*-induced nodulation in all treatments where the autochthonous soil was employed (as main substrate or as inoculum) already at 48 d.a.e., while all other treatments were virtually devoid of nodules at both harvest dates. Nodule numbers were mostly higher in the inoculated variants at 120 d.a.e.

2.3.3 Experiment II: survival of autochthonous *Rhizobium*-strains under different soil conditions and infection occurrence after inoculation using autochthonous soil and soil extract

Results are condensed in Table 25, comprising also data about rhizobial infection obtained in experiment I. Percentages of infection were very high where the autochthonous soil was used as the top layer, while mixing it with the main substrate was effective for the soil of the Meierbreite, but gave a low infection percentage for the soil of Dammhaus. Nodulation occurred in one plant each in the non-inoculated substrates from Dammhaus and from the Meierbreite.

The addition of the soil extract resulted in a complete nodulation if added to sterile vermiculite, but was less effective if introduced to the other two substrates. Only one plant in the vermiculite control exhibited rhizobial nodules.

Table 25: Percentage of plants of *Trifolium alpinum* nodulating after addition of different types of inoculum to different substrates (incubation time 48 days).

	Substrate		
Inoculum type	Dammhaus	Meierbreite	Vermiculite + nitrogen-free nutrient solution
Top layer of soil from Meran 2000	92%**	85%**	n.t.
Mix of substrate and soil of Meran 2000 (14:1 weight)	23%*	100%*	n.t.
Soil extract of Meran 2000	0%*	27%*	100%*
No inoculum (control)	0%**	0%**	3%*

* 30 observations, ** 13 observations

n.t.=not tested

2.3.4 Experiment III: Phosphor requirements of *Trifolium alpinum*

The pH of the substrates used for this experiment did not differ from each other (pH=4.0 in 0.01M $CaCl_2$).

Phosphorous supply consistently increased plant growth in the alpine autochthonous soil at both harvest dates. Differences were quite small at 48 d.a.e., while 120-day-old plants grown in P-enriched soil yielded a dry matter about 5 times higher than that achieved in the untreated soil. The number of rhizobial nodules did not differed between the treatments at the first harvest date, while at 120 d.a.e. the addition of P resulted in twice the number of nodules in comparison to the control.

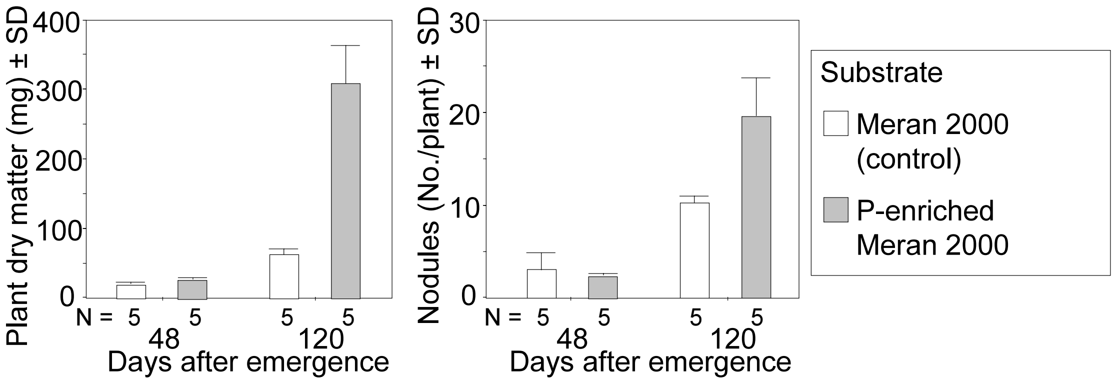

Figure 37: Plant dry matter and number of nodules in roots of 48 and 120-days-old plants of *Trifolium alpinum* grown in untreated and P-enriched alpine autochthonous soil from Meran 2000. Differences of plant dry matter against the control were significant at both harvest dates (t-test on logarithm-transformed data: at 48 d.a.e. df=8, t=-4.431, p<0.01; at 120 d.a.e. df=8, t=-14.903, p<0.001), while number of nodules differed between treatments only at 120 d.a.e. (t-test: at 48 d.a.e. equal variances not assumed, df=4.589, t=1.313, p>0.05; at 120 d.a.e. equal variances not assumed, df=4.148, t=-5.175, p<0.01).

2.3.5 Experiment IV: effect of pathogen infection

At both harvest dates the plants grown in autoclaved soil from the Meierbreite exhibited a higher plant dry matter in comparison to the untreated soil (Figure 38).

Differences between treatments increased with time and at the end of the observation period plants grown in untreated soil weighed about 1/3 less than the others, presented a reduced number of active leaves and frequent necrotic root tissues. In six out of ten plants the tap root was rotten. Two plants died between the first and the second dry period simulation. No particular signs of plant decline were detected in the other treatment.

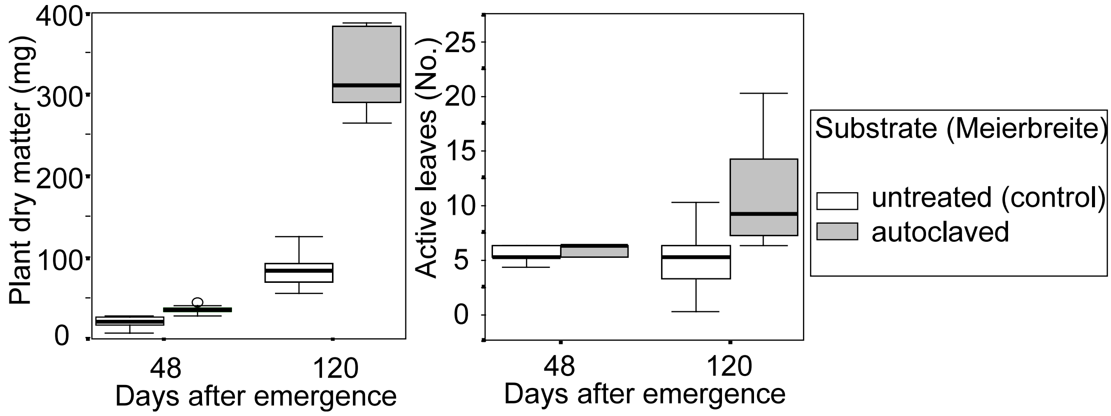

Figure 38: Plant dry matter and number of active leaves of *Trifolium alpinum* grown for 48 and 120 days in the greenhouse in untreated and autoclaved soil from the Meierbreite. N=10. Differences of plant dry matter against the control were significant at both harvest dates (t-test: at 48 d.a.e. equal variances not assumed, df=16.247, t=-6.028, p<0.001; at 120 d.a.e. equal variances not assumed, df=12.707, t=-14.651, p<0.001), while number of active leaves differed between treatments only at 120 d.a.e. (t-test: at 48 d.a.e. df=18, t=-1.987, p>0.05; at 120 d.a.e. df=18, t=-3.670, p<0.01).

A very high density of *Pratylenchus* sp. (1,185 nematodes/g fresh weight), similar to that found in experiment I, was detected at 120 d.a.e. in roots of plants grown in untreated soil. In contrast, the plants grown in autoclaved soil were devoid of nematodes. A density of 19 nematodes/g fresh weight of *Aphelenchus avenae*, a hyphal feeder, was found in root samples from the untreated soil, indicating a possible fungal infection associated with the nematode infection.

2.4 Discussion

The results of the soil analysis show that the total microbial biomass was greater in the grassland soils than in the arable soil. This is in accordance with the expectations, as the lowest carbon and nitrogen levels were found in the soil of the Meierbreite, and the microbial biomass is positively correlated with the soil organic matter (rev. in Wardle, 1992). Also the fungal biomass, as shown by the ergosterol analysis, was higher at the grassland sites. The soil of Meran 2000 contained particularly high amounts of it (8.7 µg/g). Zeller et al. (1999) found similar values in grassland soils with analogous pH and C/N in the subalpine zone of the Alps and showed that ergosterol contents increase after management cessation and are often highest towards the end of the growing season. Occurrence in the vegetation of dwarf shrubs, which are usually strongly colonised by mycorrhiza, is also thought to be an explanation for high ergosterol contents (Zeller et al., 2000).

The introduction into the allochthonous substrates of soil biota occurring in the autochthonous soil resulted in increased biomass production at both harvest dates of the present experiments. Altogether, the findings suggest that either microbial and fungal micro-organisms, or both of them, may play a beneficial role in the establishment and growth of *Trifolium alpinum*, although their influence cannot be distinguished on the basis of the present experiments. The efficiency of the rhizobial symbiosis at high altitudes was demonstrated by Jacot et al. (2000b), but the symbiosis may become inefficient at the propagation site under a different climate and dissimilar soil conditions. In the present experiments only the occurrence of nodulation was demonstrated. A role of mycorrhiza is also quite likely, as the fungal biomass in the soil of Meran 2000 was very high. Direct observations about the mycorrhizal status of *Trifolium alpinum* are not reported in the literature examined, but a widespread mycorrhizal infection has been ascertained for most species of the alpine grass heaths on siliceous soils (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Haselwandter, 1987).

Regarding the introduction of adequate rhizobia into the soil of the propagation site, the pot experiments indicate that, under the given conditions, the addition of autochthonous soil as a thin top layer was the only completely effective method in inducing the symbiosis. Mixing substrate and autochthonous soil or supplying the bacteria in form of a soil extract was partially effective or completely ineffective. A possible reason for this is that the contact between roots and bacteria can not be ensured in this way, if the bacterial charge is not adequate. Rhizobia are scarcely mobile in the soil and elevated mortality is likely to occur in the soil if the symbiosis with the plants is not achieved in a short time (rev. in Alexander, 1984). The autochthonous strain seemed to be able to survive in all substrates tested, even in a field soil with markedly different characteristics, if an adequate inoculum source was provided. The nodulation observed in one plant each in the Dammhaus- and Meierbreite-substrate 120 d.a.e. may either have been caused by contamination during watering, which is the commonest source of contamination during such experiments (Halliday, 1984), or by other *Rhizobium* strains occurring in the substrate. A partial effectiveness of allochthonous *Rhizobium* strains was indeed ascertained by Jacot and al. (2000a) using a soil extract of a location where *Trifolium alpinum* was absent.

If grown in the greenhouse, *Trifolium alpinum* exhibited in general a low biomass production (less than 1 g dry matter in four months), higher values being achieved in the allochthonous soils than in the autochthonous soil. Plants grown in the autochthonous soil yielded dry matter values about half of those assessed by other authors for plants grown in a mixture of autochthonous soil, commercial potting soil and sand (Gallmetzer and Florineth, 1996). The 5-leaf stage was achieved in the same time span (about 50 days) observed by Weilenmann (1981). A P-supply considerably increased the plant dry matter in the autochthonous soil, suggesting that, if grown under a temperature regime more favourable than in the natural environment, the growth of *Trifolium alpinum* can become phosphor-limited. The growth rate of this species was indeed found to be both temperature- and nutrient- limited in the alpine environment (Schäppi and Körner, 1996). On the basis of pot experiments in a controlled environment, in which the response of an early and of a late seral alpine species were subjected to various levels of N and P, Chambers et al. (1987b) hypothesized that late seral forbs are in general more sensitive to P than to N. In the case of *Trifolium alpinum*, this can be quite easily explained, as under natural conditions N supply is ensured by rhizobial symbiosis. Henry et al. (1986) found an analogous response of *Dryas integrifolia*, a N-fixing actinorhizal forb (Paschke, 1997), to N and P in a fertilisation study of tundra plant communities. Applications of ammonium nitrate had no effect on dry matter production increases in dry matter production, while increases were achieved if also P was supplied. Moreover, the results of the present experiments indicate that the production of rhizobial nodules in *Trifolium alpinum* is considerably enhanced by P-fertilisation so that increases in P-availability may result in higher nitrogen fixation. Phosphor deficiency is indeed regarded as the most important single limiting factor for N_2 fixation and legume production for tropical *Leguminosae* growing on acidic soils, as P plays a critical role in energy transfer and a large quantity of energy is required for the reduction of N_2 to NH_3 (Freire, 1984). The phosphor requirements of *Trifolium alpinum* seem to be quite high, as the values found in the autochthonous soil amounted to near 8 mg of plant-available P_2O_5 per 100 g of soil.

Concerning the suitability of the two allochthonous substrates investigated for the cultivation of *Trifolium alpinum*, the soil of the Meierbreite gave the best growth values at the first harvest date. At 120 d.a.e. they were still comparable with those of the soil from Dammhaus (about 1/4 lower). At this time the nematode density in the soil from the Meierbreite was already extremely high, and plant decline became evident. *Pratylenchus*-caused root damage results in fact in an increased water stress for the plants (Dropkin, 1980). The plants were probably still alive, because an optimal water supply was ensured throughout the experiment. This hypothesis is corroborated by the fact that at similar nematode densities, damage was much more marked in experiment IV, in which water stress conditions were induced. The unsuitability of the site at the Meierbreite for the cultivation of *Trifolium alpinum*, clearly demonstrated by the failure of the field trial, seems therefore to be mainly due to the nematode infection. *Trifolium alpinum* seems to represent a very suitable pabulum for *Pratylenchus* sp., as *Trifolium resupinatum*, which had been grown all around the field trial, did not show any symptom of plant decline. The susceptibility of *Trifolium alpinum* may be explained by the fact that phytophagous nematodes do not occur at the site of provenance in the Alps, and therefore this species may lack resistance against such pathogens. As plants grown in the autoclaved soil exhibited satisfactory growth and none of the symptoms observed in the field, it can be concluded that the chemical composition of the soil from the Meierbreite is not responsible for the plant decline observed in the field trials. The satisfactory growth performance in the soil from Dammhaus confirms the feasibility of the cultivation of *Trifolium alpinum* in allochthonous soils.

An increase in growth rate of the plants inoculated with the autochthonous soil represent an important advantages for the cultivation of *Trifolium alpinum* in lowlands. Assessments of the efficacy of the mechanical weed controls showed that a crucial point is the slow growth of the crop plant, which can be easily overgrown by the weeds, especially by those located inside the rows. A successful cultivation strategy should therefore promote a rapid fillings of gaps within the rows, in order to avoid germination, establishment and growth of weeds within the rows. An increased growth rate should therefore be regarded as a fundamental priority in the strategy for cultivating this species. The introduction of beneficial soil biota (rhizobia, mycorrhiza) into the cultivation soil may be achieved through suitable inoculation techniques and an adequate P-availability should be ensured.

3 Ecological restoration of ski runs in mountain regions

3.1 Introduction

Terminology

The terminology used for indicating interventions aiming at re-establishing vegetation cover at a site following disturbance has been repeatedly debated. Different meanings have been attributed to the word restoration by different authors. Bradshaw (1997b) and Haselwandter (1997) reserve the use of the word restoration to cases, in which the return to the original state, which is perfect and healthy, is possible. In the case of machine-graded ski runs, which are the subject of the present research, ecosystems are subjected to severe disturbance (mechanical destruction of the vegetation, alteration of the soil), and a return to pre-disturbance conditions appears to be most unlikely, or would require a very long time. Muller et al. (1998) adopted the word rehabilitation, meaning an improvement from a degraded state, for the revegetation of ski slopes, in which the autochthonous vegetation has been destroyed. However, Meyer (1996) argues that restoration should initiate processes that will lead to the establishment of a native ecosystem with diversity, resiliency, and capacity for continued evolution, rather than re-create static, idealized pre-disturbance vegetation. In the present work, the term ecological restoration will be used following Jackson et al. (1995), who define this process as the action of repairing damage caused by human beings to the diversity and dynamics of indigenous ecosystems. In this process are also included the assessment of needs (referred to natural reference models), an ecological approach, setting of aims and success evaluation, which should be possible within a decade. Also Keammerer (1986) recommends the use of reference areas among the monitoring parameters for assessing restoration success.

Consequences of the construction of ski runs

Enlargement and correction of the ski run profile are usually undertaken in order to improve the capacity, the attractiveness and the security of the ski slopes (Delarze, 1994), and in order to remove physical obstacles and thus allow a rational use of the implements used for the preparation of the ski run in winter (Schönthaler, 1980). The construction of machine-graded ski runs implies the destruction of the vegetation cover and severe alteration of the soil profile. Depending on the gap between the morphology prior to site modification and the planned one and depending on the modality of execution of the interventions, even substantial modifications of the site characteristics can be provoked (i.e. through the use of allochthonous soil for modelling the ski slope profile). Such alterations can preclude, even long-term, the return to ecosystems resembling the pre-disturbance situation and impede the contribution of the surrounding, intact ecosystem to the succession toward an healthy ecosystem. Machine-graded ski runs usually represent environments hostile to the re-establishment of vegetation. Problems are caused by the alteration of the ecosystem during the construction of the ski slope, by the climatic conditions of the mountain environment, and by the ski run management.

Concerning the first issue, major problems are caused by the disruption of the soil profile and by the mixing of topsoil and deeper soil layers and can be summarized as follows:

- decrease of the soil depth and thus smaller soil volume available for the growth of root systems. In machine-graded ski runs the soil depth usually range between 5 and 10 cm (Cernusca, 1986);
- strong decrease of water capacity. This is usually a consequence of the reduction of the content of humus and organic matter, whose water capacity can be three to four times that of a mineral soil (Bradshaw, 1997a), and of the increase of the particle size and of the size of the soil pores (Mosimann, 1983). Likelihood of occurrence of water stress is therefore enhanced;
- considerable decrease of the humus content and thus of total organic carbon, nitrogen and of the other main nutrients (Mosimann, 1983; Flüeler, 1992; Ellenberg, 1996);
- decrease of the mycorrhizal infection potential because of the dilution of the propagule bank, which decreases with increasing soil depth, in surface layers in which topsoil and subsoil are mixed (Miller and Jastrow, 1992);
- increase of skeletal material at the soil surface, with a subsequent increase of the particle size of soil (Bradshaw, 1997a). As a consequence, seedling establishment may be hampered. In an alpine environment, Chambers (1995) observed satisfactory seedling survival on substrates with particle size below 2 mm, although emergence occurred on substrates with particle size up to 8 mm.

On the basis of what exposed above it becomes clear that most of the detrimental consequences of the construction of a ski slope are connected with the removal (partial or complete) of the topsoil and of the humus contained therein.

Further difficulties for the revegetation are determined by the climate of the mountain regions. With increasing altitude, climatic conditions become progressively unfavourable for plant growth. The mean yearly air temperatures decrease of about 0.6°C/100 m of altitude increase (Landolt, 1992; Krautzer, 1996c; Tappeiner, 1996), while the mean yearly soil temperatures decrease more slowly because of the insulating effect of the snow (Ellenberg, 1996). The duration of the growing season shortens of about one week every 100 m (Reisigl and Keller, 1990), because of the decrease of the days with temperatures suitable for plant growth and because of the late snow melt (Harlfinger and Knees, 1999).

Nocturnal radiation losses raise with increasing altitude, thus increasing the risk of frost occurrence (Landolt, 1992). The absolute air moisture decreases and therefore transpiration of plants is enhanced (Landolt, 1992). Diurnal soil temperature amplitudes are increased if the vegetation cover is scarce, as in such situations a higher proportion of the solar radiation reaches the soil surface during the day and radiation losses by night are higher (Bradshaw and Chadwick, 1980; Tappeiner, 1985 cit. in Tappeiner, 1996). Also wind speed at the soil surface increases so that mechanical damage to the plants is possible and transpiration is stimulated (Bliss, 1962; Landolt, 1992).

Finally, difficulties for the vegetation can be caused by the activities for the preparation of the ski run during the ski season. Snow melt is usually retarded on ski slopes in comparison to the surrounding vegetation as a consequence of the mechanical preparation and, eventually, of the artificial snowing (Cernusca et al., 1990; Kammer and Hegg, 1990; Hegg and Kammer, 1991; Trockner and Kopeszki, 1994; Rixen et al., 2001). As a consequence, the duration of the growing season may be reduced. If during piste preparation with heavy implements, free water contained in the pores of wet snow is pressed downwards into the deeper snow layers, which are still very cold, it can freeze and ice layers can arise at the soil surface (Newesely at al., 1994). In case of insufficient thickness of the snow cover, the soil can be therefore exposed to repeated freezing-thawing cycles depending on air temperature, as the thermic insulation provided by the ice layers is considerably lower than that of the snow. Thus, damages to hypogeal organs of the plants, and particularly to fine roots, may occur (Cernusca, 1986). Furthermore, ice layers were found to cause, at relatively low altitudes, CO_2-increase and a parallel oxygen shortage in a ski run at the end of the winter/beginning of spring, as favourable temperature induces the resumption of metabolic activities of plants and micro-organism, oxygen is consumed for respiration (and CO_2 is produced), but the ice layers impede gaseous exchanges with the external environment (Newesely, 1997). It is controversial, whether such oxygen shortage can also lead to decrease of frost resistance (see Cernusca et al., 1990; Newesely et al., 1994 and the relative objections of Lichtenegger, 1992). Also mechanical damages can hamper a durable re-establishment of the vegetation. They can be caused, in case of insufficient snow height, by the implements used for piste preparation or by skiers. They damage the vegetation and the soil and create microhabitats, which can be re-colonised only by species with pioneer attitudes (Spatz, 1978). Various authors observed an inverse relationship between vegetation cover and altitude following revegetation interventions (Bayfield, 1980; Schiechtl, 1980; Delarze, 1994; Gottardi, 1997; Krautzer et al., 2001) or spontaneous re-colonization (Bayfield et al., 1984; Frain et al., 1986a). The timberline is usually indicated as the limit, above which the climatic conditions become unfavourable to the establishment and growth of plants and therefore restoration by use of seed mixtures with lowland forage species becomes problematic. Mosimann (1984) made a synthesis of own data and others published by Schauer (1981) and argued that three altitudinal ranges can be distinguished with respect to the achievement of a satisfactory vegetation cover, if conventional revegetation methods are considered. Up to an altitude of 1,600 m revegetation was usually not problematic, between 1,600 and 2,200 m the number of successful revegetation attempts decreased rapidly and above 2,200 m only recently sown areas exhibited an acceptable vegetation cover.

Importance of vegetation re-establishment

The re-establishment of vegetation after the construction of a ski slope is extremely important, in order to prevent erosion of the fine soil particles and organic matter caused by water runoff (Mosimann, 1981; Dietl, 1995). Florineth (1988) reported soil losses of up to 5 kg/m² in areas devoid of vegetation following an intense precipitation event of 60 mm during a storm, while soil losses were considerably lower in revegetated ski runs (between 2.4 and 8.1 g/m²), and absent in the undisturbed vegetation. Also erosion experiments, artificially simulating intense precipitation events (around 100 mm/hr), confirm that erosion does not take place if cover is nearly 100 %, even if surface runoff is high (Bunza, 1984; Karl, 1985). In revegetated ski slopes, soil losses are considerably lower than in nude areas (Markart et al., 2000), but extremely high values can be sometimes achieved despite of the occurrence of vegetation (Karl et al., 1985). Various studies have demonstrated that vegetation cover is the decisive factor, from which soil erosion depends. Linse et al. (2001) found that total cover explained more than 70% of the variability in sediment yield, for cover values above 30%. Mosimann (1981) observed that the erosion intensity in revegetated ski runs, assessed in form of an index based on erosion phenomena, followed a curvilinear relationship with the vegetation cover, decreasing rapidly with increasing cover and becoming negligible at cover values of 70% below the timberline and 80% above it. Similar, non-linear relationships between sediment production and vegetation cover were obtained in experiments with simulation of intense precipitation events (Copeland, 1965; Stocking, 1984; Linse et al., 2001), showing that soil losses are virtually absent for cover values of 70% or more. To the same conclusion came Tappeiner et al. (1998) on the basis of the results of simulations of intense precipitation in the Alps. In contrast to the experimental results cited above, a synthesis of 91 literature data available for the Alps about soil losses related to the vegetation cover (Bunza, 1984; Schauer, 1988; Bunza, 1989; Cernusca, 1984 cit. in Tappeiner et al., 1998), following rainstorm simulation (about 100 mm in one hour), a linear relationship is obtained (Figure 39), but results must be interpreted with caution, as only few data at low cover values could be found.

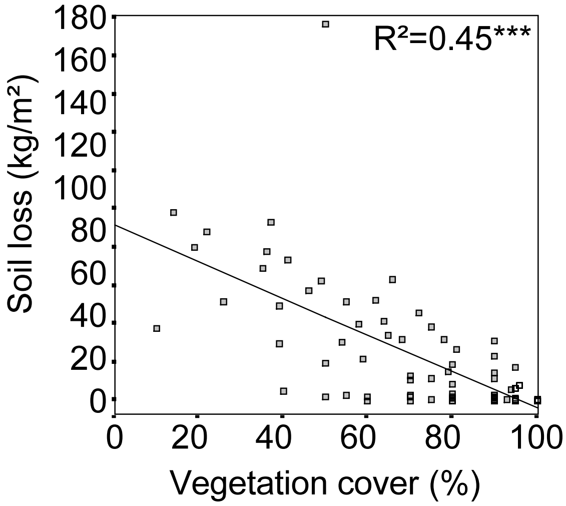

Figure 39: Relationship between soil loss and vegetation cover as obtained by literature data relative to rainstorm simulation in the Alps (Bunza, 1984a; Bunza 1984b; Schauer, 1988; Bunza, 1989; Cernusca, 1984b cit. in Tappeiner et al., 1998). Correlation was tested with Spearman's test.

According to Bradshaw (1997a), the establishment of a vegetation cover represents also the first step towards natural succession, and towards the restoration of nutrient cycles in soil. After establishment, nutrient uptake by plants can prevent losses by volatilisation or leaching (Reeder, 1990). Roots explore diffusely the soil and nutrients are concentrated in the plants, shed in dying plant parts and then accumulated near the soil surface, where they are made readily available through mineralization. Accumulation of organic matter promotes also the re-establishment of soil micro-organisms, such as fungi or bacteria, which are involved in nutrient turnover and promote the formation of soil structure (Tisdall, 1994).

Importance of topsoil conservation

The soil seed bank is considered a colonization source for a secondary succession after disturbances occurred, such as erosion, particularly adverse climatic conditions (Stimpfl, 1985). Numerous studies were performed in the past about the soil seed bank, many of them about undisturbed or agriculturally managed soils. Some of them concern disturbed soils, mainly following fire events or after artificial removal of the pre-existent vegetation (Thompson et al., 1997), and a few of them dealt with cases where the topsoil was completely removed for building a ski run (Urbanska and Fattorini, 1998a and 1998b). As most seeds are usually contained in the first 5 to 10 cm soil depth (Urbanska, 1992; Frey and Lösch, 1998), the attempt to take advantage of the soil seed bank for speeding up re-colonization processes of the autochthonous vegetation implies a conservation of the topsoil. Various authors suggest removing the topsoil layer, stockpiling it, and re-distributing it later in the area to be restored, in order to achieve a faster restoration of the disturbed site (Brugger, 1979; Schiechtl, 1980; Mosimann, 1983;

Neugirg, 1986; Naschberger, 1988; Holaus and Köck, 1989; Chambers et al., 1990; Flüeler, 1992; Lichtenegger, 1994b; Holaus and Partl, 1996; Chambers, 1997; Holaus, 1998). Otherwise, the original site conditions may be altered to such an extent that the re-establishment of the species occurred at the site prior to disturbance would become impossible (Spatz et al., 1987). The topsoil layer contains the largest part of the hypogeal propagation organs, which can shoot again after topsoil re-application (Lichtenegger, 1994b). Topsoil conservation is also a cheaper alternative to the distribution of substances ameliorating the soil (Brugger, 1981; Cernusca, 1986), as it represents a suitable source of nutrients, which are not subjected to leaching losses (Bradshaw, 1997a). In particular, a number of studies were devoted to the effect of the topsoil storage for the restoration of mine sites (Bradshaw and Chadwick, 1980), investigating the effects of the modality of storage and of the duration of the stockpiling on mycorrhiza (Rives et al., 1980; Gould and Liberta, 1981; Abdul-Kareem and McRae, 1984; Miller et al., 1985), on microbial biomass (Visser et al., 1984; Harris et al., 1989) and on the content of viable seeds (Dickie et al., 1988). There is a body of evidence that adequate practices of topsoil conservation would allow to exploit the self-healing potential occurring *in situ* and would reduce the time required for site restoration. In the particular case of Sudelfeld, where no allochthonous soil was added, and the top soil, even if disrupted and mixed with deeper mineral soil layers, was still present on the plots, investigations about the biotic resources (seed bank, vegetative propagules, vegetation rests) re-introduced through topsoil conservation seem to be particularly interesting, in order to evaluate the advantages of this practice.

Restoration by use of seed mixtures

The seed mixtures usually employed for upland revegetation include cultivars of valuable forage species, which have been selected and bred for high forage production and quality. These commercial varieties are not always suitable for the revegetation under severe climate and/or in situations with low availability of soil nutrients or extreme soil conditions (i.e. soil pH) (Schiechtl, 1972; Köck, 1975; Spatz, 1985; Urbanska, 1990). The unsuitability of such plant material is partly due to intrinsic ecological characteristics of the species, and partly to the accentuation in the bred varieties of traits typical of crop species, with a consequent increase also of environmental demand (i.e. temperature, nutrient supply). Ecotypes of forage species were found to be more productive and to have a better persistency than commercial varieties of the same species at a montane site (Paoletti et al., 1996). In the course of a comparative test of several varieties of *Festuca rubra* for resistance to the effects of the high altitude, Florineth (1982) found that best results were achieved by the least intensively bred plant material. Mehnert et al. (1985) observed a rapid decline of a variety of *Cynosurus cristatus* bred for agricultural purposes at a site where this species was well represented in the autochthonous vegetation. A similar situation is reported for commercial varieties of *Festuca rubra* and *Agrostis tenuis* employed in revegetation of ski slopes in the Austrian Alps (Köck, 1975).

The current method for the restoration of ski slopes consists in the use of cheap commercial seed mixtures, relatively intensive use of fertilisers, high seeding rates. The high productivity of the sown species requires regular cut and fertilisation, in order to avoid thinning out of the plant stands because of inter- and intraspecific competition for light and nutrients. These measures can be quite expensive in areas that are hardly accessible. As the state of the vegetation shows often a middle- or long-term decline, re-seeding is necessary in the following years (Keigley, 1988; Gottardi, verb. comm.). In extreme cases, if the erosion achieves a large extent because of the insufficient vegetation, new modelling of the soil profile and reseeding might be necessary. Long-term costs can therefore become very high.

Many authors advocated the use of indigenous seed (i.e. Schiechtl, 1967; Schiechtl, 1972; Partsch, 1980; Schönthaler, 1980; Florineth, 1982; Spatz et al., 1987; Florineth, 1997; Odermatt, 1998) and/or plant material (i.e. Urbanska, 1988; Urbanska, 1989; Urbanska, 1990; Tappeiner, 1996) for the revegetation at high altitude, retaining that only autochthonous species would be adapted to the harsh climatic and edaphic conditions of the mountain regions and would ensure a stable, durable vegetation cover. Some promising results seem to confirm the validity of this hypothesis (i.e. Florineth, 1988; Florineth, 1992; Hasler, 1992; Mannhart, 1993; Grabherr, 1995; Ruoss et al., 1995; Wittmann and Rücker, 1995; Krautzer, 1996a; Holaus, 1997; Krautzer, 1997b; Wittmann and Rücker, 1997; Holaus, 1998; Pröbstl et al., 1998; Reyneri and Siniscalco, 1999).

Transplantation of indigenous material (sods, tussocks, container-grown plantlets propagated vegetatively (i.e. Single Ramet Cloning according to Urbanska, 1989) or from seed) was found to be effective, but implies considerable man labour, and therefore presents, in general, higher costs in comparison to seeding (Fahlselt, 1988; Lichtenegger, 1994b; Krautzer, 1998). Nevertheless, the use of sods seems to be the only practicable way for a successful restoration in the high alpine area because of the extremely slow plant growth in this environment (Grabherr, 1978; Grabherr et al., 1987; Grabherr et al., 1988).

Considerable seed rates, above 20 g/m² and up to 50 g/m² are used or recommended for ski run revegetation with commercial seed mixtures (Schiechtl, 1973; Karl, 1974; Guillaume et al., 1986; Reist, 1986; Neugirg, 1986; Carbonari and Mezzanotte, 1993; Anonymous, 1993; Holaus, 1997; Antonini, 2001). Nevertheless, there is evidence that lower amounts would suffice. In a field trial in the Austrian Alps, Holaus (1997) found that increases of the seed rate brought about clear improvements of the vegetation establishment up to a upper limit of 10 g/m², further increases being of minor importance and quantities in excess of 20 g/m² being completely useless. At low seed rates (below 5 g/m²), improvements of the vegetation cover were achieved with increasing seed rates of indigenous species in attempts to re-create calcareous grassland on former farmland (Stevenson et al., 1995). Dietl (1995) suggests 8 g/m² as upper limit for the establishment of pasture and meadows in mountain regions, but Brown et al. (1984) found this seed amount to be insufficient in restoration trials carried out at high altitudes with indigenous species. In contrast, seed rates between 2.8 and 11.1 g/m² were found to be adequate by Brown et al. (1976 cit. in Brown et al., 1978) in an alpine environment, and satisfactory cover was long-term achieved by Guillaume et al. (1986) sowing 8.6 g/m².

Fertilisation

Following machine-grading, plant-available nutrient levels decrease (Mosimann, 1983; Claassen and Hogan, 1996). The skeletal-rich, humus-poor material usually contains sufficient mineral nutrients, but they are not in a directly assimilable form and cannot be made available for plants because of lack of biological activity in the soil and because of insufficient development of fine roots in such substrates (Lichtenegger, 1994b; Bradshaw, 1997a). An initial nutrient input is therefore particularly important for enhancing vegetation establishment (Flüeler, 1992; Ciotti et al., 1994). Organic fertilisers, and especially those strongly promoting the activity of the soil micro-organisms, were found to be suitable for the mountain environment (Naschberger, 1988; Holaus, 1996). In contrast to mineral fertilisers, they have the advantage that nutrients are made available for plants through mineralization, which takes place at relatively high temperatures, at the same time the nutrients are needed by plants, and therefore losses by leaching and volatilisation are considerably reduced (Holaus and Köck, 1989; Naschberger and Köck, 1983). Leaching losses may greatly endanger the groundwater quality (Partsch, 1980; Köck and Naschberger, 1991). Nevertheless, mineral fertilisation is still very frequent, especially if hydro-seeding is chosen as application method.

Protracted periodical fertilisation of plant stands obtained by seeding commercial seed mixtures is considered by various authors indispensable for maintaining satisfactory vegetation cover through time, particularly at high altitude (Schiechtl, 1980; Brugger, 1981; Köck et al., 1982; Cernusca, 1986; Frain et al., 1986b; Holaus and Köck, 1989; Holaus and Partl, 1996; Lichtenegger, 1999; Bozzo et al., 2000). Nevertheless, detrimental effects of repeated fertilisation were observed on the immigration of species from the surrounding vegetation, as sown forage species are more responsive to nutrients input than indigenous species and are therefore advantaged in competition processes (Frain et al., 1986a; Florineth, 1988; Tappeiner, 1996). Also the root biomass of sown indigenous grasses was found to be negatively influenced by repeated fertilisation (Brown et al., 1984).

Nitrogen supply can be effectively carried out in the first restoration stages by nitrogen-fixing legumes (Bradshaw, 1987). After vegetation establishment, a sustained, satisfactory percentage of legumes in the vegetation is particularly important at high altitudes, as Jacot et al. (2000b) demonstrated that in the montane vegetation belt mineralization is the main nitrogen source for sward growth, while in the alpine vegetation belt nitrogen supply is ensured through wet deposition and fixation through leguminous plants.

In the present experiment, the addition of a mycorrhizal inoculum in combination with an organic fertiliser was tested as alternative to a mineral fertilisation. Mycorrhiza fungi are known to improve nutrients and water uptake of plants through connection to the hyphal network in soil and thus increase of the absorbing surface area, mobilization of scarcely available nutrients or excretion of chelating compounds or ecto-enzymes (Abbott et al., 1992; George et al., 1992; Marschner and Dell, 1994; Galli and Schuepp, 1996). Furthermore, adequate levels of mycorrhizal infection may be important for a successful re-establishment of late seral species in restoration sites. Such species are more dependent on the mycorrhizal symbiosis than ruderal species and colonists (Francis and Read, 1994) so that high levels of mycorrhizal infection should be ensured if they are included in the seed mixtures. Most of the alpine species used for the indigenous seed mixtures of the present experiment are indeed typical components of species-rich closed grassland communities in the subalpine and low alpine vegetation belt, which were found to have the highest intensities of mycorrhizal infection in several plant communities over an altitudinal gradient in the Alps (Read and Haselwandter, 1981). Mycorrhiza have also advantageous effects on the physical stabilization of macroaggregates in soil (Tisdall, 1994). Organic fertilisation has been shown to increase mycorrhizal infection densities of dominant grass species at a restoration site, if compared to unfertilised or minerally fertilised treatments (Haselwandter, 1997).

Conceptual aspects for a site specific, ecological restoration

In consideration of what summarised above, needs, aims and evaluation criteria were worked out[2] for the planning and assessment of the present experiment. Commonly used restoration methods (seed mixture with lowland forage species, hydro-seed containing mineral fertiliser) were

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[2] Most of the conceptual aspects presented here, including also the experimental design of the trial, have been conceived in the planning phase of the EU-project ALPEROS, co-ordinated by B. Krautzer. A series of experiments, of which the present trial is part, has been established over a wide altitudinal and geographical range in the Alps.

compared to site-specific, low-impact methods (seed mixtures containing alpine species, hydro-seed containing inocula of soil micro-organisms and organic fertilisers). Site factors (biotic and abiotic) were assessed and analysed in detail.

Three main basic needs were defined and applied to the whole trial. In first place, topsoil was first removed and then re-incorporated into the substrate. The fertilisation input was limited to a sole initial application. The seed rate was maintained within an acceptable range (15 g/m²), also taking account of the deterioration of the seed germination in the field known for a number of binders often included in the application techniques (Schönthaler, 1979; Badany and Schönthaler, 1983).

Priorities were assigned to the aims of restoration. First, a long-term protection against erosion (defined as a minimum cover of 70%) was regarded as the main objective; all others were subordinated to this one. Second, a biotic interaction with the surrounding vegetation, and thus succession towards more or less self-sustainable vegetation types, resembling the autochthonous ones, was desired. Third aim was the achievement of plant stands suitable for agronomic utilisation or for protection against erosion in absence of maintenance care (reduction of costs).

Evaluation criteria were defined for the diverse restoration aims: vegetation cover, total cover and root biomass (protection against erosion), cover of indigenous species and species number (biotic interaction with the undisturbed surrounding vegetation), vegetation dynamics and fertility (sustainability and succession towards indigenous vegetation types), dry matter yield and potential forage quality (suitability for agronomic utilisation).

3.2 Materials and methods

3.2.1 Experimental site

The experimental site was located at the ski resort Sudelfeld, in the Upper Bavaria district (Oberbayern), at the northern range of the Bavarian Alps, about 20 km SE of the Schliersee, at the border between the territories of the townships Oberaudorf and Bayrischzell (Figure 40).

The Sudelfeld is one of the favourite ski resorts of Oberbayern, particularly popular among the inhabitants of Munich and Rosenheim, because of its relative proximity to these towns (80 km from Munich, 35 km from Rosenheim). It is known as ski resort since 1896; the first lifts were built at the beginning of the 50´s. There are currently 18 lifts for a total ski run length of about 25 km at an altitude ranging from 880 to 1,520 m a.s.l.. Only two ski runs, located in the lower part of the ski area and included the experimental trial, are artificially snowed. The ski season has an approximate duration of four months (beginning of December-end of March).

The whole area is used during the summer from mid June to mid September as pasture for heifers. Because of its favourable morphological conformation and because of the low woodland-proportion (the land had been already cleared in the past to obtain the pastures) only small areas were machine-graded through time and this occurred mainly for the construction of the lifts and, more recently, of water pipelines for the artificial snowing.

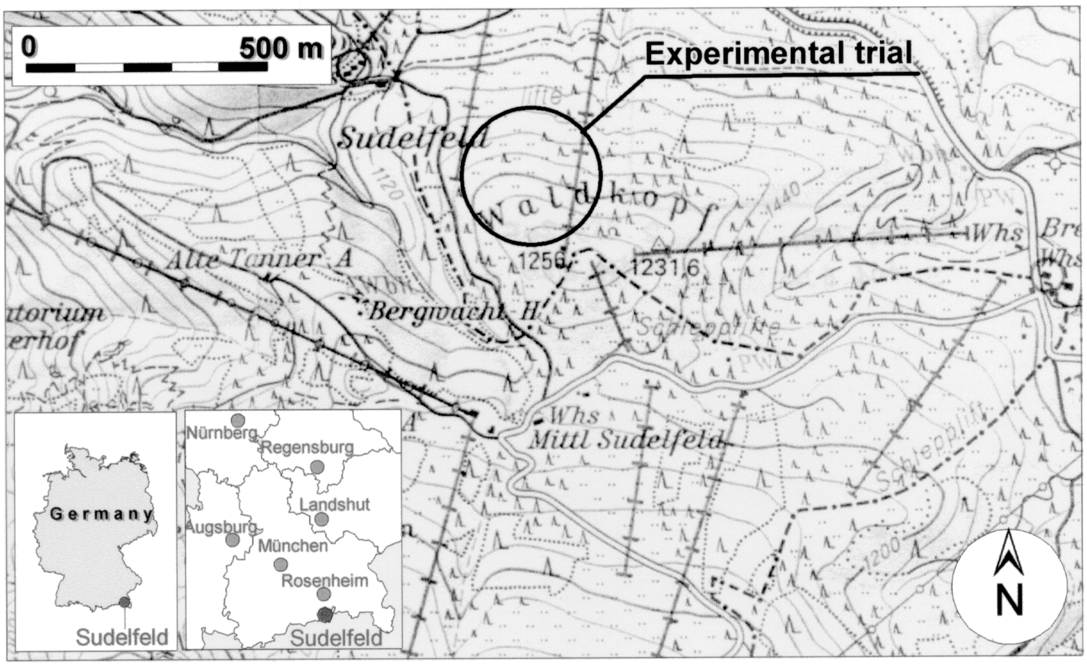

Figure 40: Location of the experimental trial at the Sudelfeld.

The experimental trial (47° 40' 52'' N, 12° 02' 27'' E) lies on the upper portion of the ski run Waldkopf, situated on the northern slopes of the homonymous hill (1,256 m), at an altitude of 1,245 m. The exposition of the trial is NNE and its inclination is about 16°. The portion of the ski slope around the field trial was not machine-graded, and was covered by semi-natural, autochthonous grassland.

3.2.2 Meteorological survey

On the 5th of July 1999, one day before the establishment of the experimental plots, a meteorological station was installed[3] at the experimental site. The meteorological station was placed few metres outside the ski run, at 1,245 m a.s.l. altitude by 8 ° inclination and exposition NE, about 30 m from the experimental plots. The meteorological station was protected by a barbed wire-fence against possible damage deriving from cattle. See location of the meteorological station in Figure 42.

All parameters were measured every 10 seconds and an hourly average was recorded and stored in a data logger (Starlogger, model 6004-1, Unidata, Australia). The weather station was inspected monthly and the sensors were checked for accuracy. Besides, the data stored on the data-logger were monthly downloaded by use of a lap top, to prevent accidental losses of data in case of damage to the data logger.

Table 26: Climatic parameters measured by the meteorological station at the experimental site Sudelfeld.

Parameter	Measurement unit
1. Rainfall	mm
2. Wind speed	m/s
3. Air temperature 2 m above ground	°C
4. Air temperature 5 cm above ground	°C
5. Soil temperature 2 cm below ground	°C
6. Soil temperature 15 cm below ground	°C

Rainfall and wind speed were measured during the whole growing season from 1999 until 2002; the pluviometer and the anemometer were dismantled at the end of the growing season at the end of October and re-installed in the following spring at the beginning of April, in order to avoid mechanical damage to the sensors. Temperatures were measured during the growing season in 1999 and then continuously protracted in the following years starting from April 2000. See Figure 41 for the definition of the observation periods.

[3] The installation was carried out by the BAL Gumpenstein. Part of the equipment was kindly placed at disposal by the above-named institution.

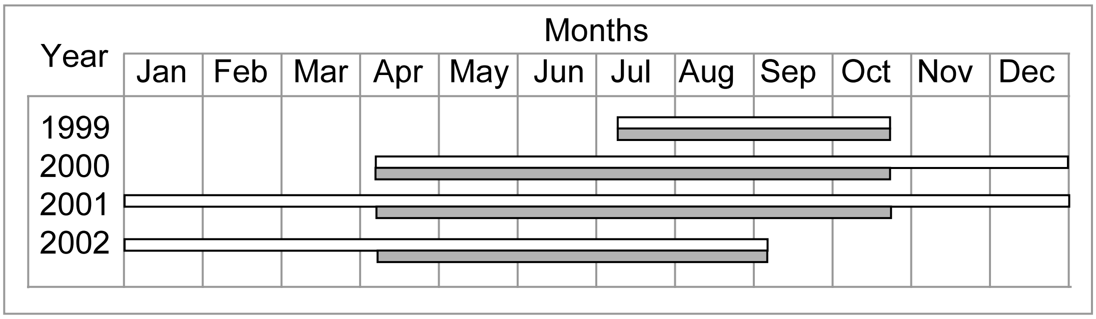

Figure 41: Observation periods of temperatures, precipitation and wind speed at the experimental site Sudelfeld. Open bars represent temperature measurements, filled grey bars precipitation and wind measurements. Because of mechanical breakdown of the anemometer and of the pluviometer following a hail storm, wind data between 3rd of July 2000 and 24th of July 2000 and precipitation data between 3rd of July 2000 and 5th of July 2000 are missing.

The data collected by the meteorological station installed at Sudelfeld were not wholly representative of the climatic conditions within the experimental field. The station was installed outside the machine-prepared, artificially snowed ski run, and therefore snow melt occurred earlier than in the experimental field. In order to determine the time period in which data are representative, the experimental site was regularly surveyed at the beginning of spring and the date on which the snow had melted on the whole experimental trial (100% of the surface was free from snow) was regarded as snow melt date. The influence of the ski run preparation and of the artificial snowing on the duration of the snow cover was calculated as the number of days between the snow melt at the meteorological station (first day with diurnal temperature oscillation 5 cm above ground bigger than 5°C) and the snow melt date. Soil temperature data (2 and 15 cm below ground) were checked for the occurrence of soil freezing and -thawing processes at the time of the snow melt.

The beginning of the growing season was defined as the first day after the snow melt with a daily mean temperature (5 cm above ground) above the 5°C-threshold, while the first day with a daily mean temperature below 5°C was regarded as the end of the growing season (Harlfinger and Knees, 1999). After the snow melt, days with at least one hourly value (2 m aboveground) below 0°C were defined as frosty days (Harlfinger and Knees, 1999), while days with a daily mean temperature (2 m above ground) below 0°C were defined as icy days. Rainy days were defined as days with a rainfall of at least 1 mm in 24 hrs (Harlfinger and Knees, 1999), while hourly precipitation amounts of at least 15 mm were regarded as intense precipitation events.

3.2.3 Geology

The "Sudelfeld" belongs to the Mangfall-mountains, in the calcareous zone of the Bavarian Alps. The prevalent geological substrate is the main dolomite, which has in this area a light-grey to brownish-grey colour and is evidently bedded. The layers' thickness is between 10 and 50 cm. The rock material is very fragile and breaks up to a polygonal, sharp-edged debris (Mehnert et al., 1985).

3.2.4 Soil

3.2.4.1 Surrounding area

The description of two adjoining soil profiles was performed, reflecting small-scale variations observed in the most frequent vegetation type (*Festuco-Cynosuretum*) occurring in the surroundings of the experimental trial[4]. For each soil layer, soil texture, soil colour and chemical properties were assessed. The soil texture was assessed in the field by finger assessment (McRae, 1988). Soil colour was determined in the field according to the Munsell® Soil Color Chart (Anonymous, 1994). The chemical analyses were performed according to the methods listed in Table 27.

Table 27: Methods used for the soil analysis. Each method refers to the respective ÖNORM-guideline.

Soil parameter	Methods
pH	Measurement in 0.01 M $CaCl_2$-solution
Humus (%)	Combustion
$CaCO_3$	Scheibler-method
C (%)	58% of humus content
N (%)	CNS-analyser
Mg	Extraction in 0.1 M $BaCl_2$-solution
P, K	CAL-extraction
Cl, S	Extraction in water 1:10
CEC	Measurement in 0.1 M $BaCl_2$-solution
Fe, Mn	EDTA-extraction

3.2.4.2 Experimental field

Chemical properties of the disturbed soil of the experimental field were investigated just before application of the restoration mixtures. On the 5th of July 1999, after the area chosen for the experimental field had been

[4] The description of the soil profiles was performed by A. Bohner.

machine-graded and just before the application of the seed mixtures, a mixed soil sample for each block of the field trial was collected with an auger up to 10 cm depth. Soil analyses of air-dried, 2-mm-sieved samples were performed according to Table 27[5].

3.2.5 Surrounding vegetation

Relevés of vegetation and survey of the relative site factors were performed in the surrounding of the experimental trial. The main vegetation types were investigated. Relevés were positioned on natural or semi-natural grassland. One relevé was also performed in a small-sized, fragmented woodland-area.

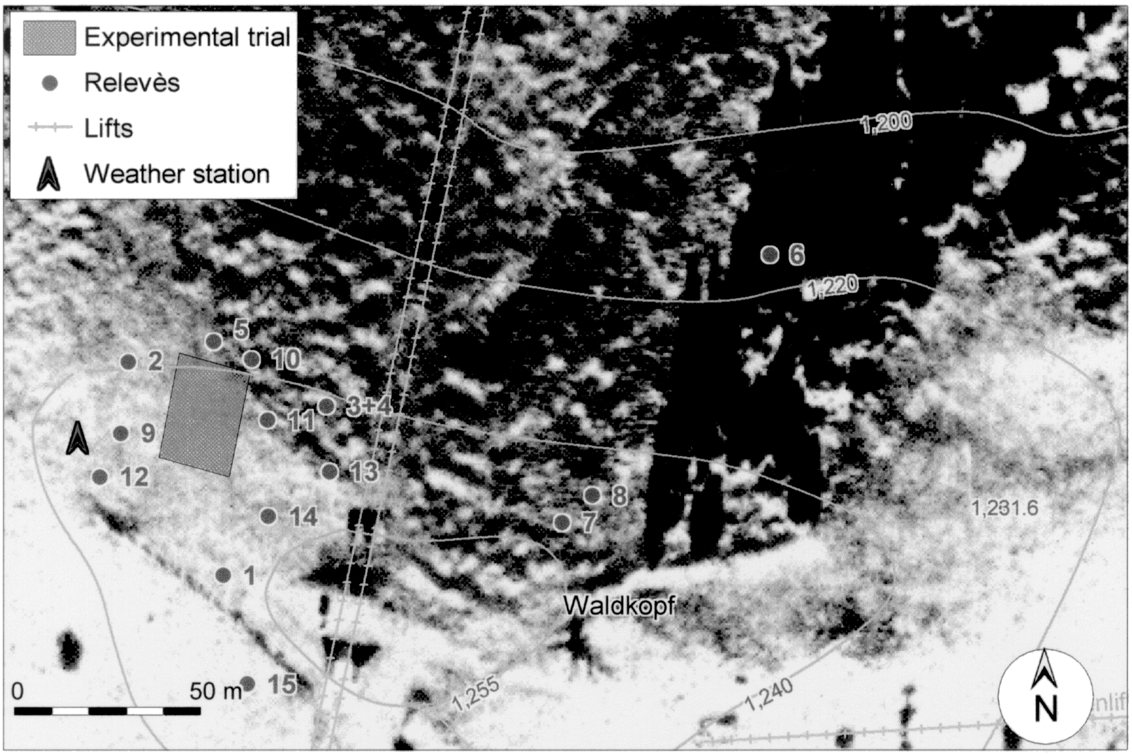

Figure 42: Location of experimental trial, relevés and meteorological station at the experimental site Sudelfeld.

The relevés were carried out according to the Braun-Blanquet method (Braun-Blanquet, 1964) with the following adjustments: cover of species was expressed as percentage of the total cover; mosses and lichens were not determined, but their total cover was assessed. The size of the sampled areas ranged from 15 to 100 m² for the grassland relevés, depending on the maximum available extension of uniform sampling units. The botanical composition was assessed, if possible, more than once during the growing season, to get a complete list of species. The

[5] The soil samples were taken and analysed by the BAL Gumpenstein.

cover was instead assessed only once, when the vegetation achieved its maximum development. Species were determined and named according to Rothmaler (2002). Species of the genus *Festuca* were determined by examination of cross-sections of the leaves at the microscope, as described by Pignatti (1982b). The nomenclature of the plant communities follows that of Oberdorfer (1977, 1978, 1990, 1992).

Exposition was measured using a compass, soil inclination with an inclinometer, altitude on a cartographic basis. Soil depth is expressed as the mean value of 5 measurements of the depth reached by plunging a metallic staff, at randomly selected points within the relevé area, into the soil up to the parent rock or to rock debris.

3.2.6 Trial establishment

3.2.6.1 Seed mixtures

A commercial seed mixture containing highly productive varieties of forage species was compared with two so-called indigenous seed mixtures.

The commercial seed mixture (type B3, Samen Schwarzenberger, Völs, A) is one of the mixtures most frequently used for the revegetation of ski runs in Austria because of its low price (Krautzer, verb. comm.). Most of the components have extra-European origin (*Phleum pratense* Alma, CDN; *Lolium perenne* Nui, NZ; *Agrostis capillaris* Highland bent, USA; *Lotus corniculatus* Leo, CDN; *Trifolium repens* Huia, NZ; *Trifolium hybridum* Aurora, USA; *Achillea millefolium*, trading goods, CDN) or are foreign European varieties (*Festuca rubra* Echo, DK; *Poa pratensis*, Senu, DK; *Festuca ovina* Ridu, DK; *Vicia sativa* Jose, F).

The two indigenous mixtures were assembled with currently available seed of alpine species[6], obtained through seed propagation in valley locations or in lowlands. Addition of bred varieties of forage legumes (*Lotus corniculatus* Oberhaunstädter, D and *Trifolium repens* Milka/Milkanova, DK) was allowed in the indigenous mixtures up to 10% seed weight. The composition of the indigenous seed mixtures was determined on the basis of the botanical composition of the surrounding vegetation, of the site factors, and of the management planned for the plant stands (further utilisation through periodical mowing or no kind of utilisation).

[6] Species available from seed propagation programmes carried out by the partners involved in the EU-project ALPEROS (FAIR CT98-4024).

Table 28: Percent weight composition (W) of the seed mixtures used at the experimental site Sudelfeld and seed germination (G) of the single species[7].

	Seed mixture					
	1 Commercial		2 Indigenous for further utilisation		3 Indigenous without further utilisation	
	W	*G*	W	*G*	W	*G*
Festuca rubra	29.6	*88*				
Phleum pratense**	19.9	*93*				
Lolium perenne	15.7	*91*				
Poa pratensis**	10.6	*86*				
Agrostis capillaris*	4.6	*93*				
Festuca ovina	2.5	*75*			10	*74*
Festuca nigrescens*			28	*91*	20	*91*
Poa alpina*			25	*81*	25	*81*
Poa violacea			10	*48*	10	*48*
Phleum hirsutum			10	*68*	5	*68*
Poa supina**			5	*92*	5	*92*
Phleum alpinum			5	*40*	5	*40*
Lotus corniculatus*	5	*94*	3	*99*	4	*99*
Trifolium repens*	4.2	*80*	6	*98*	6	*98*
Trifolium hybridum	2.4	*92*				
Vicia sativa	3.4	*85*				
Anthyllis vulneraria*			3	*90*	4	*90*
Trifolium badium			1	*96*	2	*96*
Achillea millefolium*	0.7	*75*				
Leontodon hispidus*			2	*58*	2	*58*
Plantago lanceolata*			1.9	*62*		
Crepis aurea*			0.1	*26*		
Silene vulgaris					2	*87*
Chaff	1.4					

* species occurring in the surrounding vegetation under similar ecological conditions,
** species occurring in the surrounding vegetation under different ecological conditions

The two indigenous mixtures were quite similar to each other. *Festuca nigrescens* and *Poa alpina* were chosen as main components of both mixtures. That for further utilisation contained as a difference small amounts of *Crepis aurea* and *Plantago lanceolata*, two species of

[7] Germination experiments were carried out by the BAL Gumpenstein.

medium to high fodder value according to Spatz et al. (1979), found also in the pastures surrounding the field trial. The percentage of *Festuca nigrescens* was higher than in seed mixture 3. In the mixture without further utilisation there was a higher proportion of seed of pioneer species, such as *Anthyllis vulneraria* and *Silene vulgaris*. A moderate amount (10%) *of Festuca ovina*, whose fodder value is quite low, was also enclosed in this seed mixture.

Concerning the germinative capacity of the single species (Table 28), satisfactory values of at least 75% were achieved by all species included in the commercial seed mixture, while this threshold was not achieved by 7 out of the 15 species included in the indigenous seed mixtures. Only two of them (*Poa violacea* and *Phleum alpinum*) had weight proportions in the mixture above 3%. In particular, the seed germination of three species (*Poa violacea*, *Phleum alpinum* and *Crepis aurea*) was below 50%.

The indigenous seed mixtures were slightly species-richer (13 species against 11 species in the commercial seed mixture). Considering the results of the investigations of the surrounding vegetation (see paragraph 3.3.3), it is possible to point out some remarks about the composition of the seed mixtures in comparison to the vegetation occurring on the surroundings of the experimental plots. The percentage of species in common between the seed mixtures and the vegetation surrounding the field trial is in all cases below 70% (54% in mixture 1, 69% in mixture 2, 54% in mixture 3), and it is slightly higher for the indigenous seed mixture for further utilisation. Five of the twelve species included in mixture 1 (*Achillea millefolium*, *Agrostis capillaris*, *Lotus corniculatus*, *Phleum pratense* and *Trifolium repens*) occur spontaneously in the surrounding vegetation. Most of them have quite a large ecological amplitude and are distributed from the plane up to the montane or subalpine vegetation belt. With exception of *Trifolium repens* and *Lotus corniculatus*, the opposite situation occurs in mixtures 2 and 3, where part of the species are typical of the subalpine and alpine vegetation belts, and some of the species in common with the surrounding vegetation are here on the lowest limit of their altitudinal distribution, such as *Poa alpina* or *Festuca nigrescens*.

The weight percentage of seed of autochthonous species, however, showed a large difference between commercial (45%) and indigenous mixtures (74% and 66%). This difference further increases, if only the species occurring in the surrounding vegetation under ecological conditions similar to those of the experimental trial are considered. *Phleum pratense*, *Poa pratensis* and *Poa supina* were found only on flatter, deeper, hollow grounds in stands with a larger proportion of species with relatively high nutrient demand. If they are not regarded as site-specific indigenous species, just 15% seed weight of mixture 1 is to ascribe to indigenous species, while seed mixtures 2 and 3 exhibit relatively high percentages (69% and 61% respectively).

A seed rate of 15 g/m² was used for each seed mixture.

Each seed mixture was coupled with an utilisation measure corresponding to the aim the seed mixture was planned for. While no kind of management was undertaken for mixture 3, the others were mown once at the end of the second growing season, and later on twice a year, at the flowering time of *Festuca nigrescens* and towards the end of the growing season (beginning to mid September). The factor "seed mixture" is therefore a combination of a seed mixture and of the utilisation connected with it.

3.2.6.2 Application techniques

A conventional hydro-seed-technique (A) containing mineral fertilisers was compared with two innovative techniques (B and C) lacking of mineral fertilisation and containing a mycorrhiza-inoculum, a fungal inoculum (*Penicillium* sp.), algal spores (dried blue and green algae) and low levels of nutrients input. Techniques B ad C differed from each other only for the additional soil protection with a straw mulch in technique C.

The three techniques are defined as follows:

A. Conventional hydro-seed: 15 g/m² of seed were thoroughly mixed together with 80 g/m² cellulose, 15 g/m² Curasol (synthetic binder), 20 g/m² of mineral fertiliser (15 N: 15 P: 15 K), 5 g/m² Rekuform® (Proagro GmbH, Abenberg, D) and water.

B. Mycorrhiza-hydro-seed: 15 g/m² of seed were thoroughly mixed together with 80 g/m² of mature compost, 65 g/m² organic nutrient conveyor (provide Verde®, Siegfried Agro AG, Zofingen, CH), 3.5 g/m² organic binder (GSA 2000, Siegfried Agro AG, Zofingen, CH), 65 g/m² mycorrhiza inoculum (mykoVAMP, Siegfried Agro AG, Zofingen, CH) and water.

C. Mycorrhiza-hydro-seed with straw mulch: the same ingredients used for application technique B. After seeding, the plots were additionally protected with 500 g/m² of straw, which was fastened to the ground with 500 g/m² of unstable bituminous emulsion.

After trial establishment, no kind of fertilisation input was supplied to the plots in the following years.

Table 29: Composition and expected effects of brand products (binders, fertilisers and inoculants) enclosed in the application techniques used at the Sudelfeld. Information about most products was obtained from the producers.

Product name	Product type	Composition	Expected effect (according to the producer)
Curasol [1]	Synthetic Binder	Polyvinyl acetate	Binding of seed to the soil and fastening of the soil itself (protection against erosion)
Rekuform® [2]	Synthetic-organic N fertiliser	38% N (4% urea, 34% methylen-urea)	Slow-release N-fertilisation
provide Verde® [3]	Fungal and algal inoculum, nutrient conveyor	25 g/kg of *Penicillium* sp. and spores of blue and green algae; 920 g/kg organic nutrient concentrate (58 g/kg N, 28 g/kg P_2O_5, 16 g/kg K_2O)	Root colonization of *Penicillium* sp. with production of organic acids dissolving P, which becomes available for plant uptake; production of organic matter by the algae; soil fertilisation
GSA 2000 [4]	Organic binder	Starch- and polysaccharides-concentrate	Binding of seed to the soil and fastening of the soil itself (protection against erosion)
MykoVAMP [5]	Vesicular-arbuscular mycorrhiza-inoculum	Infection units (spores and hyphae) of *Glomus etunicatum*, *Glomus intraradices* and *Glomus fasciculatum*, carried on expanded clay	Mycorrhizal infection of plants and, subsequently, improved nutrient supply and promotion of soil aggregation

Information sources:
[1] www.nwmissouri.edu/~soils/WIND-E.doc (12.03.2003),
[2] http://www.proagro-gmbh.de/produktseite.htm (12.03.2003),
[3] www.geoverde.ch/provideVerdee.pdf (12.03.2003),
[4] www.geoverde.ch/gsa2000.pdf (12.03.2002),
[5] www.geoverde.ch/mykoVamp.pdf (12.03.2003)

3.2.6.3 Experimental design, field preparation and maintenance

On the week before trial establishment, an area of about 30 x 22 m was machine-graded near the top station of the ski-lift "Waldkopf", enlarging an area that had already been excavated in early spring 1999 in order to build a water pipeline for the artificial snowing of the ski run. The topsoil was removed with an excavator and later re-incorporated into the surface.

The seed mixtures and the application methods were used in all possible combination with three replicates. The experimental design (Figure 43) was arranged as a two-factorial split-plot[8], each level of the factor "application technique" being assigned to whole plots and the levels of the factor "seed mixture" being assigned to the sub-plots. The area of each plot was 21 m² (7 x 3 m); 20-cm-wide corridors separated the plots from each other.

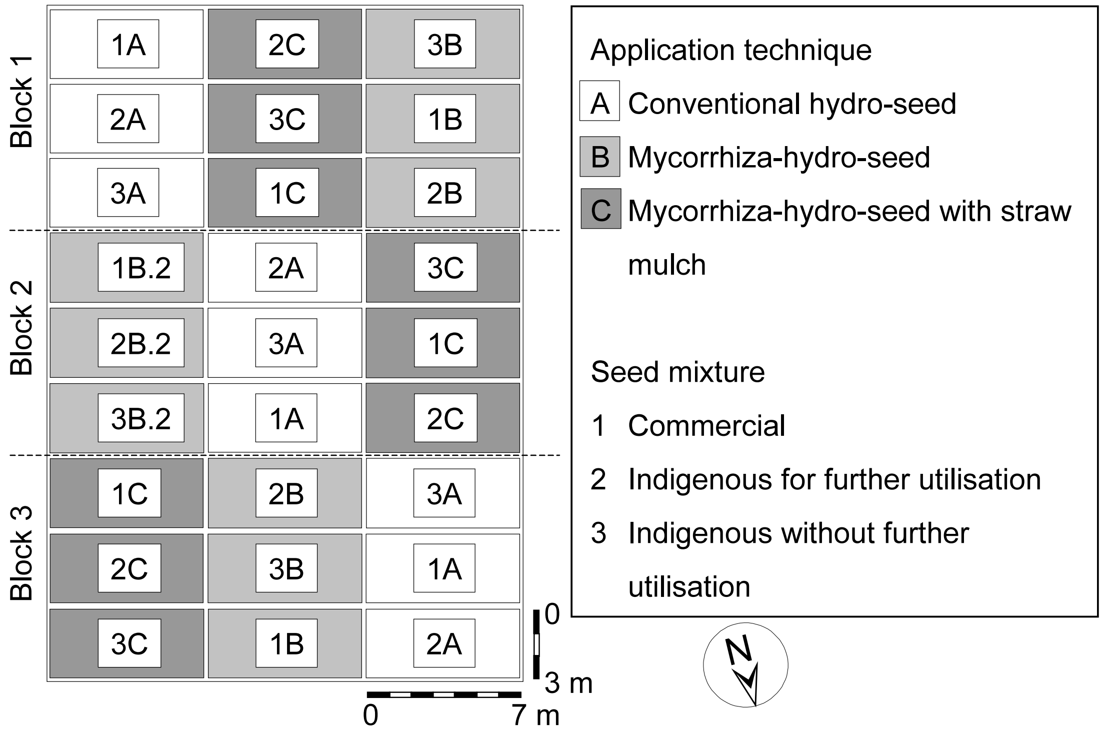

Figure 43: Experimental design of the field trial at the Sudelfeld.

The restoration intervention took place on the 6th of July 1999 and was carried out by a restoration company (Hydrogreen Landschaftsbau, Eschenau, A). The treatments were sprayed onto the plots by use of a small-sized hydro-seeder (prototype). The tank was charged every time with the amount of restoration mixture needed for the three plots with the same combination of seed mixture and application technique, and then washed with water, to prevent mixing up of residuals with the further combination. As an intense precipitation with strong wind took place before the completion of application technique C, it was necessary to delay the application of straw and bituminous emulsion to the next day.

8 The design was improperly randomised, as a systematic order was recognisable in the spatial distribution of the plots. A spatial analysis was performed for some variables measured in 1999 and 2000, assigning x- and y-coordinates to each plot. Attempts to estimate the covariance using the coordinates as covariates failed, probably because of the insufficient amount of data. The hypothesis, that the lack of randomisation would affect the results could be neither rejected nor accepted. Results must be therefore considered with caution.

In each plot, two opposite corners were marked by round magnets (30 mm diameter x 16 mm thickness, Josef Attenberger GmbH, Sankt Wolfgang, D) buried at about 15 cm depth, so that their position could be found again in the following growing seasons using a metal detector (mod. GA-52 Cx, Harrer Wassertechnik, Vienna, A) and the outline of the plots could be traced. Additionally, plastic rods (Baumann Saatzuchtbedarf, Waldenburg, D) stuck into the soil at each corner and cut about 2 cm above soil level helped recovering precisely the position of the plots. The plots were marked during the growing season with further plastic rods, in order to rapidly find their position.

An electrical fence (8 V voltage) was built up all around the trial, in order to prevent entry and trampling of animals (cows, hares) into the experimental field. Wires were positioned in the first year at 100 cm height and starting from the second year at three additional heights (10 cm, 20 cm, 45 cm). The vegetation under the fence was cut every month during the growing season, in order to avoid voltage drops due to plants touching the electric wire.

The fence and the plastic rods marking the plots were dismantled every year at the beginning of the autumn and rebuilt in the following spring.

3.2.7 Cover of residual fragments of the pre-existent vegetation

As fragments of the pre-existent autochthonous vegetation were still quite abundant within the machine-graded experimental field, their overall cover was assessed by sight prior to the application of the seed mixtures. Furthermore, all species occurring within the plots were recorded, and those substantially contributing to the overall cover (more than 1% cover) were separately listed, in order to identify those possibly affecting the vegetation dynamics within the experimental field.

Species were named according to Rothmaler (2002).

3.2.8 Soil seed bank

The day before the trial establishment, after the field had already been machine-graded and prepared for the application of the restoration mixtures, one soil core (exceeding 10x10x10 cm) was collected per each plot, transported immediately to Witzenhausen and stored in cold room at 6°C in the dark for 18 weeks. The samples were then reduced to a volume of 1 l and sieved. Roots, stems and leaves were discharged, in order to avoid vegetative multiplication. The remaining soil was thoroughly mixed and then spread in 1 to 2 cm thick layers on 20x30 cm plastic trays, whose bottom was punctured to prevent water stagnation. The samples were transferred to a greenhouse cabinet conditioned as follows: 20°C temperature; 12 hours light. These settings have to be considered purely indicative, as the measurements performed in the cabinet indicated that diurnal temperature fluctuations depending on external temperature and solar radiation as well as general temperature increases in the warm months were experienced by plants (Figure 44).

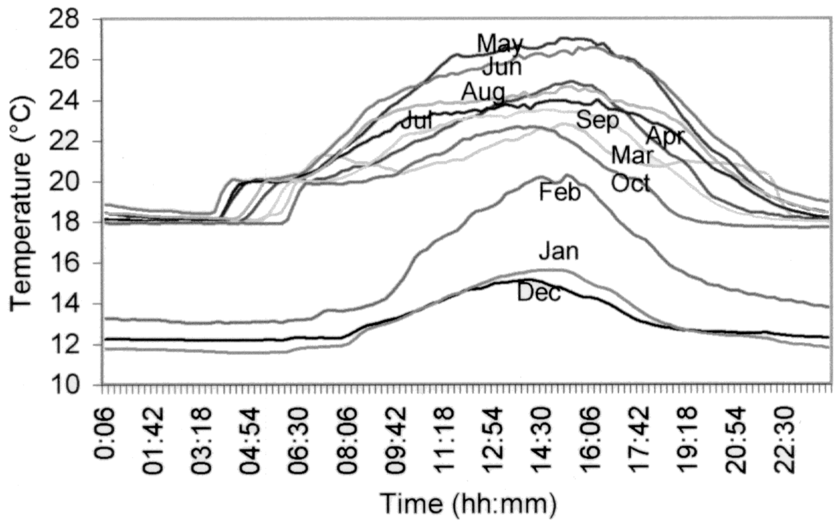

Figure 44: Mean temperature daily patterns in the greenhouse on a monthly basis (data relative to the first and the last month in which the experiment took place are missing).

Samples were kept wet by periodical watering with rain water. Three times per week they were inspected and seedlings emerged were recorded. When in two weeks no new seedling appeared in any of the samples, all of them were left dry, the soil was stirred in order to bring seeds with light requirements for germination to the soil surface, and then watered again. This procedure was repeated altogether 5 times. After 12 months the experiment was definitively dismantled.

Fourteen-day-old seedlings were transferred to multipot-trays (Romberg, Ellerau, D) and grown in gardening substrate until species determination was possible. Some species that did not flower in the first year, but whose flowering was required for their identification, were transplanted in the open field in Autumn 2000 and determined in the following growing season. Species identification was impossible for some seedlings that died before they achieved a developmental stage suitable for identification. They were simply grouped as grasses, graminoids, legumes and forbs. The species nomenclature of Rothmaler (2002) was followed. Data about emerged seedlings were converted to seedlings per square metre.

3.2.9 Vegetative re-colonization of the autochthonous vegetation

Two months after the establishment of the experimental field, the vegetative re-colonization of the autochthonous vegetation within the plots was studied according to the quadrat-charting method (Müller-Dombois and Ellenberg, 1974). Each plot was delimited using plastic strings with marks every metre. The plot was then divided with further strings in 21 1-m²-squares. A square frame (1x1 m) sub-divided with strings in 100 square decimetres was positioned on the ground with the aid of the 1-m²-grid and the crown outlines of the plants that took origin from residual of the pre-existing vegetation or from vegetative organs contained in the soil were drawn in scale 1:18.2 on paper. The whole area of each plot was charted by subsequent placing of the square frame. At the time of the assessment, the sown vegetation was clearly distinguishable from the rest of the vegetation because of the different size and developmental stage of plants. Tillers originated by stolons were represented as circles with an area approximately equivalent to the real one. The charts on paper were reduced in size by copying them to an A4-format and then scanned. The graphic files were imported in a GIS program (MapInfo Professional, release 4.1, MapInfo Corporation, 1996), geo-referenced, digitized, and the total cover achieved by the autochthonous vegetation through vegetative re-colonization was calculated.

3.2.10 Cover and botanical composition

The cover was measured in the first year (1999) at the end of the growing season and in the following years at the flowering time of *Festuca nigrescens* (25% of plants flowering), in order to link the time of the assessments to an indicator of the developmental stage of the vegetation, thus minimizing in the comparisons between years the effects of different meteorological condition, which may determine an earlier or later start of the growing season. The cover was measured with the point intercept method (Levy and Madden, 1933, cit. in Goodall, 1952; Müller-Dombois and Ellenberg, 1974). Wire pins were vertically lowered downwards through regularly-spaced holes (every 20 cm) in a woody frame, having one extensible side and mobile stands to keep it stationary. Frame verticality was achieved with the aid of a water level (Figure 45). The first species intercepted by the tip of the pin was recorded. Altogether 100 points were recorded along the diagonals and the symmetry axis of the plots (30 points along each diagonal and the major axis, 10 along the minor axis). Measurements were started 68 cm from the corners (for points along the diagonals) and 50 cm from the margins of the plot (for assessments along the symmetry axis), in order to minimize margin effects and allow measurements at the same sampling points each year.

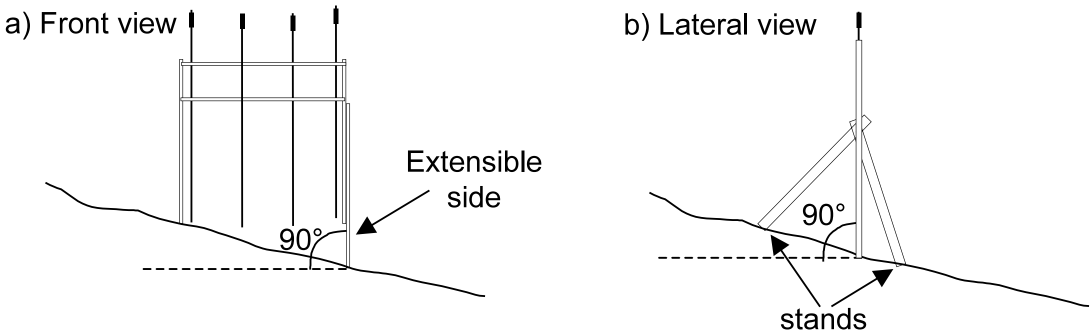

Figure 45: Positioning of the point quadrat frame for cover assessment.

The number of contacts of a certain species gave its percent cover. In the first observation year the grasses contained in the seed mixtures 2 and 3 were recorded in undifferentiated manner as "grasses" because their size and their early developmental stage made it impossible to determine them. Mosses were generically recorded as "mosses". If no plant was intercepted, soil, litter, stones, rocks or straw (in plots with application technique C) were recorded. Attention must be paid to the fact that vegetation cover in plots with application technique C may have been slightly underestimated in the first growth phase (plants masked by straw) and straw may have been underestimated when plants achieved large size (straw masked by plants).

Further species, occurring in the plots but not intercepted by the metal rod, were listed and a percent cover 0.1 was assigned to species with more than one individual. The cover of rare species was recorded as 0.05%. The percent cover of the species was then adjusted, starting from the species with the highest cover scores, in order to get altogether (covered + uncovered surface) a value between 99.5% and 100.5%. The species nomenclature of Rothmaler (2002) was followed, with the exception of *Poa violacea*, absent in Germany and named according to Ehrendorfer (1973). The determination of plants in a vegetative stage was performed according to a self-made compendium of various keys (Kiffmann, 1978; Hubbard and Boeker, 1985; König and Mott, 1986; Doree, 1987; Klapp and Opitz von Boberfeld, 1995a and 1995b; Dietl et al., 1998).

The partial cover given by species, here defined as indigenous, occurring both in the experimental plots and in the semi-natural plant communities around the field trial, was calculated on the basis of the results of the investigations of the surrounding vegetation. As the soil of the field trial had been severely disturbed, and thus low nutrient levels were expected, the sole species found in the scanty, typical aspect of the pre-existent surrounding vegetation were included in the calculation (see the botanical composition of cluster B in paragraph 3.3.3, only species occurring at least in 4 of the 5 relevés).

Additionally, the cover of the straw mulch used in the application technique C was estimated by sight each year two weeks after the snow melt and at the end of the growing season. Such data were used, together with those of the point quadrat assessments, for determining the persistence of the mulch material.

3.2.11 Species frequency

At the time of the flowering of *Festuca nigrescens* the frequency of the species in the plots was measured through linear analysis, according to the methodology of Daget and Poissonet (1971). This method, relying on the point quadrat method (Levy and Madden, 1933 cit. in Goodall, 1952), was originally developed for an indirect assessment of pasture productivity in the alpine region, but it is regarded by some authors as a measurement of species frequency (Kreeb, 1983; Ostermann, 1991). The linear analysis was shown to be a reliable method for the precise assessment of vegetational changes, if the absolute number of contacts is used (Stampfli, 1991), the so-called specific frequency (Daget and Poissonet, 1971). The same frame described in paragraph 3.2.10 was used for this assessment (see there details about the frame and its positioning). At regular distance intervals (each 20 cm) along the diagonals and the symmetry axis of the plots, a metal rod (5 mm Ø) was driven vertically downward. At each rod positioning, the species intercepted were recorded. Each species was recorded once per each rod positioning, even if more parts of the same plants or more plants of the same species were touching the rod at the same time. As plants intercepted at different heights are recorded, the linear analysis allows to measure the frequency of all species, also those of small size, that are usually screened by the large-sized ones (Daget and Poissonet, 1971). Altogether 100 observations were performed in each plot. Mosses were generically recorded as "mosses". The species nomenclature of Rothmaler (2002) was followed, with the exception of *Poa violacea*, absent in Germany and named according to Ehrendorfer (1973).

Changes of the specific frequency of sown species through time were analysed separately within each seed mixture, as species were contained in different proportions within each seed mixture. An exception was made for non-sown, indigenous species, assuming that the same amount of vegetative propagation units (propagules and parts of plants able to shoot again) and seeds occurred in each treatment.

3.2.12 Ellenberg's ecological indicators

The weighted mean scores of the ecological indicators of Ellenberg (1996) were calculated for each plot, on the basis of the cover data, as suggested by Spatz et al. (1979). The indicators for some missing species (*Glyceria declinata*, *Polygala amarella*, *Thymus praecox* ssp. *polytrichus*, *Willemetia stipitata*) were mutuated from those proposed by Karrer for the Austrian flora (2002).

Although ecological indicators are not cardinal numbers, the calculation of mean values has become usual in the practice (Briemle, 1997), and there is general agreement about their quasi-cardinal nature (Ellenberg et al., 1992). Means are usually calculated in order to characterise ecologically a site. In the present work they were instead used to monitor changes of the proportion between species with different ecological characteristics. In the first phase after sowing, plant stands are mainly influenced by the composition of the seed mixtures and by the competitiveness of the single species, while ecological factors play an important role, together with intra- and inter-specific competition, in determining vegetational changes (Voigtländer and Voss, 1979). The increase of a certain indicator would be caused either by a quantitative increase of the proportion of species having high values of that indicator or by a decrease of those with a lower value. Evident variations indicate ongoing adaptation of the vegetation to the site conditions and, indirectly, which ecological factors are mainly affecting the succession. Ecological indicators are therefore not used in this case to detect changes in site conditions, but rather to individuate indirectly ecological factors influencing the vegetation dynamics.

3.2.13 Above-ground biomass

The dry matter production was assessed in 2000 at the end of the growing season, and in the following years also at the time of flowering of *Festuca nigrescens*. In each plot where seed mixtures 1 and 2 were sown, an aluminium-frame (1x1 m) was randomly placed four times on the ground and the area marked by the frame was accurately cut with electric scissors (model Accu 6, Gardena Kress and Kastner GmbH, Ulm, D) at an height of about 3 cm. Plot margins were avoided for this assessment. The fresh biomass was weighed in the field, as well as a sample of it, which was later oven-dried (36 hrs at 105°C) and re-weighed to determine the water content of the fresh biomass, and in turn the dry matter production. The remaining area of each plot was cut with portable motor scythes (mod. FS 85T, Stihl, Waiblingen, D and mod. SRM-2015L, Echo, Metzingen, D) and the resulting biomass was removed from the field trial.

3.2.14 Below-ground biomass

In the third observation year, two root samples were taken within each experimental plot at the end of the growing season (24th October) according to the auger method of Böhm (1977), avoiding spots where residual of the pre-existing vegetation were charted in the first growing season. A cylindrical steel auger (8 cm diameter) with a sharp edge was plunged into the soil to a depth of 5 cm and a soil volume of 250 cm^3 was extracted. This sampling depth was chosen because this was the maximum soil depth achievable in most of the lower part of the experimental field (block 3). The samples were frozen and stored until analysis[9]. The soil was then removed from the samples with the aid of a root-washing machine (Smucker et al., 1982) and the so-obtained below-ground biomass was oven-dried at 70°C for 12 hours. For each plot, the average of two measurements was used for statistical analysis.

3.2.15 Potential forage quality

In order to compare the forage quality of plant stands deriving from different seed mixtures and to monitor quality changes through time, the

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[9] The measurement of the root biomass was carried out by A. Bohner at the BAL Gumpenstein.

Klapp's value score (Wertzahlen) was calculated according to Klapp et al. (1953) for each plot on the basis of the specific contribution. The Klapp's value scores allow the evaluation of the potential forage quality, which can be achieved with an adequate management (Voigtländer and Jacob, 1987). A relatively close relationship between the measured (expressed as energy content) and the potential forage quality (expressed as Klapp's value score) was found by Franke (2002) for a number of plant communities, with exception of sedges- and rushes-dominated stands. Value scores of single species were obtained from the database of the program Oeksyn (Spatz et al., 1979) containing those proposed by Klapp (1971) and others added by the program's authors. Missing values were mutuated from Park (1984; *Aster bellidiastrum*, *Carduus defloratus*, *Carex sempervirens*, *Galium anisophyllon*, *Gentiana asclepiadea*, *Leucanthemum vulgare*, *Phleum alpinum*, *Ranunculus montanus*, *Selaginella selaginoides*, *Soldanella alpina*, *Viola biflora*, *Willemetia stipitata*), Klapp and Opitz von Boberfeld (1995; *Poa supina*) or assigned according to the results of the forage analysis of Stebler and Schröter (reported in Hegi, 1964; *Trifolium badium*, 7). *Festuca nigrescens* was assimilated to *Festuca rubra*. The specific contribution was obtained as quotient of the specific frequency and the sum of all contacts (see paragraph 3.2.11) and can be considered a relative expression of the biomass. Poissonet and Poissonet (1969 cit. in Daget and Poissonet, 1971) demonstrated that there is a linear relationship between the specific contribution and the weight contribution (relative weight contribution of each species to the yield), which can be considered the most accurate method for determining species proportion in the grassland sward.

3.2.16 Fertility

The species included in the seed mixtures were repeatedly examined during the growing seasons 2000 and 2001 and the proportion of plants which had flowered and produced ripe seed was estimated by sight. Species were considered having produced ripe seed if their fruits or glumes contained filled seeds. Seeds of grasses must have at least attained the wax-ripe stage; a fingernail-test was used to check it. Small seed samples of the species ripening at that time were randomly hand-harvested within the experimental field. The seed was allowed to dry on paper at room temperature for about two weeks, then it was further oven-dried at 30°C for 36 hrs. After storage at 6°C for about 4 months the seed was manually cleaned using reflected light as aid.

Table 30: Test conditions of the germination tests carried out on seeds harvested at the experimental site Sudelfeld (2000 and 2001).

Species	Pre-treatment [a]	Temperature (°C)	Light (hrs)	Reference
Agrostis capillaris	p-c; KNO_3	20-30	12	ISTA, 1996
Anthyllis vulneraria	p-c	20	12	ISTA, 1996; Krautzer, 1995a
Crepis aurea		20	12	Krautzer, 1995a
Festuca nigrescens	p-c; KNO_3	20	12	Krautzer, 1995a
Leontodon hispidus		20-10	16	Schütz, 1988
Lolium perenne	p-c; KNO_3	20	12	ISTA, 1996
Lotus corniculatus	p-c	20	12	ISTA, 1996; Krautzer, 1995a
Phleum alpinum	p-c; KNO_3	20	12	Krautzer, 1995a
Phleum hirsutum	p-c; KNO_3	20	12	Krautzer, 1995a
Phleum pratense	p-c; KNO_3	20-30	12	ISTA, 1996
Poa alpina		20	12	Krautzer, 1995a
Poa supina		20	12	
Poa violacea	p-c; KNO_3	20	12	
Plantago lanceolata		20	12	ISTA, 1996
Silene vulgaris	m.s.	20-10	16	Schütz, 1988; Schütz, 1990
Trifolium badium	p-c; m.s.	20	12	Krautzer, 1995a
Trifolium hybridum	p-c	20	12	ISTA, 1996
Trifolium repens	p-c	20	12	ISTA, 1996

a p-c: pre-chilling (storage of the seeds in contact with the moist substrate for 7 days at 4-6°C in the dark); KNO3: 0.2% KNO3 as medium (P 8291, Sigma-Aldrich Chemie GmbH, Deisenhofen, D); m.s.: mechanical scarification (razor blade).

3.2.17 Statistical analysis

Concerning the relevés performed in the surrounding vegetation, classification of the vegetation was performed through cluster analysis (Wildi and Orloci, 1990). Percent cover data were transformed with the van der Maarel´s scale (van der Maarel, 1979), which resembles a logarithmic scale and therefore matches the human perception of cover abundances (Müller-Dombois and Ellenberg, 1974). Relevés were grouped by mean of cluster analysis using the similarity ratio (Westhoff and van der Maarel, 1978) as similarity coefficient and the minimum variance clustering (Orloci, 1967) as clustering technique. The minimum variance clustering is considered to be a suitable classification method for vegetation data with relatively continuous variations, such as in the case of the main vegetation type occurring at the Sudelfeld, because it tends to minimize the heterogeneity within groups while enhancing that between groups (Feoli et al., 1982).

For data assessed within the experimental field, fulfilment of the assumptions of analysis of variance was checked prior to analysis of variance. Normal distribution of residuals was tested with the Kolmogorov-Smirnov's test (Köhler et al., 1996). Variance homogeneity, according to the suggestions of Sachs (2002), was tested with the Cochran's test (Camussi et al., 1990) in case of lightly skewed distribution; in case of normal distribution, the Cochran's test was employed for less than ten samples, otherwise the Bartlett's test (Sokal and Rohlf, 1995) was performed; for distributions flatter or higher than the normal one, the Cochran's test was used up to ten samples, and the Levene's statistic (Sachs, 2002) for ten or more samples. As at least 10 observations per sample are recommended for the Levene's test (Sachs, 1990), where less observations were available the Cochran's test was performed instead. All above-mentioned tests were performed at the 10% level.

In case the assumptions of the analysis of variance were met, analysis of variance (ANOVA) was performed. ANOVA and all below-described tests were carried out at $\alpha=0.05$. If main effects or their interactions were significant, multiple comparisons were performed with Duncan's Multiple Range Test (DMRT) at $\alpha=0.05$. Correlation between variables was investigated with the Pearson's parametric correlation test after positive testing of residuals for normal distribution with the Kolmogorov-Smirnov's test (Sokal and Rohlf, 1995).

Cover percentages and density counts were generally transformed, if necessary, using respectively the arcsine transformation function and the logarithm function, in order to meet variance assumptions before submitting the data to variance analysis (Sokal and Rohlf, 1995). Fulfilment of the assumptions of analysis of variance was checked afterwards as above described.

If, even after transformation, data violated the assumptions of the variance analysis, non-parametric tests (the Kruskal-Wallis-test for several independent samples, the Mann-Whitney-U-test for two independent samples) were used instead. Several related samples were investigated with the Friedman test; for multiple comparisons the Wilcoxon-Wilcox-test was used (Köhler et al., 1996). The tests were performed assuming that no interaction between the main factors occurred.

Ordination of the cover assessments performed within the experimental plots from 2000 to 2002 was performed through Principal Component Analysis (PCA), in order to make visible vegetation shifts through time. Prior to analysis, data were arcsine-transformed according to Goodall (1952), in order to achieve normality of data and variance homogeneity. As data contained quite a large amount of zeroes, the chord distance (Orloci, 1967), corresponding to the Euclidean distance computed after scaling the relevé vectors to length 1 was chosen, as it was demonstrated to be a suitable distance measure in such cases (Legendre and Gallagher, 2001).

Multivariate analyses were performed with the software MULVA-5 (release 1.04, Wildi and Orloci, 1994), all other analyses with the software SPSS (release 7.0, SPSS Inc. 1995).

3.3 Results

3.3.1 Meteorological survey

The analysis of the air temperatures near the ground in the first four weeks after sowing showed that the mean daily temperature remained throughout almost the whole period above 10°C, being therefore suitable for seedling establishment (Figure 46). Starting from the second week, daily temperature peaks of 20°C or more were a common feature, ensuring suitable conditions for seed germination. Adverse events such as frost were not observed. Considering the daily precipitation, water stress periods were not present, as rainfall was quite evenly distributed, with exception of the fourth week. An almost continuous rainfall, including also an intense precipitation event, occurred in the first week after sowing.

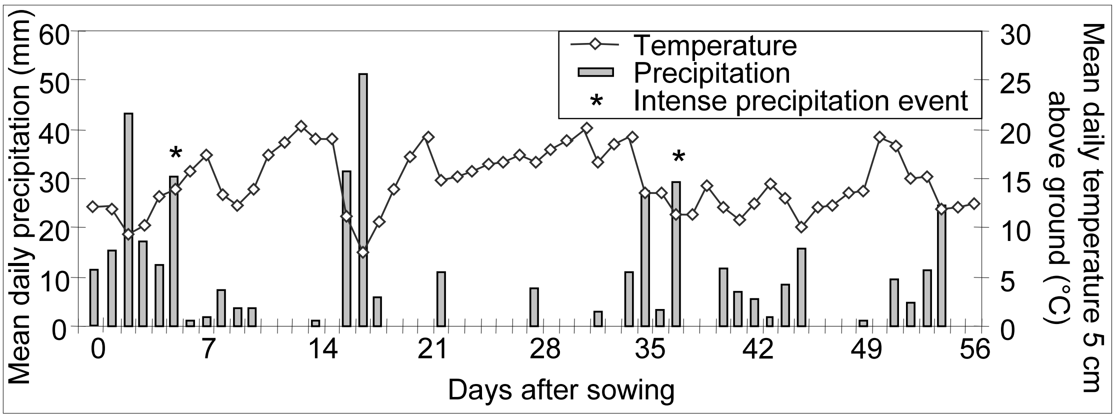


Figure 46: Temperature and precipitation during vegetation establishment (first eight weeks after sowing) at the experimental trial Sudelfeld.

Concerning the duration of the growing season, a certain variability was observed in the four investigation years (Figure 47).

A theoretical duration of almost six months, based on the 5°C-temperature-threshold, was observed outside the ski run, which was instead prepared and artificially snowed. Snow cover periods caused by late snowfall were observed within the growing season. The field trial became instead completely snow-free at the end of April or at the beginning of May, with a delay of more than one month in comparison to the non-prepared adjacent slope. After the snow melt, no further snow precipitation was observed at the Sudelfeld. The duration of the growing season within the ski run ranged therefore between four and five months. In this time period, just one frosty day (June 2001) and no icy days were recorded. The flowering time of *Festuca nigrescens* seemed to be affected by the date of snow melt and it showed a close relationship with the number of hourly mean temperature above 5°C recorded between the snow melt and the flowering date (1,247 in 2000, 1,280 in 2001 and 1,232 in 2002).

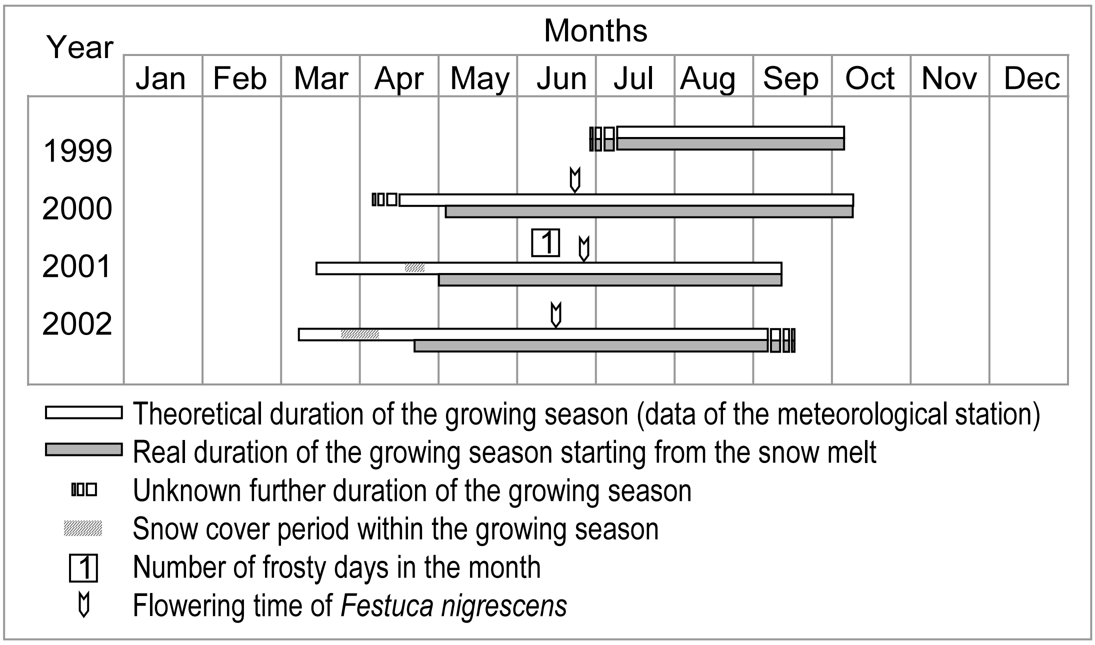


Figure 47: Duration of the growing season, occurrence of climatically adverse events (frosty days) and flowering time of *Festuca nigrescens* at the experimental site Sudelfeld.

Diurnal patterns of alternated soil-freezing and -thawing, which have a detrimental effect on plants (Lichtenegger, 1990), were not exhibited by the soil temperatures recorded by the meteorological station. They are nevertheless likely to have occurred within the ski run, as the formation of ice layers was repeatedly noticed there (Figure 48b). This discrepance can be attributed to the location of the weather station outside the ski run, where no kind of mechanical preparation of the snow cover was undertaken.

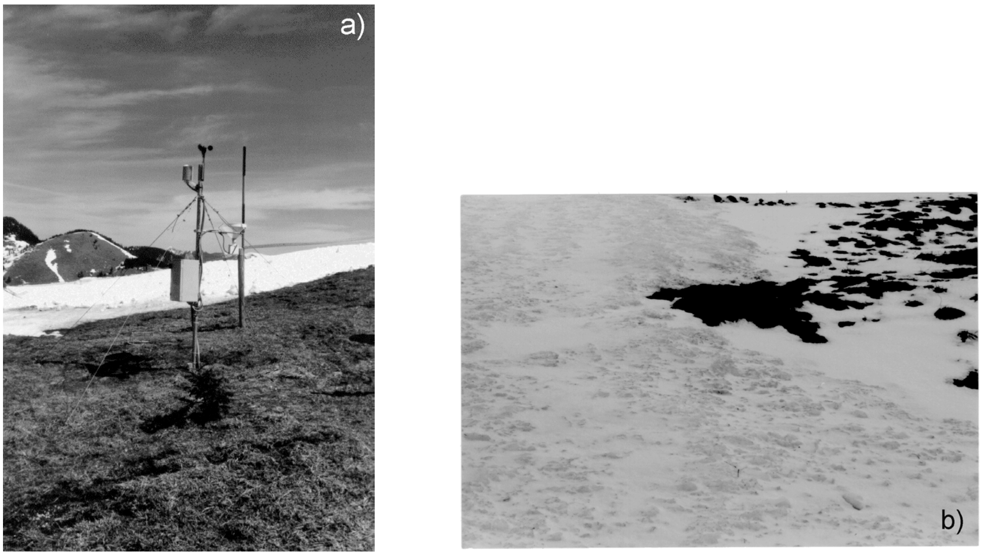


Figure 48: a) Delay of the snow melt within the ski run in comparison to the adjacent slope where the meteorological station was located (Sudelfeld, 14th April 2000) and b) occurrence of ice layers within the ski run at the beginning of spring (Sudelfeld, 5th April 2001).

The examination of the monthly mean temperatures and of the respective monthly precipitation sums (Figure 49) shows that an adequate water availability was ensured during all four growing seasons. Total rainfalls between 661 mm (1999, in 90 days) and 1,044 mm (2000, in 157 days) were recorded. Months between May and August had in all observation years a mean temperature above 10°C. Peaks up to 16°C were observed.

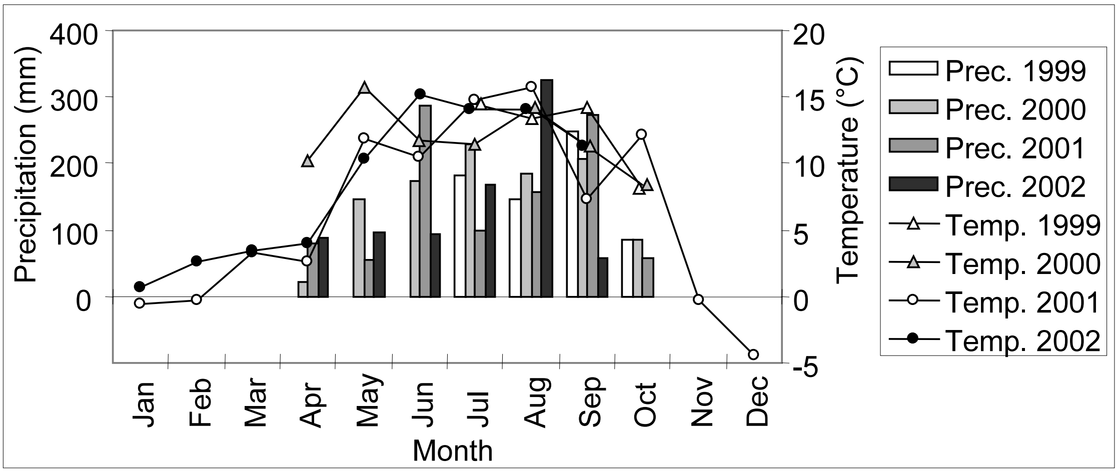


Figure 49: Monthly precipitation sums and monthly mean temperatures (2 m above ground) during the investigation period at the experimental site Sudelfeld.

Table 31: Rainy days, intense precipitation events and mean wind speed during the growing seasons 1999 - 2002 († = incomplete data).

Month	Rainy days (No.)				Intense precipitation events (No.)				Mean wind speed (m/s)			
	1999	2000	2001	2002	1999	2000	2001	2002	1999	2000	2001	2002
April	*	*	*	6/8	*	*	*	0	*	*	*	3.5
May	*	19/28	10/30	13/30	*	*	0	0	*	2.3	3.0	2.7
June	*	14/30	16/30	11/30	*	2	0	0	*	3.0	2.9	2.5
July	18/25	26/30†	12/31	16/31	1	1	0	0	1.5	2.7†	2.3	2.6
August	16/31	17/31	13/31	14/31	1	0	0	1	1.8	2.1	2.1	2.6
September	16/30	15/30	9/10	2/3	1	0	0	1	1.8	2.3	4.8	2.3
October	4/4	4/7	*	*	0	0	*	*	2.3	2.1	*	*
Whole period	**54/90**	**96/157**	**60/132**	**62/133**	**3**	**3**	**0**	**2**	**1.9**	**2.4**	**3.0**	**2.7**

In general, a favourable distribution of the precipitation was found during the growing season, as rainfall was distributed in at least 1/3 of rainy days in each month (Table 31). A relatively low number of intense precipitation events, ranging between zero and three, occurred during the growing season (Table 31). The experimental site proved to be quite windy, as relatively high mean wind speed were registered in the whole observation period (1.9 to 3.0 m/s on average) with peaks up to 37.3 m/s (Table 31).

3.3.2 Soil

3.3.2.1 Surrounding area

The description of the soil profile underlying the most frequent vegetation type showed that the soil type is a complex of a pseudo-gleyfied Rendzic Leptosol and a pseudo-gleyfied Chromic Cambisol (Figure 50), reflecting small-scale variations observed also in the vegetation.

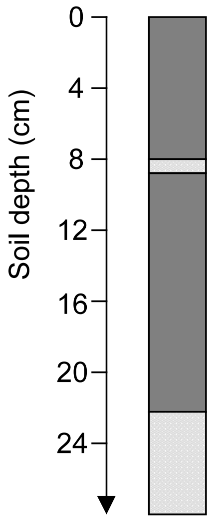
According to the results of the chemical analysis (Table 32), both soils showed a relatively high soil pH (in the carbonate buffer range), with a relatively high humus-content and a relatively small C/N ratio. A low level of P and a low saturation of K were detected. A high saturation of Mg was found in both cases. High amounts of EDTA-extractable Fe and Mn were found.

Table 32: Results of the soil analysis performed on samples from the experimental field prior to trial establishment and from the surrounding vegetation.

	Surrounding vegetation		Experimental plots
Year	1999	1999	1999
Soil type	Pseudo-gleyfied Rendzic Leptosol	Pseudo-gleyfied Chromic Cambisol	Machine-graded surface with incorporated topsoil prior to restoration
Number of samples	1	1	3
pH ($CaCl_2$)	6.7	6.7	6.9
Humus (%)	17.2	21.8	8.2
$CaCO_3$ (%)	10.2	14.7	59.5
C (%)	10.0	12.6	4.7
N (%)	0.9	1.1	0.4
C/N	11.4	12	10.6
P_2O_5 (mg/100g)	4	3.5	3.0
K_2O (mg/100g)	9.5	10.5	6.7
SO_4 (mg/100g)	7.5	9.0	4.3
Cl (mg/100g)	8.7	8.1	16.9
exchang. Ca (mval/100g)	44.0	49.7	23.9
exchang. Mg (mval/100g)	20.8	22.1	9.7
exchang. K (mval/100g)	0.2	0.1	0.1
EDTA-extr. Fe (mg/kg)	503.5	673.5	228.3
EDTA-extr. Mn (mg/kg)	372.0	306.0	274.7

Altitude:	1,245 m a.s.l.
Inclination:	15°
Exposition:	N
Position:	Upper portion of the slope
Parent rock:	Main dolomite
Soil type:	Complex of pseudo-gleyfied Rendzic Leptosol and pseudo-gleyfied Chromic Cambisol
Water supply:	Moderately variable moisture, topsoil prone to dryness

Profile A - Pseudo-gleyfied Rendzic Leptosol



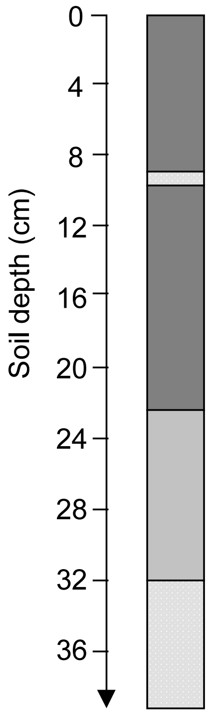
AP1 N = NF = M = 10YR 3/2
Densely rooted; very humus-rich (moist mull); few stones; slight gravel content; silt loam; clearly crumbly and indistinctly platy; numerous rust tubes; clear boundary to

C String of main dolomite debris

AP2 N = 10YR 3/2; NF = 10YR 3/1 + 3/2; M = 10YR 4/2
Densely rooted; very humus-rich (moist mull); few stones; slight gravel content; clay loam; strongly blocky and crumbly; numerous rust tubes; scarce earthworms; clear boundary to

D Very faintly rooted; main dolomite debris.

Profile B - Pseudo-gleyfied Chromic Cambisol



AP1 N = NF = M = 10YR 3/2
Densely rooted; very humus-rich (moist mull); few stones; slight gravel content; clay loam; strongly crumbly and indistinctly platy; numerous rust tubes; clear boundary to

C String of main dolomite debris

AP2 N = 10YR 2/2; NP = 10YR 2/1; M = 2,5Y 3/1
Densely rooted; very humus-rich (moist mull); few stones; slight gravel content; clay loam; strongly crumbly; numerous rust tubes; scarce earthworms; clear boundary to

B rel S N = 10YR 5/4 + 5/6; NF = 10YR 5/4; M = 10YR 5/4
Few roots; silty clay loam; strongly blocky; numerous rust and pale mottles; scarce earthworms; clear boundary to

D Very faintly rooted; main dolomite debris.

Figure 50: Description of soil profiles typical for the main vegetation type (*Festuco-Cynosuretum*) surrounding the experimental trial at the Sudelfeld (N=colour of fresh soil samples, NF=colour of soil samples moistened until achievement of a steady colour, M=colour of soil samples pasted up to the liquid limit).

3.3.2.2 Experimental field

Most of the chemical properties assessed for the mixed soil samples taken before the establishment of the field trial suggested the existence of a gradient from the upper to the lower part of the experimental field (Table 33). Humus-, nitrogen- and potassium content decreased considerably from block 1 to block 3.

Table 33: Chemical soil properties of mixed soil samples collected within the experimental field in each block prior to restoration.

	Block		
	1	2	3
Humus (%)	11.8	8.1	4.8
N (%)	0.56	0.41	0.34
P_2O_5 (mg/100g)	3	3	3
K_2O (mg/100g)	8	7	5

Comparing the results of the soil analysis of the intact soil from the surrounding area with those of the strongly disturbed, machine-graded soil of the experimental trial prior to restoration (Table 32), some differences due to the disruption of the soil and to the mixing of the topsoil with deeper mineral layers are evident. As first consequence, the $CaCO_3$-content increased 4 to 6 times. The humus content decreased of about the half in comparison to the Leptosol and of about 2/3 in comparison to the Cambisol, remaining nevertheless acceptable (around 8%). An analogous decline of the N level was observed, while the C/N remained relatively small. Concerning the other main nutrients, P decreased very slightly remaining to a low level, while the K-content decreased of about 1/3. The base saturation decreased of about the half and particularly a decline of exchangeable Ca and Mg was observed. The EDTA-extractable amount of Fe decreased considerably, while that of Mn changed only slightly.

3.3.3 Surrounding vegetation

On the basis of the cluster analysis of the 15 relevés performed in the surrounding vegetation, five grassland vegetation types (A to E) and one woodland vegetation type (F) could be defined at a similarity ratio of 0.62 (Figure 51).

The predominant plant community in the surroundings of the experimental trial (clusters A, B and D in Table 34) can be ascribed, according to Oberdorfer (1983), to the *Festuco-Cynosuretum*, a so-called "scanty-fat pasture" (Mager-Fettweide), because of the occurrence of species indicating soil scantiness (*Briza media*, *Plantago media*) together with other species found also in the fat pastures of the lowland (*Trifolium repens*, *Cynosurus cristatus*, *Dactylis glomerata*). *Potentilla erecta*, *Briza media* and *Nardus stricta* represent here differential species against the intensively managed lowland-pastures, whose main components are here missing (*Lolium perenne*), or rarely found in the fatter stands (*Phleum pratense*). In cluster B, representing a typical form of the high montane *Crepis aurea*-form of this plant community, *Crepis aurea*, *Ranunculus montanus*, *Campanula scheuchzeri*, *Trollius europaeus*, *Galium anisophyllon* and *Soldanella alpina* can be considered differential species against the other forms of this association. Some of the species characteristic of the fat alpine pastures of the *Poion alpinae*-alliance (*Poa alpina*, *Crepis aurea*) are as well present, although with low cover scores. Sedges (*Carex flacca* particularly, but also *Carex panicea*, *Carex flava* and others) largely contribute to the structure of the stands investigated. *Leontodon hispidus* is particularly abundant among the forbs. This vegetation type surrounds almost entirely the medium and lower part of the trial (Figure 52).

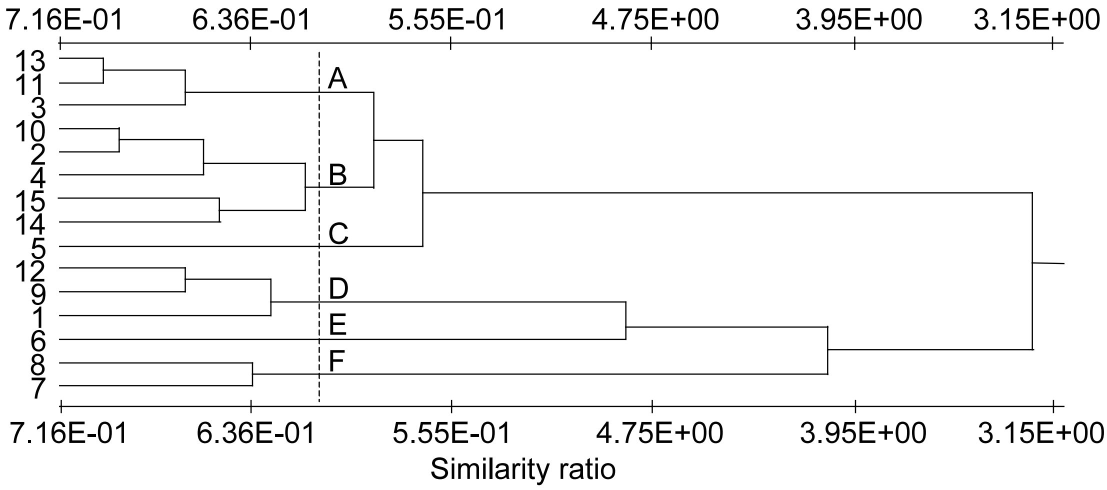


Figure 51: Dendrogram of the relevés performed in the surrounding vegetation at the Sudelfeld (similarity coefficient: similarity ratio, clustering technique: minimum variance clustering). Groups are identified by capital letters.

The relevés of group A were performed in the eastern portion of the ski run, which has an uneven morphology with numerous small hunchs, originating a characteristic steps-shape: short hollows or planes are alternated to steep declivities. In all relevés the diagnostically relevant species of the *Festuco-Cynosuretum* are still occurring, but on the steep stretches *Carex sempervirens* and *Sesleria albicans*, both absent in the flat part and quantitatively less represented in the other facies of the *Festuco-Cynosuretum*, form about 35% of the total cover. In this cluster some species fail (*Festuca pratensis*, *Alchemilla vulgaris*, *Carum carvi*, *Poa alpina*), typical of the fat meadows and pastures of the class *Molinio-Arrhenatheretea*. The botanical composition of relevé 4, carried out in a small-sized hollows adjacent to relevé 3, resembles that of the typical form of the *Festuco-Cynosuretum*. This vegetational mosaic, in which the variant with *Carex sempervirens* and *Sesleria albicans* is predominant, is contiguous to the north-eastern corner of block 3.

The relevés of group D were carried out on the upper portion and at the top of the ski run, on spots with low inclination and relatively deep soil, in places where the cattle was quite frequently observed to rest. In comparison to the relevés of group B, there is a considerable reduction of the total number of species and particularly of those indicating soil scantiness, while the proportion of plants of nutrients-rich soils, such as *Phleum pratense*, *Poa pratensis*, *Geum rivale* and *Poa supina* is increased. This fact is reflected also by the results of the cluster analysis, linking this vegetation unit to the clusters E and F rather than to the other aspects of the *Festuco-Cynosuretum*. *Mentha longifolia*, *Poa trivialis*, *Chaerophyllum hirsutum* and *Alchemilla vulgaris* agg. define the physiognomy of these stands. The upper portion of block 1 of the experimental trial is marginally contiguous to this vegetation type.

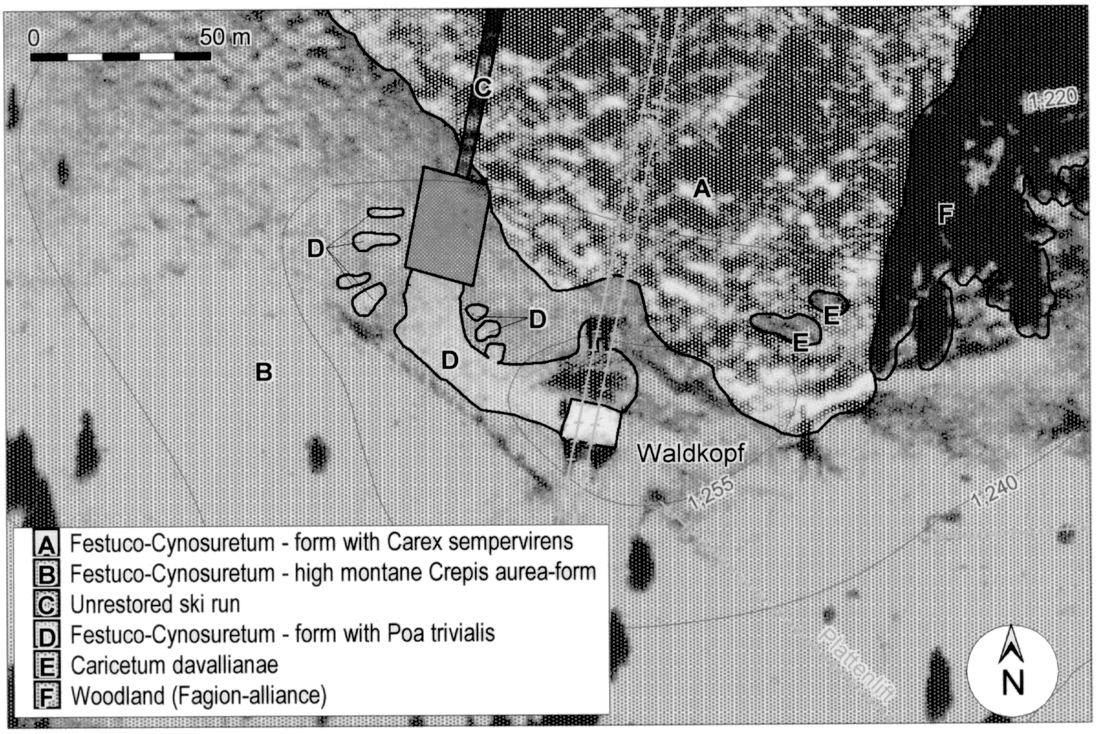


Figure 52: Surrounding vegetation at the experimental trial Sudelfeld. Small-scale alternance of different vegetation types was found (mainly small-sized stands of group D where group B is the predominant vegetation type and very small stands of group B where group A is predominant); such vegetational mosaics are represented in the map as the predominant vegetation type.

Relevé 5, which was performed immediately outside the trial on a soil stripe corresponding to the area excavated in the spring 1999 to build the new water pipe, could not be included in any of the other clusters and formed an own group (vegetation type C). As on this area (a former *Festuco-Cynosuretum*) no herbaceous vegetation was restored by sowing, the results of the vegetation analysis give some indications of which species of the *Festuco-Cynosuretum* are most efficient in colonising new available space, starting from the soil seed bank and from plants or parts of plants remained in place after disturbance. In the first year the overall cover of this stand was quite low (15%), showing little short-term re-colonization capacity of the autochthonous vegetation. It is remarkable that *Leontodon hispidus*, *Carex flacca*, *Carex panicea* and *Poa trivialis* formed about half of the vegetation cover.

Aspects of the *Festuco-Cynosuretum* similar to those found at the Sudelfeld are reported by Springer (1996) under the name of form with *Carlina acaulis* and form with *Festuca pratensis*, corresponding respectively to cluster A and D.

A further plant community observed in the area investigated was the *Caricetum davallianae* (cluster F, Table 35), a plant community typical of spring-swamps. It was observed in the upper part of the eastern slope in very small areas, where the soil was waterlogged by a spring. It was not in contact with the trial.

Small areas in the surroundings of the experimental field are still occupied by arboreal vegetation, in which the prevalent species is *Picea abies* (Table 35, relevé 6). Nevertheless, the composition of the herbaceous layer suggests that the potential forest type would be a mixed montane beech-silver fir-wood, as numerous species are characteristic of the *Fagetalia*-order (*Daphne mezereum*, *Viola reichenbachiana*, *Sanicula europaea* and others) or of the *Fagion*-alliance (*Petasites albus* ssp. *albus, Prenanthes purpurea*, *Veronica urticifolia*).

Table 34: Various aspects of the *Festuco-Cynosuretum* at the Sudelfeld. Cover of single species is expressed according to the scale of Van der Maarel (1979). For the topographic position, m=medium portion of the slope, m=medium portion of the slope, u=upper portion of the slope, h=hill summit.

Cluster	A	A	A	B	B	B	B	B	C	D	D	D
Relevé number	13	11	3	10	2	4	15	14	5	12	9	1
Altitude (m a.s.l.)	1,250	1,245	1,240	1,235	1,240	1,240	1,250	1,250	1,235	1,245	1,245	1,250
Exposition (°)	360	360	350	360	350	350	350	340	360	350	350	300
Slope (°)	25	28	25	20	17	5	13	10	19	5	5	7
Topographic position	m	m	m	m	m	u	u	u	m	u	u	h
Relevé area (m²)	30	30	14	18	100	14	25	21	50	12	50	70
Soil depth (cm)	17	27	23	30	18	33	24	25	.	33	28	26
Vegetation cover (%)	90	95	93	99	95	95	98	95	15	100	99	90
Not covered soil (%)	10	5	7	1	5	5	2	10	85	0	1	10
Grasses (%)	42	48	50	34	50	44	50	36	59	56	62	45
Legumes (%)	7	10	17	7	7	15	12	19	6	10	7	4
Forbs (%)	51	42	30	59	40	37	37	45	35	34	31	51
Mosses (%)	2	2	4	2	4	4	2	2	.	0.1	0.1	0.1
Species number	61	53	54	67	73	61	51	59	45	49	48	57
Carlina acaulis ssp. caulescens	2	4	3	2	.	.	2	.	.	.	.	.
Homogyne alpina	2	3	2	.	2	.	.	.	.	.	.	.
Hieracium bifidum	.	3	2	2	.	.	.	.	.	.	.	.
Carex sempervirens	6	6	7	2	5	.	3	3	2	.	.	.
Sesleria albicans	4	5	5	2	2	.	6	2	.	.	.	.
Aster bellidiastrum	4	3	2	4	2	.	4	5	.	.	2	.
Thymus praecox ssp. polytrichus	2	2	2	3	2	.	5	2	2	.	.	.
Crepis aurea	3	2	2	3	3	2	.	3	2	2	.	.
Scabiosa columbaria	5	5	2	4	2	2	2	2	2	.	.	.
Anthyllis vulneraria ssp. carpatica	3	3	5	2	3	3	3	5	.	.	.	.
Lotus corniculatus	3	4	4	3	3	4	3	4	2	.	.	.
Galium anisophyllon	2	2	2	2	2	2	2	2	.	.	.	.
Linum catharticum ssp. suecicum	2	2	2	2	2	2	2	2	.	.	.	.
Campanula scheuchzeri	2	2	2	2	2	2	2	2	2	.	2	.
Primula farinosa	2	2	2	2	2	2	2	5	2	.	.	.
Gymnadenia conopsea	2	2	2	2	2	2	2	2	2	.	.	.
Potentilla erecta	2	2	2	3	2	2	2	2	2	.	.	.
Selaginella selaginoides	2	2	2	2	2	.	2	2	.	.	.	.
Polygala amarella	2	2	2	2	2	.	2	3	.	.	.	.
Gentiana asclepiadea	3	2	2	.	2	2	2	.	2	2	.	.
Soldanella alpina	2	2	3	2	3	2	.	.	2	.	.	.
Anemone nemorosa	2	2	2	2	2	2	.	.	.	.	.	2
Tofieldia calyculata	2	2	2	2	3	2	.	.	.	.	.	.
Gentiana verna	2	.	2	1	2	2	2	2	.	.	.	.
Juncus articulatus	2	.	2	2	2	3	.	.	2	.	.	.
Carex flava	2	.	2	2	4	4	.	2	2	.	.	3
Carex capillaris	2	2	.	2	3	3	.	3	2	.	.	.
Pinguicula vulgaris	2	.	2	2	2	2	.	2	.	.	.	.
Carex ornithopoda	2	2	.	2	2	.	.	3	.	.	.	.
Coeloglossum viride	2	2	.	2	2	.	.	2	.	.	.	2
Carduus defloratus ssp. defloratus	2	.	2	3	2	.	2	.	2	.	.	.
Phyteuma orbiculare	2	2	.	2	.	2	.	2	.	.	.	.
Acinos alpinus	.	2	2	.	2	.	2	.	2	.	.	.
Hippocrepis comosa	2	2	.	.	.	2	4	2	.	.	.	.
Nardus stricta	2	.	2	.	.	2	3	2	.	.	.	.
Leontodon hispidus	6	5	5	6	5	5	2	5	3	3	2	5
Carex flacca	4	4	3	5	5	2	5	4	3	2	2	5
Deschampsia cespitosa ssp. cespitosa	4	4	3	4	3	5	2	2	2	4	5	4
Plantago media	5	4	3	4	3	3	4	2	2	2	2	3
Centaurea jacea	5	3	2	3	2	2	5	2	2	5	2	3
Briza media	3	3	2	4	3	3	3	5	2	5	5	3
Trifolium pratense ssp. pratense	3	3	3	4	3	5	3	2	2	4	4	4
Rhinanthus minor	2	2	3	2	3	2	2	2	2	2	2	2

Cluster	A	A	A	B	B	B	B	B	C	D	D	D
Relevé number	13	11	3	10	2	4	15	14	5	12	9	1
Primula elatior ssp. elatior	2	2	2	3	2	3	2	2	2	2	3	2
Ranunculus montanus	3	2	4	3	3	3	2	4	2	.	2	3
Festuca nigrescens	3	3	3	4	3	3	3	3	.	4	.	2
Crocus albiflorus	2	2	2	2	2	2	.	2	2	2	2	.
Carex panicea	3	3	5	5	4	3	4	5	2	.	3	3
Cynosurus cristatus	2	2	3	2	2	5	4	2	.	5	5	3
Pimpinella major	2	2	2	3	3	2	2	2	.	3	.	3
Prunella vulgaris	4	4	.	2	2	2	2	2	2	2	2	2
Leucanthemum vulgare	2	3	.	3	2	2	3	2	2	2	2	.
Trifolium repens ssp. repens	2	2	.	2	2	3	2	2	.	5	3	.
Plantago lanceolata	2	2	.	2	2	2	3	2	2	2	2	2
Dactylis glomerata ssp. glomerata	2	.	2	2	3	3	2	2	2	5	6	3
Taraxacum officinale	.	.	.	2	2	2	.	.	2	3	3	2
Alchemilla vulgaris agg.	2	.	.	2	3	4	2	5	2	5	4	4
Veratrum album ssp. album	.	.	.	2	2	3	.	.	2	3	2	3
Festuca pratensis	.	.	.	3	4	4	5	.	2	5	5	3
Carum carvi	.	.	.	4	4	4	2	2	2	4	3	4
Poa alpina	.	.	.	.	2	2	2	3	2	3	2	3
Euphrasia officinalis ssp. rostkoviana	.	.	.	.	2	.	2	2	.	.	.	.
Gentianella germanica	.	.	.	2	2	2	2	2	.	.	.	.
Gentianella ciliata	.	.	.	2	2	.	2	2	.	.	.	.
Anthoxanthum odoratum	2	.	.	2	2	2	.	.	.	3	3	2
Carex sylvatica	2	.	.	2	2	.	.	.	.	2	2	2
Galium album	.	2	.	2	2	2	.	.	.	3	2	.
Agrostis capillaris	.	.	.	2	.	.	2	.	.	2	2	.
Bellis perennis	.	.	.	2	.	2	.	.	.	.	2	2
Ranunculus nemorosus	.	.	.	.	.	.	2	2	2	2	.	2
Achillea millefolium agg.	.	.	.	.	.	2	.	2	2	2	2	3
Hypericum maculatum ssp. maculatum	.	.	.	.	2	.	.	.	.	4	2	3
Rumex acetosa	.	.	.	.	.	2	.	.	.	2	3	2
Chaerophyllum hirsutum	.	.	.	.	.	3	.	.	2	2	3	3
Poa trivialis ssp. trivialis	.	.	.	.	.	.	.	.	2	3	5	5
Ranunculus acris	.	.	.	.	.	.	.	.	.	2	3	2
Mentha longifolia	.	.	.	1	.	.	.	.	2	.	5	5
Geum rivale	.	.	.	.	.	.	.	.	.	2	2	2
Silene dioica	.	.	.	.	.	.	.	.	.	3	.	2
Phleum pratense	.	.	.	.	.	.	.	.	.	2	.	3
Poa pratensis	.	.	.	.	.	.	.	.	.	2	3	.
Ranunculus aconitifolius	.	.	.	.	.	.	.	.	.	2	2	.
Veronica chamaedrys ssp. chamaedrys	.	.	.	.	.	.	.	.	.	2	2	.
Ranunculus ficaria ssp. bulbilifer	.	.	.	.	.	.	.	.	.	.	2	2
Poa supina	.	.	.	.	.	.	.	.	.	.	2	2
Dentaria enneaphyllos	.	.	.	.	.	.	.	.	.	.	2	2
Carex caryophyllea	.	2	2	.	2	.	.	2	.	.	.	2
Agrostis stolonifera	.	.	2	.	2	2	.	.	.	.	.	2
Carex nigra	.	.	2	1	2	3	.	2	.	2	2	.
Viola biflora	.	2	2	.	.	2	.	.	.	.	.	2
Trollius europaeus	.	2	.	.	2	.	.	.	.	2	.	1
Cerastium holosteoides	.	.	.	.	2	2	.	.	.	2	.	.
Listera ovata	2	.	.	2	.	.	.	2	.	2	.	.
Acer pseudoplatanus	.	.	.	.	2	1	.	.	2	.	.	2
Carex pallescens	2	.	.	.	2	.	.	.	.	.	.	3
Cirsium eriophorum	.	.	.	2	2	.	.	.	.	.	2	.
Valeriana dioica	2	1	.	.	.	.	.	.	.	.	.	.
Juncus alpinoarticulatus	.	.	2	.	.	.	.	.	2	.	.	.

Sporadic species (cover in brackets): in rel. 13 *Traunsteinera globosa* (2), *Orchis ustulata* (2); in rel. 3 *Buphtalmum salicifolium* (2), *Antennaria dioica* (2), *Hieracium pilosella* (1); in rel. 10 *Willemetia stipitata* (2); in rel. 2 *Parnassia palustris* (2) and *Sagina procumbens* (1); in rel. 4 *Euphrasia stricta* (2); in rel. 15 *Polygala chamaebuxus* (3); in rel. 14 *Carex pulicaris* (2) *Trifolium montanum* (1), *Luzula campestris* (1); in rel. 12 *Rosa pendulina* (2), *Astrantia major* ssp. *carinthiaca* (2); in rel. 9 *Agrostis gigantea* (2); in rel. 1 *Plantago major* (3), *Vicia sepium* (2), *Veronica serpyllifolia* ssp. *humifusa* (2), *Symphytum tuberosum* ssp. *nodosum* (2), *Stellaria graminea* (2), *Dactylorhiza maculata* (2), *Cruciata laevipes* (2), *Carex hirta* (2).

Table 35: *Caricetum davallianae* and woodland at the Sudelfeld.

Cluster	E	E
Relevé number	8	7
Altitude (m a.s.l.)	1,235	1,230
Exposition (°)	360	350
Slope (°)	17	5
Topographic position	u	u
Relevé area (m²)	50	50
Soil depth (cm)	22	19
Vegetation cover (%)	70	65
Not covered soil (%)	30	35
Grasses (%)	65	79
Legumes (%)	.	+
Forbs (%)	30	16
Mosses	5	5
Species number	33	47
Carex davalliana	7	7
Mentha longifolia	5	3
Carex panicea	5	2
Caltha palustris	5	2
Juncus articulatus	3	3
Carex nigra	4	2
Hieracium bifidum	3	2
Primula farinosa	2	3
Deschampsia cespitosa ssp. cespitosa	3	2
Briza media	3	2
Carex flava	3	2
Valeriana dioica	2	3
Cirsium palustre	2	3
Sesleria albicans	2	2
Scabiosa columbaria	2	2
Aster bellidiastrum	2	2
Tofieldia calyculata	2	2
Gentiana asclepiadea	2	2
Potentilla erecta	2	2
Galium anisophyllon	2	2
Carex flacca	2	2
Festuca nigrescens	2	2
Primula elatior ssp. elatior	2	2
Alchemilla vulgaris agg.	2	2
Veratrum album ssp. album	2	2
Juncus alpinoarticulatus	2	2
Carex pulicaris	2	2
Gymnadenia conopsea	2	.
Leucanthemum vulgare	2	.
Dactylis glomerata ssp. glomerata	2	.
Geum rivale	2	.
Poa trivialis ssp. trivialis	2	.
Homogyne alpina	1	.
Glyceria declinata	.	4
Trifolium pratense ssp. pratense	.	3
Tussilago farfara	.	3
Hippocrepis comosa	.	2
Thymus praecox ssp. polytrichus	.	2
Phyteuma orbiculare	.	2
Listera ovata	.	2
Linum catharticum ssp. suecicum	.	2
Selaginella selaginoides	.	2
Polygala amarella Crantz	.	2
Gentiana verna	.	2
Lotus corniculatus	.	2
Pinguicula vulgaris	.	2
Centaurea jacea	.	2
Prunella vulgaris	.	2
Viola biflora	.	2
Ranunculus acris	.	2
Veronica beccabunga	.	2
Veronica aphylla	.	2
Cardamine amara ssp. amara	.	2

Cluster	F
Relevé number	6
Altitude (m a.s.l.)	1,230
Exposition (°)	350
Slope (°)	26
Topographic position	m
Relevé area (m²)	225
Soil depth (cm)	24
Vegetation cover (%)	80
Not covered soil (%)	20
Grasses (%)	24
Legumes (%)	0
Forbs (%)	71
Mosses	5
Species number	45
Tree layer	
Picea abies	8
Fagus sylvatica	6
Acer pseudoplatanus	5
Herbaceous layer	
Polygonatum verticillatum	5
Mercurialis perennis	5
Adenostyles glabra	5
Phyteuma spicatum ssp. spicatum	5
Poa nemoralis	5
Chaerophyllum hirsutum	5
Deschampsia cespitosa ssp. cespitosa	5
Lysimachia nemorum	4
Centaurea montana ssp. montana	4
Hieracium bifidum	3
Sanicula europaea	3
Veronica urticifolia	3
Ranunculus aconitifolius	3
Oxalis acetosella	3
Mycelis muralis	3
Fragaria vesca	3
Festuca pratensis	3
Dactylis glomerata ssp. glomerata	3
Fagus sylvatica	2
Prenanthes purpurea	2
Cardamine trifolia	2
Acer pseudoplatanus	2
Lilium martagon	2
Daphne mezereum	2
Milium effusum	2
Viola reichenbachiana	2
Anemone nemorosa	2
Homogyne alpina	2
Viola biflora	2
Gymnadenia conopsea	2
Campanula scheuchzeri	2
Trifolium repens ssp. repens	2
Carex panicea	2
Carex flacca	2
Trifolium pratense ssp. pratense	2
Cynosurus cristatus	2
Primula elatior ssp. elatior	2
Ranunculus nemorosus	2
Taraxacum officinale	2
Carex sylvatica	2
Agrostis capillaris	2
Hypericum maculatum ssp. maculatum	2
Sesleria albicans	2
Sorbus aucuparia	2
Petasites albus	2

3.3.4 Topsoil conservation

3.3.4.1 Cover of residual fragments of the pre-existent vegetation

The total cover of residuals of the pre-existent vegetation remained in place after the field trial had been machine-graded ranged between 2% and 18% and was 6% on average. In about half of the cases (13 out of 27) it was less than 4%. The cover did not differ between blocks (Kruskal-Wallis test, $p>0.05$). Among the species with cover scores above 1%, *Leontodon hispidus* was clearly the most frequent one (in 25 plots), followed by *Carex flacca*, *Carum carvi*, *Ranunculus montanus* and *Deschampsia cespitosa*. *Lotus corniculatus*, *Primula elatior*, *Trifolium pratense* and *Alchemilla vulgaris* were also very frequent, although usually with small cover scores. Up to 41 species, and at least 28, were observed in a single plot; on average 33 species per plot were found.

3.3.4.2 Soil seed bank

Most of the seedlings emerged in the first three months; only 10% of the seeds germinated thereafter. The seed density in single samples was largely variable, ranging between 0 and 5,900 seeds/m². A seed bank of 1,929 seeds/m² was found on average. The mean values were decreasing from block 1 (2,711 seeds/m²) to block 3 (1,100 seed/m²); block 2 showed an intermediate value (1,977 seeds/m²). The number of samples taken for the whole trial met the minimal requirements proposed by Thompson et al. (1997), in order to estimate the mean density with 20% standard error.

Forbs represented numerically 66% of the total seed bank, followed by grasses (21%) and graminoids (11%). A very small percentage of legumes was found (2%). Altogether 54 species could be determined (Table 36). Most of them (50 species) occurred also in the vegetation near the trial. *Urtica urens* was not recorded in the relevés performed near the experimental field, but was observed not faraway from the trial. Four species (*Poa annua*, *Trifolium hybridum*, *Chenopodium album*, *Lolium perenne*), occurring infrequently and with very low densities in the soil samples, were not found at all in the surrounding vegetation. The highest seed densities were achieved by a group of five forbs (*Mentha longifolia*, *Leucanthemum vulgare*, *Hypericum maculatum*, *Alchemilla vulgaris* agg. and *Plantago media*). Among the grasses and graminoids, the largest values were found for *Juncus articulatus*. All these species showed a seed density above 100 seeds/m². Other abundant grasses were *Deschampsia cespitosa*, *Poa pratensis* and *Poa trivialis*. Among the legumes, only *Lotus corniculatus* had a seed density above 20 seeds/m². More than one third of the species found shows a seed density below 10 seeds/m².

Table 36: Seed density of the species occurring in the soil seed bank at the experimental site Sudelfeld.

Species	Seed density (seeds/m²)	Species	Seed density (seeds/m²)
Grasses	**407**	**Forbs**	**1,252**
Deschampsia cespitosa	59	Mentha longifolia	230
Poa pratensis	44	Leucanthemum vulgare	219
Poa trivialis	41	Hypericum maculatum	178
Festuca pratensis	33	Alchemilla vulgaris agg.	137
Agrostis gigantea	22	Plantago media	137
Agrostis stolonifera	19	Cerastium holosteoides	78
Poa alpina	15	Potentilla erecta	37
Poa annua*	15	Campanula scheuchzeri	30
Cynosurus cristatus	11	Leontodon hispidus	7
Dactylis glomerata	11	Linum catharticum	22
Festuca nigrescens	11	Primula farinosa	22
Briza media	7	Plantago major	19
Lolium perenne*	7	Prunella vulgaris	19
Nardus stricta	7	Ranunculus montanus	19
Poa supina	7	Achillea millefolium agg.	15
Other grasses	96	Taraxacum officinale	15
Graminoids	**219**	Thymus praecox	15
Juncus articulatus	130	Centaurea jacea	11
Luzula campestris	26	Crepis aurea	7
Carex flava	19	Veronica chamaedrys	7
Carex panicea	11	Aster bellidiastrum	4
Juncus alpinoarticulatus	11	Caltha palustris	4
Carex ornithopoda	7	Chenopodium album *	4
Carex sp.	7	Galium anisophyllon	4
Carex caryophyllea	4	Sagina procumbens	4
Carex flacca	4	Urtica urens	4
Legumes	**44**	Veronica beccabunga	4
Lotus corniculatus	26	Other forbs	4
Trifolium repens	11		
Trifolium hybridum*	7		

* species not found in the surrounding above-ground vegetation

In general, the proportion between species did not reflect species adundances in the above-ground vegetation. Some grasses and graminoids, such as *Poa pratensis*, *Poa trivialis* and *Juncus articulatus*, which had considerable seed densities, were quantitatively scarce in the surrounding vegetation. Similarly, forbs having the greatest seed densities in the seed bank occurred with low cover values in most vegetation types (*Leucanthemum vulgare*, *Alchemilla vulgaris* and *Plantago media*) or their presence was limited to single plant communities (*Mentha longifolia*, *Hypericum maculatum*).

3.3.4.3 Vegetative re-colonization

The vegetative propagules and plant rests contained in the soil of the experimental field were observed to shoot again about two weeks after sowing and plants shortly achieved greater size than the seedlings germinated from seed. Following spontaneous re-colonization, a considerable cover of indigenous plants, originated from vegetative propagules and vegetation residuals located within the plots, was observed eight weeks after establishment of the field trial (Figure 53a). Values ranged between 9% and 42% with a mean cover of 21%. In some plots, entire sods with considerable area could establish (up to 3 m²), but most units had a small extension (median=16.6 cm²) by a density of 12.8 units/m². Cover was not affected by any of the main factors, while a significant interaction between block and application technique was found (ANOVA, $F_{4,12}$=3.93; $p<0.05$). The cover values were weakly correlated with those observed prior to the restoration intervention (Pearson correlation, R^2=0.46; $p<0.01$).

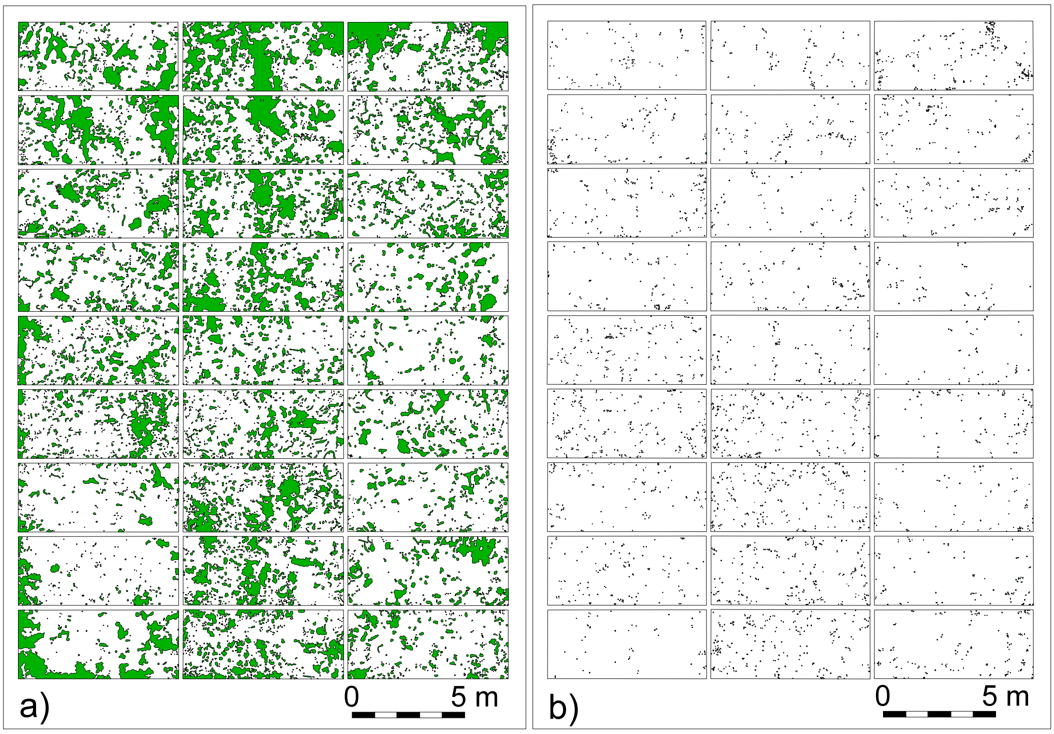


Figure 53: Spontaneous vegetative re-colonization 8 weeks after establishment of the trial. Filled areas represent the cover achieved by autochthonous plants, which spread in the experimental field starting from vegetative propagation units in the soil. a) all charted areas, b) tillers of species spreading through stolons (mainly *Carex flacca* and *Carex panicea*).

A large amount of tillers produced by species propagating through stolons (almost exclusively *Carex flacca* and *Carex panicea*) were observed within the experimental field. Such colonization units had a relatively high density (7 units/m² on average), although they represented only the 3.3% of the total charted cover (Figure 53b).

3.3.5 Cover and botanical composition

In the course of the first four observation years, the significance of the experimental factors changed through time.

About nine weeks after trial establishment a vegetation cover (phanerogams + mosses) ranging between 49% and 81% was measured (Figure 54). The analysis of variance did not show any effect of the seed mixtures on the vegetation cover, while the factor application technique was significant (Table 37). The highest vegetation cover scores (72% on average) were achieved by the plots where the conventional hydro-seed was employed, while the lowest values (56%) were recorded in plots with the mycorrhiza-hydro-seed; the mycorrhiza-hydro-seed with straw-mulch showed scores in-between (64%).

Table 37: Results of the analysis of variance of vegetation cover per each observation year depending on the main factors (AT=application technique, SM=seed mixture) and their interactions ($^{n.s.}$=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001).

			Year			
			1999	2000	2001	2002
Source		df	F Sig.	F Sig.	F Sig.	F Sig.
AT	Hypothesis	2	10.6 *	1.8 $^{n.s.}$	1.8 $^{n.s.}$	1.6 $^{n.s.}$
	Error	4				
SM	Hypothesis	2	0.6 $^{n.s.}$	1.2 $^{n.s.}$	12.6 **	32.5 ***
	Error	12				
AT x SM	Hypothesis	4	0.4 $^{n.s.}$	2.1 $^{n.s.}$	0.5 $^{n.s.}$	2.9 $^{n.s.}$
	Error	12				
Block (B)	Hypothesis	2	1.4 $^{n.s.}$	1.7 $^{n.s.}$	5.4 $^{n.s.}$	3.6 $^{n.s.}$
	Error	4				
AT x B	Hypothesis	4	1.3 $^{n.s.}$	5.3 **	2.6 $^{n.s.}$	5.3 **
	Error	12				

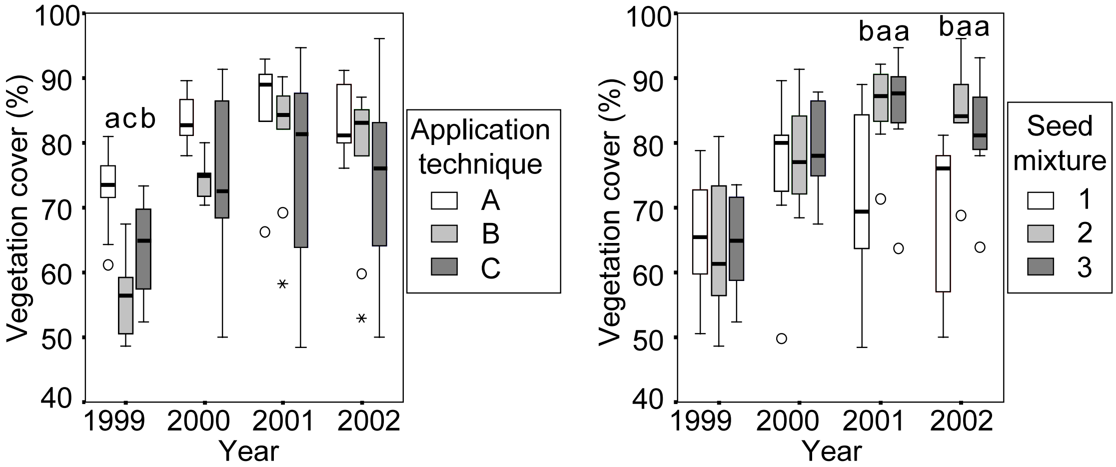


Figure 54: Effect of application technique (A=conventional hydro-seed, B=mycorrhiza-hydro-seed, C=mycorrhiza-hydro-seed with straw mulch) and of seed mixture (1=commercial, 2=indigenous for further utilisation, 3=indigenous without further utilisation) on vegetation cover through time. N=9. Mean separation within each year by DMRT at $\alpha=0.05$. Only significant differences are marked. Boxplots without common letter are significantly different.

In the second growing season none of the factors had a significant effect and a vegetation cover above 70% was achieved on average by all treatments. Considering the vegetation cover variations through time depending on the application technique, the cover increased generally in all treatments, the differences between treatments becoming smaller from the first to the second observation year. The strongest increase was observed in the mycorrhiza-hydro-seed treatments. In the third growing season the vegetation cover maintained satisfactory values for all treatments, being on average at least 70%. No effect of the application technique was detected by the statistical analysis, while that of the seed mixture was highly significant. A cover decrease was observed in plots with the commercial seed mixture, while in those with both indigenous seed mixtures further increases were observed. In the fourth growing season, the trend above described for the different seed mixtures became more evident. A highly significant effect of the seed mixture on the vegetation cover was detected by the statistical analysis. Both indigenous seed mixtures had mean cover scores between 82% and 85%, while those with the commercial seed mixture were shortly below 70%.

Significant interactions between block and application technique were found in 2000 and 2002.

No significant correlation was found between the cover of pre-existent vegetation in the plots and the vegetation cover in any of the observation years (Pearson correlation, $p>0.05$).

Table 38: Results of the analysis of variance of total cover per each observation year depending on the main factors (AT=application technique, SM=seed mixture) and their interactions ($^{n.s.}$=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001).

			Year							
			1999		2000		2001		2002	
Source		df	F	Sig.	F	Sig.	F	Sig.	F	Sig.
AT	Hypothesis	2	23.0	**	5.2	n.s.	1.0	n.s.	2.3	n.s.
	Error	4								
SM	Hypothesis	2	1.1	n.s.	0.5	n.s.	7.7	**	67.7	***
	Error	12								
AT x SM	Hypothesis	4	0.6	n.s.	0.8	n.s.	0.2	n.s.	4.5	*
	Error	12								
Block (B)	Hypothesis	2	1.4	n.s.	2.1	n.s.	12.2	*	2.8	n.s.
	Error	4								
AT x B	Hypothesis	4	1.9	n.s.	2.1	n.s.	0.6	n.s.	5.8	**
	Error	12								

Concerning the total cover, which considers also the protective action against erosion of necromass, such as litter and straw, a pattern through time similar to that observed for the vegetation cover was found (Table 38).

In the first growing season the sole application technique had a significant effect. Values were in all treatments slightly higher on average than those of the vegetation cover because of dead vegetation residuals (litter) remained on the soil surface after the preparation of the trial, and were remarkably larger where the straw cover was employed, as at that time the mean straw cover was around 20% (Figure 56). The highest total cover (85% on average) was measured in these plots. Differences between treatments became smaller with time and were no longer significant from the second growing season. Starting from the second growing season, the same pattern already described for the vegetation cover was observed between treatments; at this time the straw cover had already had a marked decline and was around 10%. The reduction of the cover given by straw was compensated by the increase of the vegetation cover, as the total cover did not change from year to year (Figure 55).

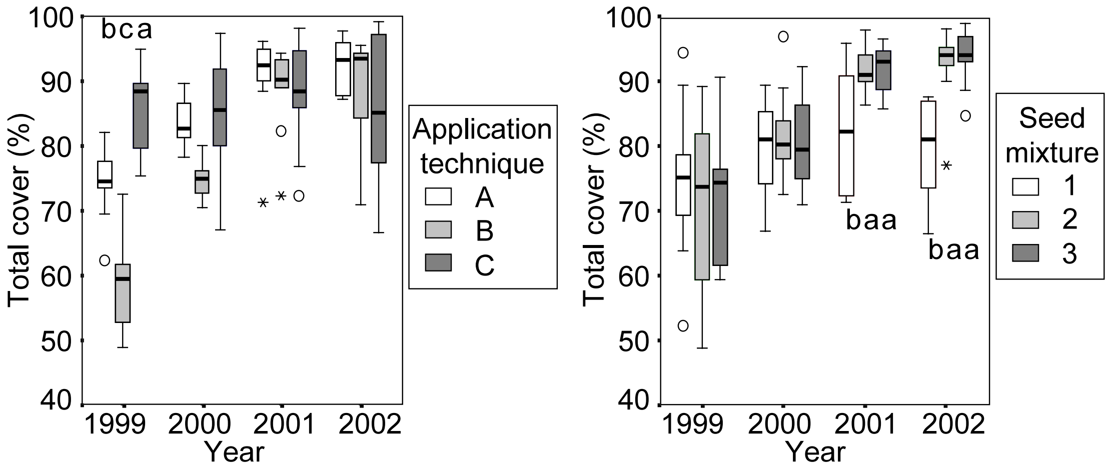


Figure 55: Effect of application technique (A=conventional hydro-seed, B=mycorhhiza-hydro-seed, C=mycorrhiza-hydro-seed with straw mulch) and of seed mixture (1=commercial, 2=indigenous for further utilisation, 3=indigenous without further utilisation) on total cover through time. N=9. Mean separation within each year by DMRT at α=0.05. Only significant differences are marked. Boxplots without common letter are significantly different.

In 2002, a significant interaction between application technique and seed mixture was detected by ANOVA (Table 38). The commercial seed mixture achieved in general lower values than the indigenous ones within each application technique, and exhibited lower cover scores if used in combination with both mycorrhiza-hydro-seed techniques. Furthermore, plots with the indigenous seed mixture for further utilisation exhibited lower values in combination with the mycorrhiza-hydro-seed with straw mulch (Table 39).

Table 39: Effect of the interaction between seed mixture and application technique on total cover in 2002. N=3. Mean separation by DMRT at α=0.05. Means without common letter are significantly different.

Seed mixture	Total cover (%) Conventional hydro-seed	Mycorrhiza-hydro-seed	Mycorrhiza-hydro-seed with straw mulch
Commercial	87.3 ^{c}	76.9 ^{d}	73.8 ^{d}
Indigenous for further utilisation	94.3 ^{a}	94.3 ^{a}	88.6 ^{bc}
Indigenous without further utilisation	95.6 ^{a}	92.2 ^{abc}	93.7 ^{ab}

Analysing the cover of straw residuals and litter, which represent the difference between vegetation cover and total cover, a significant effect of the seed mixture was detected by ANOVA ($F_{2,12}$=4.50, p<0.05), and higher cover values of necromass were found for the commercial seed mixture and for the indigenous seed mixture without further utilisation (Table 40).

Table 40: Cover of necromass (litter + straw) depending on the seed mixture in 2002. Average of 9 measurements. Mean separation by DMRT at α=0.05. Means without common letter are significantly different.

Seed mixture	Cover of necromass (%)
Commercial	11.4 [a]
Indigenous for further utilisation	7.2 [b]
Indigenous without further utilisation	12.0 [a]

The straw cover decreased rapidly in the first 10 weeks after the restoration, achieving about 1/4 of the initial value, then it slowly declined (10% in 2000, 5% in 2001). Data of 2002 are not completely representative of the straw cover, because the advanced stage of biological degradation of the straw made impossible to distinguish it from other kinds of litter during the assessments.

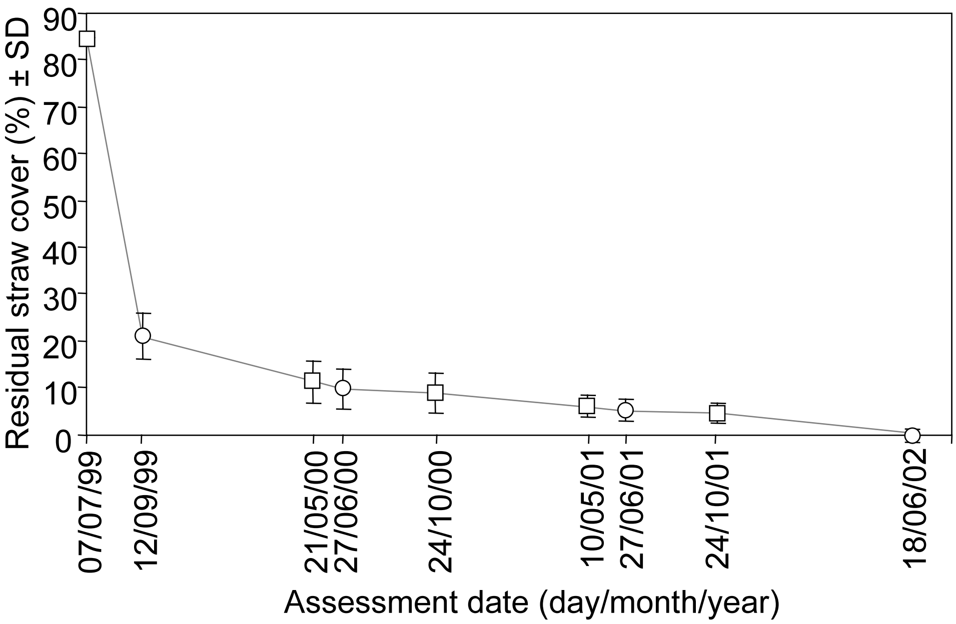


Figure 56: Cover decrease through time of the straw used in application technique 3 (mycorrhiza-hydroseed with straw mulch). Average of 9 measurements ± SE. Circles represent point-quadrat-measured values, squares sight-estimated cover scores.

Considering the partial cover given by indigenous species in common with the surrounding vegetation, namely the species found both in the plots and in the relevés of cluster B (typical aspect of the high montane *Crepis aurea*-form of the *Festuco-Cynosuretum*), which presents ecological conditions similar to those of the experimental plots (the species bound to higher levels of nutrient availability do not occur in these relevés), a significant effect of the seed mixture was detected by the statistical analysis, while the application technique had no influence on this variable. The highest values were consistently achieved by plots with the indigenous seed mixture for further utilisation, closely followed by those with the indigenous seed mixture without further utilisation (Figure 57).

In the second growing season, the cover of the indigenous species in plots with seed mixture 2 was about double than that achieved in plots where the commercial seed mixture was used. In the following year the cover of indigenous species increased in all treatments and the differences observed between the seed mixtures remained more or less unchanged. In this observation year peaks were achieved by plots with the indigenous seed mixture with further utilisation (75%) and with the indigenous seed mixture without further utilisation (65%), while the commercial seed mixture showed a further increase in the fourth growing season, achieving a mean value of 47%.

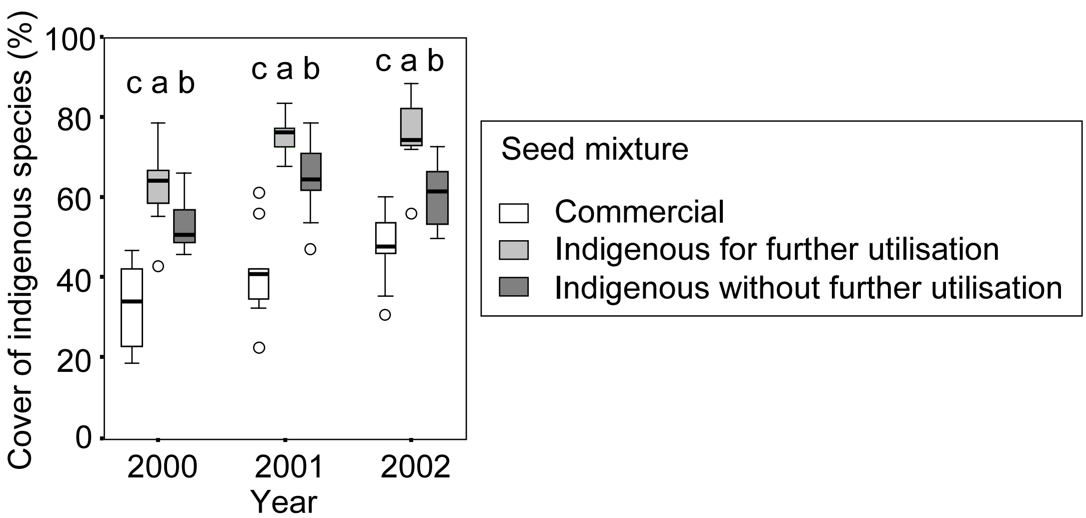


Figure 57: Effect of the seed mixture on the cover of indigenous species (species in common with the surrounding vegetation) through time. N=9. Mean separation within each year by DMRT at α=0.05. Boxplots without common letter are significantly different.

A similar pattern through time was observed for the species number within the plots. This parameter increased in all treatments from the second to the third growing season, but in plots with the indigenous seed mixtures it peaked already in the third growing season, while a further increase occurred in plots with the commercial seed mixture (Figure 58). Significant effects of the seed mixture were found only in

2001, the indigenous seed mixtures showing values higher than those of the commercial seed mixture.

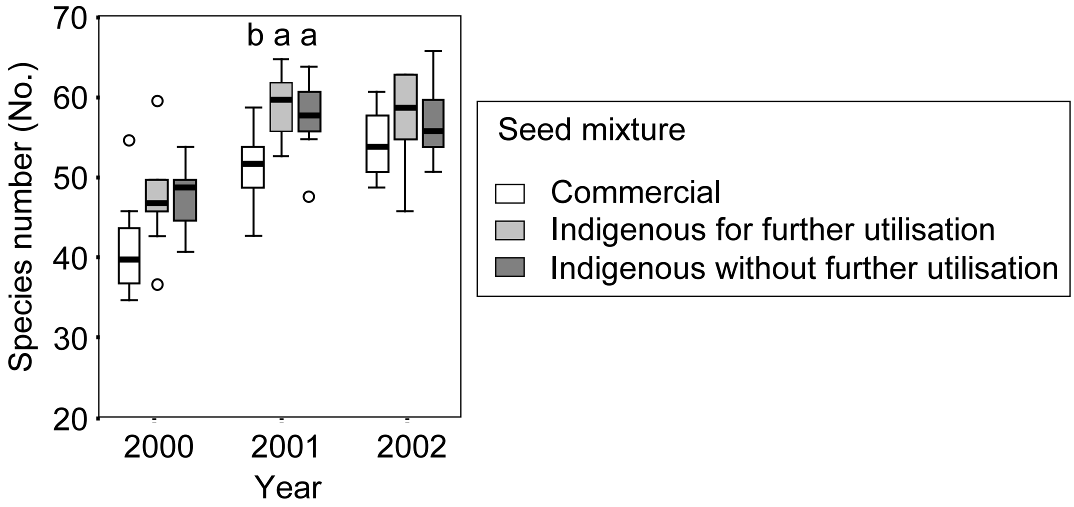


Figure 58: Effect of the seed mixture on the species number through time. N=9. Mean separation within each year by DMRT at α=0.05. Only significant differences are marked. Boxplots without common letter are significantly different.

These differences were mainly due to different species numbers contained in the seed mixtures, as the factor seed mixture did not have any effect (ANOVA, $F_{2,12}=0.22$, $p>0.05$) on the number of species not sown (see Table 41 for the definition of non-sown species). In the fourth growing season mean values between 54 and 57 species were achieved, with a considerable increase with respect to the number of sown species.

Table 41: Species not considered for calculating the number of non-sown species occurring within the plots at the Sudelfeld and reasons assumed for their exclusion.

Species	Species excluded from the "non sown species" in plots with seed mixture			Reason
	1	2	3	
Lolium perenne		x	x	transported by surface run-off after trial set up
Phleum pratense		x	x	
Trifolium badium	x			
Trifolium hybridum		x	x	
Brassica oleracea	x	x	x	impurity in the seed mixture
Bromus hordeaceus		x	x	
Matricaria chamomilla		x		
Silene latifolia		x		
Alopecurus geniculatus		x	x	
Polygonum lapathifolium	x			
Hordeum vulgare		x	x	contained in the straw mulch

3.3.6 Vegetation dynamics

A marked vegetation dynamics took place within each treatment.

In plots with the commercial seed mixture, the valuable lowland forage species (*Lolium perenne*, *Phleum pratense*), which were very abundant in the first two growing seasons, decreased rapidly (Figure 59). The frequency of *Phleum pratense* peaked in the second growing season, in which it was over 50%, and then decreased to 20% in the two following years. *Lolium perenne*, after exhibiting a considerable frequency of 36% in 2000, became quite rare in the fourth growing season (below 3%). *Festuca rubra* gained instead steadily in abundance and achieved in the fourth year about 60% frequency. *Trifolium repens* was the most successful species among the legumes; it was well represented in the first two years and declined rapidly thereafter. The frequency of *Achillea millefolium,* the only forb included in the mixture, increased more or less linearly throughout the observation period.

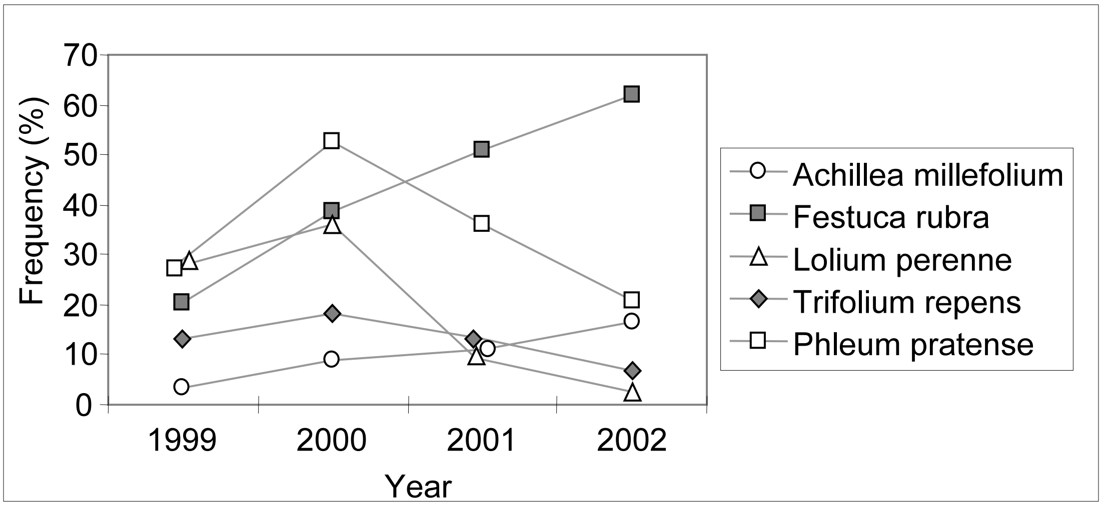


Figure 59: Frequency variations through time of the main species included in the commercial seed mixture. Average of nine measurements.

Other grasses (*Poa pratensis*, *Agrostis capillaris*) exhibited since the beginning of the experiment a poor establishment and very low frequencies throughout the observation period (Table 42), although their seed represented altogether 15% weight of the mixture. Further species (*Festuca ovina*, *Lotus corniculatus*, *Trifolium hybridum*) had initially frequencies between 5% and 10% and showed a negative trend in the following growing seasons. *Vicia sativa* established very poorly and was since 2000 virtually absent in the plots.

Table 42: Frequency variations through time of the species included in the commercial seed mixture. Significance of the factor year was tested with the Friedman test ([n.s.]=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001). Separation of yearly mean frequencies by Wilcoxon-Wilcox-test at α=0.05. Average of 9 measurements. For each species, values without common letters are significantly different.

	Friedman test			Frequency (%)			
Species	df	Chi²	Sig.	1999	2000	2001	2002
Festuca rubra	4	19.99	***	20.4 [a]	38.6 [ab]	51.1 [bc]	61.8 [c]
Lolium perenne	4	23.29	***	28.8 [ab]	36.1 [a]	9.7 [bc]	2.7 [c]
Phleum pratense	4	20.66	***	27.1 [bc]	52.6 [a]	36.2 [ab]	20.8 [bc]
Poa pratensis	3	14.06	**	n.m.	5.6 [a]	2.6 [ab]	0.6 [b]
Agrostis capillaris	3	4.46	n.s.	n.m.	1.6 [a]	0.2 [a]	1.3 [a]
Festuca ovina	3	18.00	***	n.m.	10.2 [a]	3.8 [ab]	0.8 [b]
Lotus corniculatus	4	17.22	**	4.4 [a]	7.9 [a]	4.0 [ab]	1.3 [b]
Trifolium repens	4	13.28	**	13.0 [ab]	18.4 [a]	13.1 [ab]	6.8 [b]
Trifolium hybridum	3	6.75	*	n.m.	6.9 [a]	3.0 [a]	3.3 [a]
Vicia sativa	4	24.43	***	1.2 [a]	0.1 [b]	0.0 [b]	0.0 [b]
Achillea millefolium	4	13.18	**	3.1 [b]	8.9 [b]	11.2 [ab]	16.4 [a]

n.m. not measured because the species could not be determined

In plots with the indigenous seed mixture for further utilisation the frequency increase of *Festuca nigrescens* was analogous to that found for *Festuca rubra* (Figure 60). *Poa alpina* and *Poa violacea* decreased quite clearly after a satisfactory establishment, while the frequency of *Phleum alpinum* and *Phleum hirsutum* remained low but constant through time.

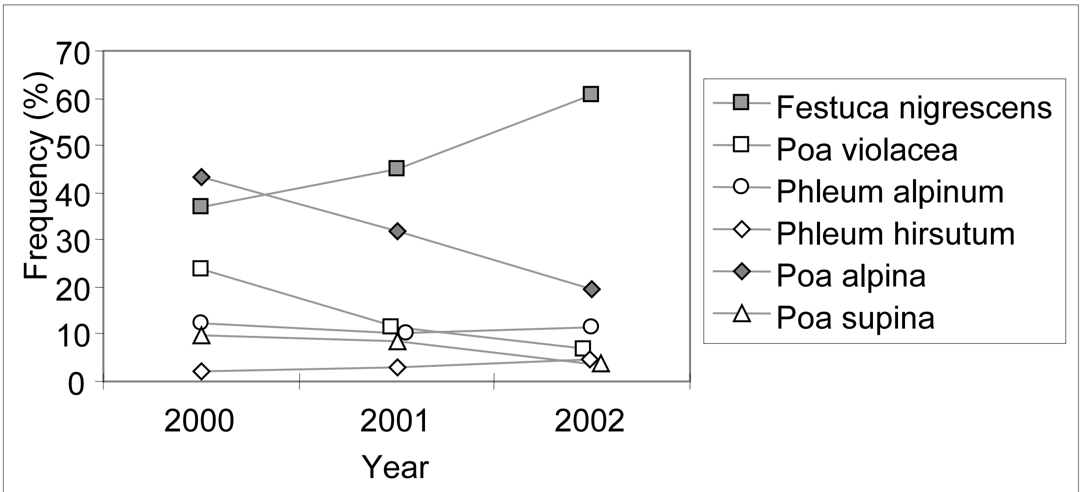


Figure 60: Frequency variations through time of grasses in the indigenous seed mixture for further utilisation. Average of nine measurements.

Trifolium repens showed the same trend already seen in plots with the commercial seed mixture. *Trifolium badium* exhibited low, more or less unvaried frequencies, while the frequency of *Anthyllis vulneraria* peaked in the second year and the species virtually disappeared thereafter.

Other species, such as *Lotus corniculatus* or *Plantago lanceolata* showed low, more or less stable values around 5%. Among the forbs, the most successful in gaining frequency was *Leontodon hispidus*, which achieved a frequency of more than 30% in the fourth year. *Alopecurus geniculatus*, a lowland species of wet soils, contained in the seed mixtures as impurity (Krautzer, verb. comm.) and not found in the surrounding vegetation and in the soil seed bank, showed frequencies up to 6% in the second growing season and then became extremely rare (Table 43).

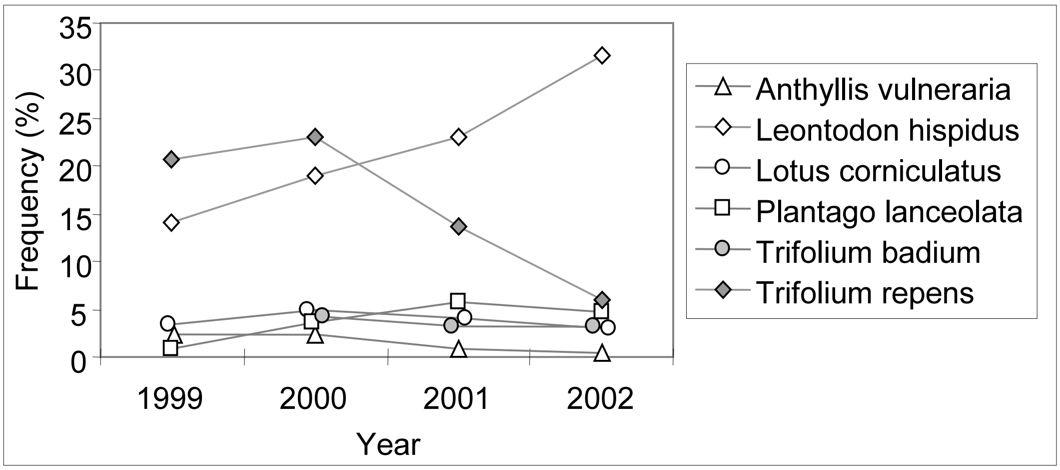


Figure 61: Frequency variations through time of legumes and forbs in the indigenous seed mixture for further utilisation. Average of nine measurements.

Table 43: Frequency variations through time of the species included in the indigenous seed mixture for further utilisation. Significance of the factor year was tested with the Friedman test ([n.s.]=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001). Separation of yearly mean frequencies by Wilcoxon-Wilcox-test at α=0.05. Average of 9 measurements. For each species, values without common letters are significantly different.

	Friedman test			Frequency (%)			
Species	df	Chi²	Sig.	1999	2000	2001	2002
Festuca nigrescens	3	14.11	**	n.m.	36.9 b	45.0 b	60.7 a
Poa alpina	3	17.00	***	n.m.	43.2 a	32.0 ab	19.7 b
Poa violacea	3	14.89	**	n.m.	23.6 a	11.3 b	7.0 b
Phleum hirsutum	3	4.94	n.s.	n.m.	2.1 a	3.0 a	4.6 a
Poa supina	3	10.89	**	n.m.	9.8 a	8.4 a	3.2 b
Phleum alpinum	3	2.69	n.s.	n.m.	12.1 a	10.1 a	11.4 a
Lotus corniculatus	4	2.41	n.s.	3.4 a	4.9 a	4.1 a	3.0 a
Trifolium repens	4	17.16	**	20.7 a	23.1 a	13.6 ab	6.0 b
Anthyllis vulneraria	4	10.36	*	2.0 a	2.3 a	0.8 a	0.3 a
Trifolium badium	3	0.75	n.s.	n.m.	4.3 a	3.1 a	3.1 a
Leontodon hispidus	4	23.35	***	14.0 b	18.9 b	23.0 ab	31.7 a
Plantago lanceolata	4	9.61	*	0.9 b	3.7 ab	5.8 a	4.1 ab
Crepis aurea	3	3.25	n.s.	n.m.	1.0 a	0.4 a	1.2 a
Alopecurus geniculatus	3	16.71	***	n.m.	6.2 a	0.6 b	0.0 b

n.m. not measured because the species could not be determined

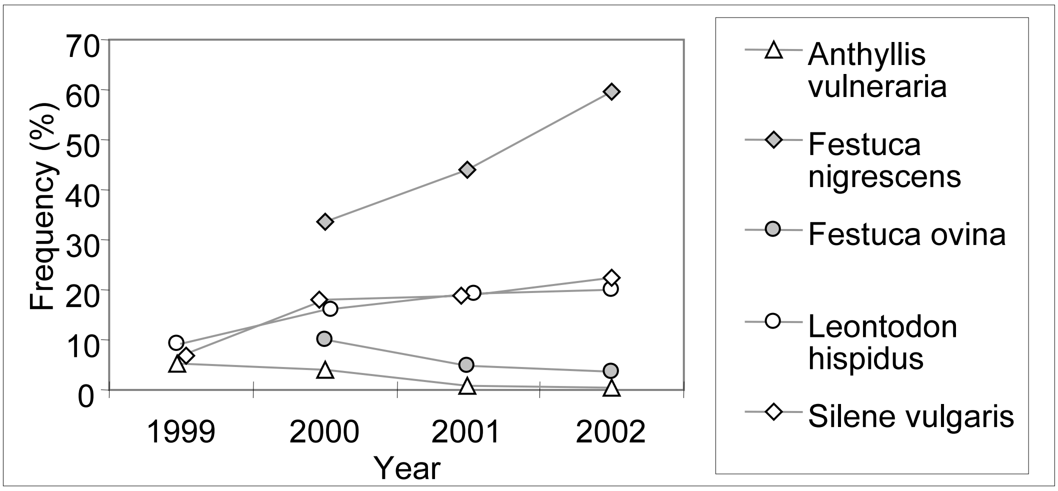


Figure 62: Frequency variations through time of selected species in the indigenous seed mixtures without further utilisation. Average of nine measurements.

Table 44: Frequency variations through time of the species included in the indigenous seed mixture without further utilisation. Significance of the factor year was tested with the Friedman test ($^{n.s.}$=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001). Separation of yearly mean frequencies by Wilcoxon-Wilcox-test at α=0.05. Average of 9 measurements. For each species, values without common letters are significantly different.

	Friedman test			Frequency (%)			
Species	df	Chi²	Sig.	1999	2000	2001	2002
Festuca nigrescens	3	14.89	**	n.m.	33.4 ^{b}	44.0 ^{b}	59.8 ^{a}
Poa alpina	3	16.22	***	n.m.	43.6 ^{a}	28.9 ^{a}	12.0 ^{b}
Poa violacea	3	7.60	*	n.m.	19.6 ^{a}	12.4 ^{ab}	10.0 ^{b}
Festuca ovina	3	13.41	**	n.m.	10.1 ^{a}	4.8 ^{ab}	3.6 ^{b}
Phleum hirsutum	3	12.79	**	n.m.	0.7 ^{b}	2.3 ^{ab}	3.4 ^{a}
Poa supina	3	12.40	**	n.m.	7.7 ^{b}	4.6 ^{ab}	1.6 ^{a}
Phleum alpinum	3	1.23	n.s.	n.m.	12.6 ^{a}	10.2 ^{a}	9.3 ^{a}
Lotus corniculatus	4	8.49	*	3.3 ^{ab}	4.4 ^{a}	3.2 ^{ab}	1.4 ^{b}
Trifolium repens	4	22.73	***	20.8 ^{a}	26.9 ^{a}	8.9 ^{ab}	2.0 ^{b}
Anthyllis vulneraria	4	19.23	***	5.1 ^{a}	3.9 ^{ab}	0.8 ^{bc}	0.1 ^{c}
Trifolium badium	3	13.41	**	n.m.	8.2 ^{a}	4.7 ^{ab}	1.4 ^{b}
Leontodon hispidus	4	9.63	*	9.2 ^{b}	15.9 ^{ab}	19.1 ^{a}	19.9 ^{ab}
Silene vulgaris	4	16.67	**	6.8 ^{b}	17.9 ^{ab}	19.0 ^{a}	22.4 ^{a}
Alopecurus geniculatus	3	17.43	***	n.m.	6.9 ^{a}	0.1 ^{b}	0.0 ^{b}

n.m. not measured because the species could not be measured

The two indigenous seed mixtures had many species in common, although with slightly different weight percentages. Most of the species in common showed therefore trends similar to those above described, as shown by Table 44. Differences were observed for *Leontodon hispidus*, whose frequency was about 2/3 of that measured in plots with the other indigenous seed mixture and became more or less stable from the third growing season. *Silene vulgaris* performed in a very similar manner, showing frequencies through time resembling very closely those of *Leontodon hispidus* (Figure 62).

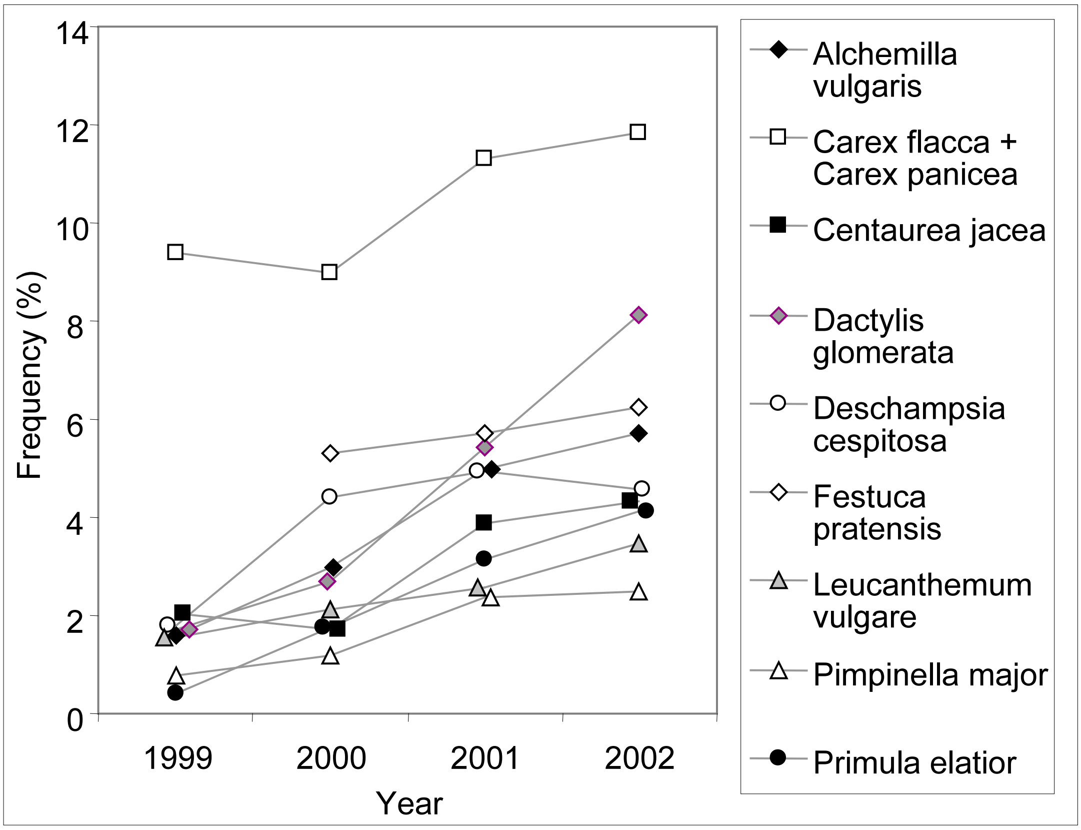


Figure 63: Frequency variations through time of selected autochthonous species not enclosed in the seed mixtures. Average of 27 measurements calculated across different seed mixtures. *Carex flacca* and *Carex panicea* were merged in a single variable, as no difference was made in their measurement in the first growing season.

Considering the mean frequency of autochthonous species not included in the seed mixtures, calculated across all seed mixtures (significant effects of the seed mixture were excluded by statistical analysis), steady increasing courses of a number of grasses and forbs was ascertained (Figure 63), indicating that their abundance was increasing everywhere in the experimental field. *Dactylis glomerata*, *Carex flacca*, *Carex panicea* and *Alchemilla vulgaris* agg. were found to be particularly successful.

Leontodon hispidus, contained in the indigenous seed mixtures, but being also quite abundant in the surrounding vegetation and in the residuals of the pre-existing vegetation within the plots, showed impressive increases through time in plots with the commercial seed mixture (from 10% in 1999 to 17% in 2002).

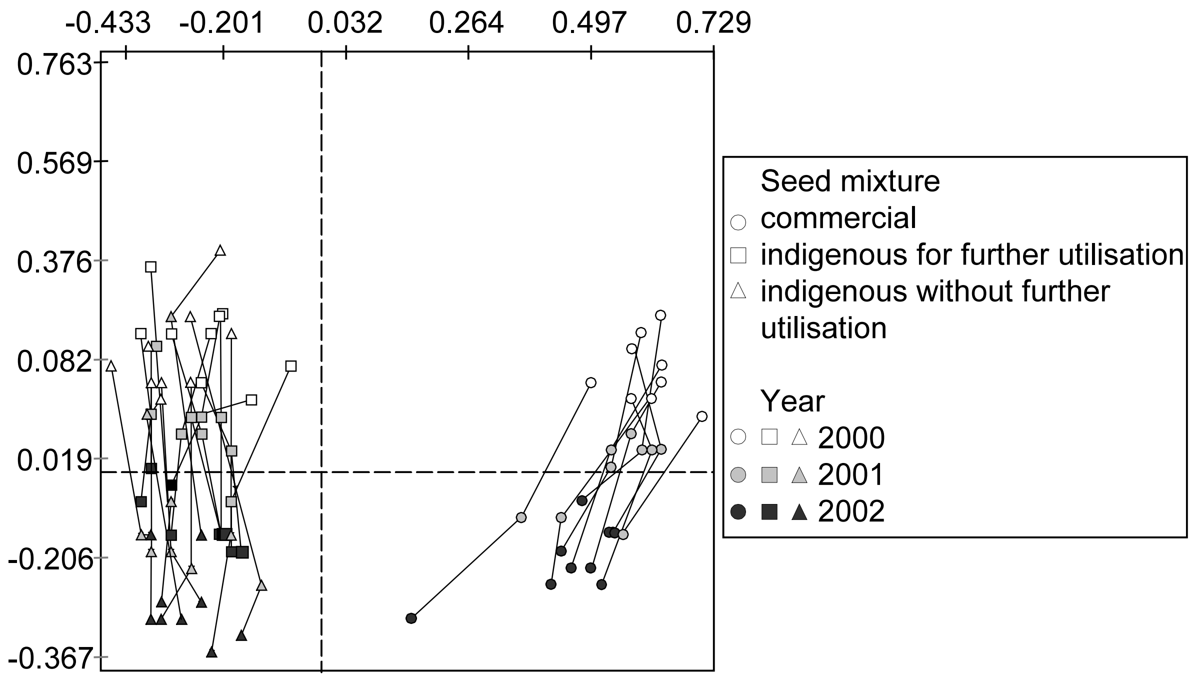


Figure 64: Ordination plot obtained through principal component analysis of the cover assessments performed in the plots from 2000 to 2002. Cover values were arcsine-transformed prior to ordination; chord distance was used instead of Euclidean distance. Lines indicate shifts from one year to the next one.

The ordination of the relevés performed through cover assessment from 2002 to 2002 (Figure 64) showed a clear separation of the commercial seed mixture from the indigenous seed mixtures along the first axis. Mixtures 2 and 3 were instead unclearly separated. Along the second axis, a consistent shifting downwards was observed for all plots through time. Commercial and indigenous seed mixtures exhibited a tendency to converge through time, as plots sown with the commercial seed mixture moderately shifted through time along the first axis, while the plots with the indigenous seed mixtures did not. However, separation remained distinct during the whole observation period.

Some indications about ecological factors possibly influencing the vegetation dynamics within the experimental plots were obtained by the analysis of the weighted mean scores of the ecological indicators of Ellenberg (Figure 65). Small changes were found for light, continentality and moisture, which tended to decrease through time in plots with the indigenous seed mixtures, while maintaining constant values in those with the commercial seed mixture. The soil reaction indicator did not change significantly through time in any of the treatments. A large decrease, concerning only plots with the commercial seed mixture, was detected for the temperature indicator. A clear negative trend of the nitrogen indicator was consistently observed for all treatments.

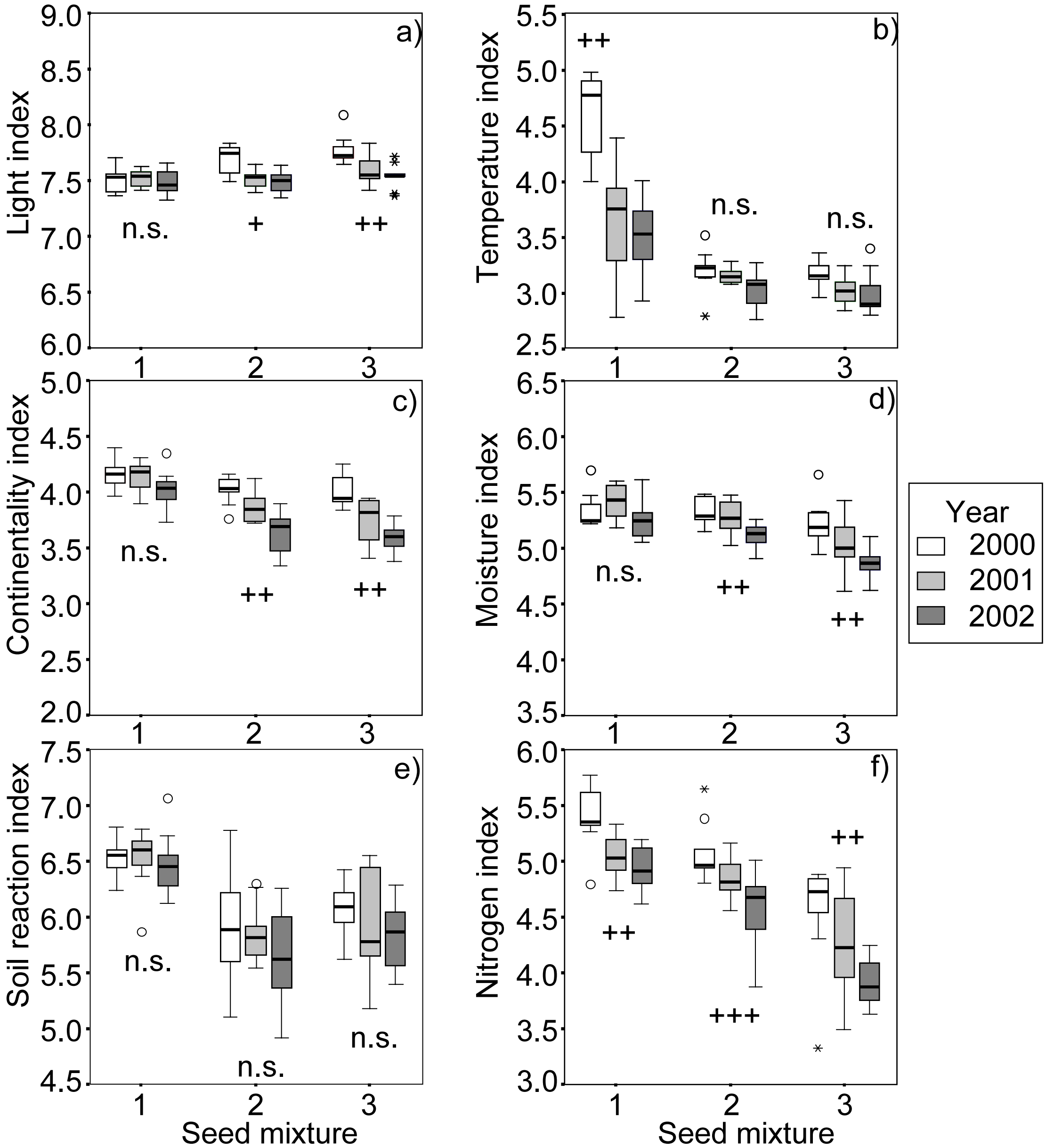


Figure 65: Weighted mean scores of the ecological indicators of Ellenberg in the period 2000-2002 at the experimental site Sudelfeld: (a) light, (b) temperature, (c) continentality, (d) moisture, (e) soil reaction, (f) nutrients. N=9. Within each seed mixture, significance of time effect was tested with the Friedman test at α=0.05 (n.s.=not significant, + =p<0.05, ++ =p<0.01, +++ =p<0.001).

3.3.7 Dry matter yield and potential forage quality

The seed mixture affected significantly the dry matter in the sole first harvest year (Mann-Whitney-U-test, $p<0.01$), while no effect of the application technique was observed (the Mann-Whitney-U-test consistently yielded not significant effects).

In 2000 the commercial seed mixture produced about 1/3 more dry matter than the indigenous seed mixture for further utilisation (3,000 against 2,100 kg/ha). Later on, differences between seed mixtures were no longer significant. The dry matter yield of the indigenous seed mixture exhibited a tendential increase through time, while that of the commercial seed mixture showed an opposite trend (Figure 66).

The indigenous seed mixture without further utilisation presented on average a dry matter yield of 2,252 kg/ha and 1,635 kg/ha in 2001 and in 2002 respectively (data not included in the statistical analysis), which was about 20% to 30% lower than that of the other treatments.

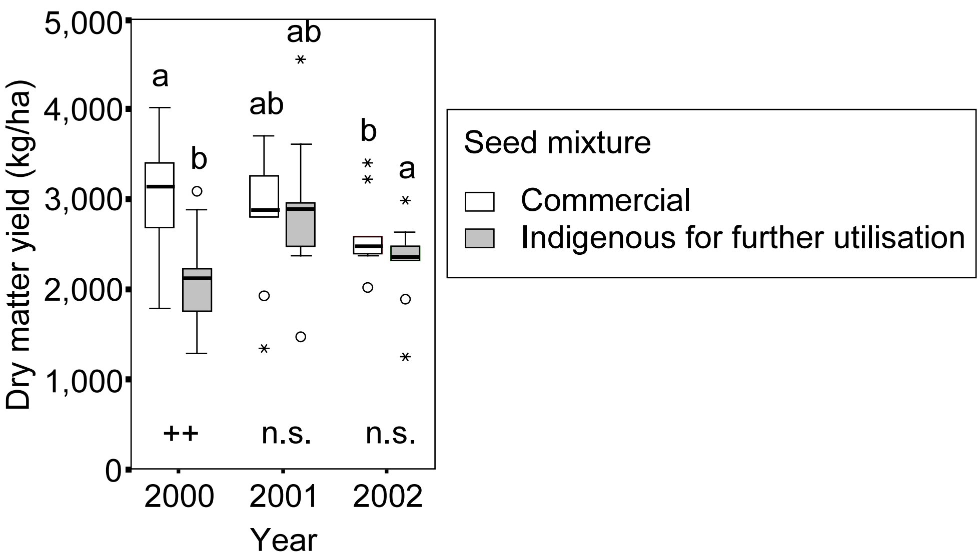


Figure 66: Dry matter yield at the experimental site Sudelfeld in the period 2000-2002. N=9. Significance of the effect of the seed mixture on the dry matter yield was tested for each year by Mann-Whitney-U-test at $\alpha=0.05$ (n.s.=not significant, ++ =$p<0.01$). Within each seed mixture, boxplots without common letters are significantly different (separation by Wilcoxon-Wilcox-test at $\alpha=0.05$).

The potential forage quality decreased through time for each seed mixture. Higher values were initially found for the commercial seed mixture, but differences became smaller with time and already in 2001 were no longer significant.

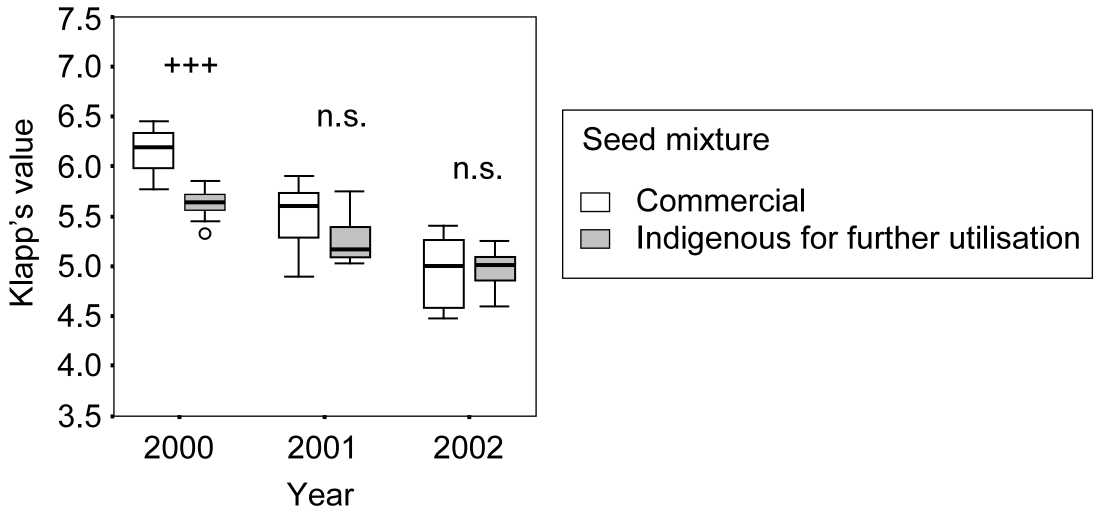


Figure 67: Potential forage quality at the experimental site Sudelfeld in the period 2000-2002. N=9. Significance of the effect of the seed mixture on the potential forage quality was tested within each observation year with Mann-Whitney-U-test at α=0.05 (n.s.=not significant, +++ =p<0.001).

3.3.8 Below-ground biomass

None of the main factors was found to affect significantly the below-ground biomass, while a significant interaction of seed mixture and application technique was detected by the analysis of variance (see Table 45).

Table 45: Results of the analysis of the variance of the below-ground biomass at the experimental site Sudelfeld in 2001 (n.s.=not significant, * =p<0.05, ** =p<0.01, *** =p<0.001). N=9.

Source		df	F	Sig.
Application technique (AT)	Hypothesis	2	0.5	n.s.
	Error	4		
Seed mixture (SM)	Hypothesis	2	1.0	n.s.
	Error	12		
AT x SM	Hypothesis	4	3.8	*
	Error	12		
Block (B)	Hypothesis	2	2.5	n.s.
	Error	4		
AT x B	Hypothesis	4	1.4	n.s.
	Error	12		

Root dry weights ranging between 0.9 and 1.6 g/100 cm² were measured. The highest below-ground biomass was found for the combination between the indigenous seed mixture for further utilisation and the conventional hydro-seed, while the lowest was found for the indigenous seed mixture without further utilisation if applied with the mycorrhiza hydro-seed (Figure 68). In plots with the indigenous seed mixture with further utilisation, the use of the mycorrhiza-hydro-seed with straw mulch resulted in root dry matter lower than where a conventional hydro-seed was performed. Further significant differences in root dry matter were found between plots with the indigenous seed mixtures, if the mycorrhiza-hydro-seed with straw mulch was used.

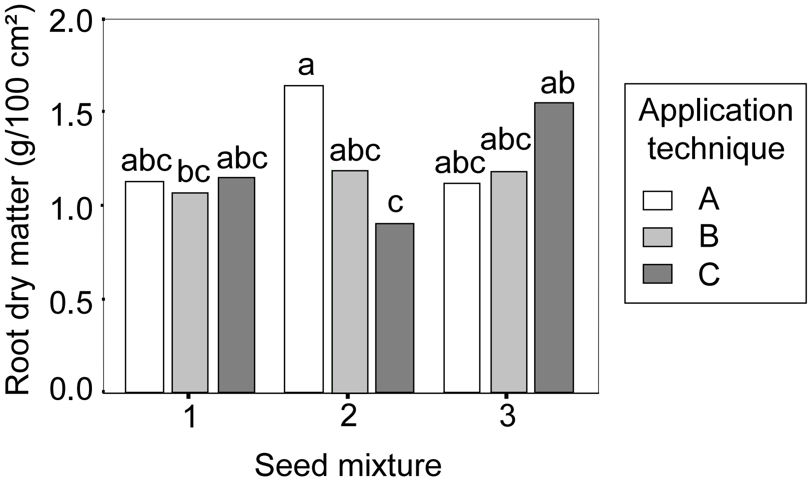


Figure 68: Root dry matter at the experimental site Sudelfeld in 2001. Average of 3 measurements. Mean separation by DMRT at 5%. (application technique: A=conventional hydroseed, B=mycorrhiza-hydroseed, C=mycorrhiza hydroseed with straw mulch; seed mixture: 1=commercial, 2=indigenous for further utilisation, 3=indigenous without further utilisation).

3.3.9 Fertility

With the exception of *Vicia sativa*, which was not found anymore within the plots after the first winter, except for one plant, all species included in the seed mixtures flowered in 2000 and 2001.

Only few of the sown species produced ripe seed already at the beginning of July (*Poa alpina*, *Poa supina*, *Crepis aurea* and, in the third growing season, *Trifolium badium*). A relatively numerous group, formed mainly by indigenous species, attained seed ripeness between the end of July and the end of August. A further group, containing almost exclusively lowland species, and particularly those being dominant in the seed mixture and in the plant stands (*Phleum pratense*, *Lolium perenne*), produced ripe seed from the beginning of September. As exceptions to the above-described distribution of lowland- and indigenous species within the groups, *Trifolium repens* was found to ripen relatively early in the third growing season (end of July), while *Anthyllis vulneraria* did not produce ripe seed until September. Some species (*Trifolium badium*, *Trifolium repens*, *Silene vulgaris*) attained seed ripeness in the third growing season earlier than in the previous year.

Table 46: Fertility of the species included in the seed mixtures used at the experimental site Sudelfeld assessed in the second and third growing season. Degree of flowering and ripe seed production are expressed in percentage of plants that flowered or attained seed ripeness on the total number of plants of each species found in the plots (° =0%; * =less than 5%, ** =5-35%, *** = 36-70%, **** =71-100%). Seed germination refers to the seed sample taken in the date in which ripeness was assessed. Species that were not enough abundant to determine the fertility (*Festuca ovina*, *Poa pratensis*, *Vicia sativa*) or that did not attain seed ripeness because of the first cut (*Achillea millefolium*, *Festuca rubra*), are not included in the table.

Species	Degree of flowering		Ripe seed production		Date in which seed ripeness was assessed		Seed germination (%)	
	2000	2001	2000	2001	2000	2001	2000	2001
Poa supina	****	****	****	****	05 Jul	23 Jun	n.t.	33
Poa alpina	****	****	****	****	05 Jul	02 Jul	96	98
Trifolium badium	***	***	***	***	24 Oct	02 Jul	100 †	100 †
Crepis aurea	****	****	****	****	05 Jul	27 Jul	n.t.	52
Leontodon hispidus	****	****	****	****	22 Aug	27 Jul	86	38
Trifolium repens	***	***	***	***	06 Sep	27 Jul	12	96 †
Silene vulgaris	****	****	****	****	06 Sep	27 Jul	98	93
Festuca nigrescens	*	****	°	****	n.a.	27 Jul	n.t.	68
Poa violacea	*	***	°	***	n.a.	27 Jul	n.t.	87
Lotus corniculatus	**	***	**	**	06 Sep	24 Aug	n.t.	98 †
Phleum alpinum	**	***	**	*	06 Sep	24 Aug	52	96
Phleum hirsutum	*	***	°	***	n.a.	24 Aug	n.t.	93
Plantago lanceolata	***	***	***	***	28 Aug	13 Sep	67	48
Phleum pratense	***	*	***	*	06 Sep	13 Sep	5	81
Lolium perenne	***	*	*	*	06 Sep	13 Sep	88	92
Agrostis capillaris	**	*	**	*	06 Sep	13 Sep	n.t.	67
Trifolium hybridum	***	***	***	***	06 Sep	13 Sep	37	98 †
Anthyllis vulneraria	***	***	***	***	06 Sep	13 Sep	67	99 †

n.a. not attained; n.t. not tested; † after mechanical scarification

Among the grasses, considerable changes in the seed production were observed between the two growing seasons investigated. In the indigenous seed mixtures, a high proportion of plants of *Poa alpina* and *Poa supina* produced ripe seed in both years, while *Festuca nigrescens*, *Poa violacea* and *Phleum hirsutum* entered the reproductive phase just in the third growing season. An inverse pattern was observed for the main components of the commercial seed mixture: *Lolium perenne* and *Phleum pratense* had a high percentage of ripening plants in the second growing season (plots were mown in September), while a poor seed production was assessed in the following year (plots were cut also in June). Among the forbs, an abundant seed production was assessed for *Crepis aurea*, *Leontodon hispidus* and *Silene vulgaris*. The two latter germinated also in very high percentages.

All species tested for seed germination germinated, although some of them achieved only low (*Leontodon hispidus*, *Poa supina*) or very low (*Phleum pratense* in the second growing season) percentages. All legumes germinated very poorly in the tests of the first observation year, but after mechanical scarification in the following year a virtually complete germination was achieved. Seed germination decreases, in comparison to the germination values of the seed included in the restoration mixtures, were observed among the indigenous grasses for *Festuca nigrescens* and *Poa supina*, while increases were found for *Poa violacea*, *Phleum alpinum* and *Phleum hirsutum*. For some species, whose ripening occurred in the second growing season at the end of the growing season, seed ripeness was achieved in the third growing season earlier than in the year before. *Trifolium badium*, *Leontodon hispidus*, *Trifolium repens*, *Silene vulgaris* were the most evident cases, with differences ranging between 1 and more than 3 months.

3.4 Discussion

3.4.1 General evaluation of the experimental site Sudelfeld

Climate

In accordance with the expectations based on the altitudinal location of the experimental site with respect to the timberline, the climate was found to be relatively favourable to plant growth.

During vegetation establishment, water stress did not take place and cold periods, potentially endangering the seedlings, were not observed. However, the high frequency of precipitation and the occurrence of intense precipitation events during and immediately after the trial establishment, may have negatively affected the fastening of the binders used in the different application techniques, as rainfall started during the completion of the field trial establishment. The onset of surface runoff in this initial phase may have caused a downward transport of seed or other materials by water.

After establishment, adverse temperature events (frost) were extremely rare during the growth phase. Water supply seemed to be adequate throughout the whole observation period.

In comparison to the slopes contiguous to the ski run, a considerable delay of the snow melt (up to 6 weeks) was observed, which caused a retarded start of the growing season. Snow melt delays between prepared ski run and non-managed surrounding slopes increase in general with decreasing altitude (Hegg and Kammer, 1991). Delays resembling those observed at the Sudelfeld were assessed elsewhere at relatively low altitudes (Kammer and Hegg, 1990), while at high altitudes delays of few days are reported (Rixen et al., 2001). Nevertheless, a wide range of values, between one and three weeks, is reported by other authors (Cernusca et al., 1990; Trockner and Kopeszki, 1994) regardless of the altitude, as the duration of the snow cover depends to a large extent from the management and the preparation of the ski run. However, the actual duration of the growing season at the Sudelfeld (at least five months), although reduced by the mechanical preparation of the ski run and by the artificial snowing, does not represent a limiting factor for the vegetation.

The high wind speed can be mentioned as climatic factor possibly affecting negatively plant growth at the Sudelfeld. Also the onset of ice layers on soil surface, observed towards the beginning of spring, is indirectly related to the climate of the experimental site. If the mechanical preparation of the ski run is carried out with an insufficient snow depth, they are relatively frequent at low altitudes, as their formation is favoured by an increasing number of thawing periods (Lichtenegger, 1992).

Soil

The differences found between the experimental trial and the undisturbed surrounding vegetation for most soil chemical properties confirm what already observed by other authors following the construction of a ski run. Flüeler (1992) found at a calcareous alpine site clear decreases of organic carbon (10 times less than the initial content), nitrogen and phosphorous (1/3 of the initial content) and a very strong increase of calcium carbonate (15 times more than the initial content). Analogous changes were observed at the Sudelfeld, but they were less pronounced because of the partial re-incorporation of the topsoil into the substrate of the machine-graded ski run. For this reason, the organic matter content at the Sudelfeld was still relatively high, and most main nutrients were consequently on satisfactory levels. In particular, nitrogen level was above the minimum content needed by plant communities for self-sustained growth in severely disturbed lands without periodical external supply (1,000 kg/ha according to Bradshaw et al., 1982, corresponding roughly to 0.05%, according to Claassen and Hoogan, 1996). The humus content found in the soil (8.2%) was within the wide range (1.3% to 10%) reported for machine-graded ski-runs at calcareous sites (Holaus and Köck, 1989; Holaus and Partl, 1996). Also the low P_2O_5-content resembled values reported by the same authors, ranging between 1 and 3 mg. However, a direct comparison between sites may not to be fully reliable, as at calcareous sites the content of skeleton in the substrate is very variable and can achieve high values. The values measured in the 2-mm-sieved samples are therefore not completely representative of the situation in the field, where the contents measured may be diluted in larger volumes. Similarly, comparisons between the contents in the upper layer of the undisturbed soil profile and those in the substrate of the machine-graded ski run should be considered with caution and differences observed are likely to underestimate the real gap between the two situations. Particle-size analyses were not performed at the Sudelfeld, but it can be assumed that ski slopes obtained artificially contain more skeletal material than undisturbed profiles, as superficial and deep layers are mixed together during construction of the ski slope and skeleton content increases with increasing soil depth (Mosimann, 1983). The increase in calcium carbonate content measured after trial establishment at the Sudelfeld is evidence of the mixing of superficial and deeper soil layers.

Surrounding vegetation

Also from the point of view of the surrounding vegetation, the experimental site Sudelfeld represents a relatively favourable situation. The presence of a well-established autochthonous vegetation in contact with the experimental field is an advantage for the immigration of autochthonous species into the experimental plots, as the surrounding vegetation act as source of seed and vegetative propagules. As alteration of the site conditions was considerable but not substantial, thanks to topsoil conservation, the use of the surrounding vegetation as reference for evaluating restoration success seems to be plausible, as in such cases a secondary succession is expected (Bradshaw, 1987).

The relevé of the vegetation in a disturbed but unrestored area gave some indications about species that were able to spread and re-colonize machine-graded area if revegetation was not carried out. As *Leontodon hispidus*, *Carex flacca* and *Carex panicea* were found to be quite rare in the soil seed bank, it is very likely that their spreading occurred vegetatively starting from vegetative propagation units contained in the soil. *Poa trivialis* was instead well represented (41 seeds/m² on average) in the soil samples obtained from the experimental trial before the restoration, and it is also cited by Schauer (1981) for the Bavarian Alps among the species dominating in revegetated ski runs after decline of the commercial seed mixtures.

3.4.2 Topsoil conservation

Major problems connected with topsoil stockpiling during mining activities have been described by several authors. Through time, a reduction of the microbial biomass (Harris et al., 1980; Visser et al., 1984), of the mycorrhizal infection potential (Rives et al., 1980; Gould an Liberta, 1981; Abdul-Kareem and McRae, 1985; Visser et al., 1984; Miller et al., 1985) and of the viable component of the seed bank (Dickie et al., 1988) are likely to occur, particularly in the deeper layers of the stores. Such detrimental processes were observed to occur after a pluriennal storage, while minor changes were found in recent stockpiles. They are therefore unlikely to occur during the short storage time necessary for the construction of a ski run, which normally lasts less than one season. If, in the most cost-intensive and favourable case, the vegetation was removed carefully in form of sods, storage was demonstrated to result in minor damages to the plants contained therein, as high survival rates and reprise of the vegetative growth occurred (Stimpfl, 1985). Moreover, if topsoil conservation would be regarded as a priority for the restoration of ski run by managers and authorities defining criteria for approval and acceptance procedures of ski slopes, the organisation of the field works could be arranged in order to minimize the delay between removal of the topsoil and its re-application.

3.4.2.1 Vegetative re-colonization

The vegetation charting performed at the Sudelfeld provides evidence that the fragments of native plants contained in the topsoil are able, at least in climatically favourable conditions, to shoot again and allow a rapid re-colonization, as already stated by Bradshaw (1997a). They carry out various beneficial functions:

- In first place, they directly contribute to the achievement of satisfactory vegetation cover in short time. The results of the vegetation charting indicate that roughly one third of the vegetation cover measured two months after trial establishment was achieved through vegetative re-colonization of the autochthonous vegetation.
- Second, they act as nurse plants for the seedlings of species sown or originated from the soil seed bank, providing a suitable micro-environment for their establishment (Bradshaw, 1987). A positive influence of neighbouring plants on the establishment in vegetation gaps was found by Ryser (1990) in a limestone grassland. Here, seedling establishment of species vulnerable to water stress was enhanced under moderate vegetation cover, as the canopy of the neighbouring plants effectively protected the topsoil from desiccation. In revegetation attempts in mountain regions, such effects are sometimes pursued through addition of seed of fast-growing species to the mixtures, such as winter wheat or winter rye (Ferchau, 1998), with the disadvantage that these plants are destined to disappear after the first winter and were shown to be ineffective at high altitude (Wild and Florineth, 1999).
- Third, the fragments of native plants represent possible seed sources for the further re-colonization of the disturbed area through sexual reproduction, reducing the travelling distance of the diaspores to reach the restoration site. This is a very important function, as it was demonstrated that the seed of numerous species disperse few meters from the nearest source (Boot and Hutchings, 1990). In the alpine environment, Urbanska (1997) found that 50% of the seeds of a number of species was retrieved within one metre from the nearest possible source, while a further 20% travelled to a distance of up to 5 m and distances exceeding 18 m were very rare. To similar conclusions came Marchand and Roach (1980), who observed that seed frequency decreased very rapidly at distances in excess of 0.5 m and became virtually zero at more than 1 metre of distance. For example, seeds of *Biscutella laevigata* and *Silene vulgaris* ssp. *glareosa* germinate in characteristic clusters within a short distance from the mother plant (Urbanska, 1988).

At high altitude, the use of sods of autochthonous vegetation, coupled with seeding of alpine species, was successfully tested at high altitude by Witmann and Rücker (1995; 1997). The situation of the experimental trial at the Sudelfeld may be roughly assimilated to that obtained with this method, as the plants originated by the vegetation fragments in the topsoil acted in a similar manner such as the transplanted sods. The present findings proved that the contribution of the vegetative organs in the soil was particularly important for the spreading of *Carex*-species within the experimental field, as seeds of these species were very rare in the soil seed bank. Furthermore, the seed of most sedges found at the Sudelfeld (*Carex flacca*, *Carex panicea*, *Carex sylvatica*, *Carex* *pallescens*, *Carex nigra*, *Carex capillaris*) germinates poorly if untreated and requires cold-wet stratification (at 4° for 6 months in presence of diurnal light cycles) and alternating temperatures (22/10°C) under light conditions for achievement of satisfactory germination (Schütz and Rave, 1999; Schütz, 2000). Such features, which indicate that these species are adapted to colonize vegetation gaps occasionally occurring in late spring (Schütz, 2000), make quite difficult their use in seed mixtures. Concerning the spontaneous immigration into disturbed areas, the cover of sedges remains considerably lower in restored areas than in the undisturbed vegetation, even long time (25 years) after restoration (Bayfield, 1996).

The relatively weak correlation (R^2=0,46) between the cover of the residuals of pre-existing vegetation prior to restoration and that achieved through vegetative re-colonization, suggests that the visible above-ground vegetation alone is only a partially suitable parameter for forecasting the contribution of the vegetative resources contained in the topsoil to the vegetation cover after restoration, and that also buried plant residuals in soil play also an important role. These results are in accordance with those of Bayfield (1974), which demonstrated in the course of an experiment simulating vegetation burial with different debris thickness that recovery was possible for most plant species up to a burial depth of 5 cm and started to occur already two months after burial.

3.4.2.2 Soil seed bank

Although a very large amount of data is available about the seed bank of West Europe (Thompson et al. 1997), a limited number of experiments were carried out until now about this topic at high altitudes in the alpine region (Stimpfl, 1985; Hatt, 1991; Diemer and Prock, 1993; Prock et al., 1998; Urbanska and Fattorini, 1998a; Urbanska and Fattorini, 1998b) or in North America (Chambers, 1993;. Ingersoll and Wilson, 1993). Where the topsoil had been completely removed and discarded, extremely low seed bank densities (around 50 seeds/m²) were assessed in 10-years-old un-restored ski runs (Urbanska and Fattorini, 1998a). Under the same premises, seed bank densities comparable to those of the undisturbed vegetation are reported by Chambers (1993) for a 35-year-old gravel borrow area at high altitude; however, seed densities and number of species were still significantly higher in the seed bank of the undisturbed area. Following restoration with clonal transplants of indigenous species, a 5-year-old ski run was found to be virtually devoid of seed bank (Hatt, 1991), while 10 years after restoration seed bank densities were in most cases about half of that assessed at the Sudelfeld, and only a little number of species was found, one of them contributing for about 80% to the total seed density (Urbanska and Fattorini, 1998b). This suggests that the reconstitution of a species-rich, large seed bank is a long-term process, which can not be easily accomplished by human intervention. The present findings indicate that even after a partial mixing of the topsoil with mineral layers, which are devoid of a seed bank, seed densities are still considerable. The conservation of the topsoil should therefore be regarded as an important task when new ski runs are planned. Discarding the topsoil during the construction of a ski slope represents a waste of valuable autochthonous plant material, which is available in place for a site-specific, low-impact restoration.

In comparison to the densities reported for the seed bank of intact grassland vegetation by Prock et al. (1998) in the subalpine zone and by Urbanska and Fattorini (1998a) in the alpine zone, the values observed for Sudelfeld are considerably lower. This is in accordance with the expectations, as the topsoil was severely disturbed by machine-grading. The seed densities assessed in the experiment do not represent those occurring in the undisturbed soil.

Concerning the composition of the seed bank, differences of qualitative and quantitative composition were observed between the seed bank and the surrounding above-ground autochthonous vegetation. Only 37% of the species found in the relevés performed around the trial were detected in the seed bank, while, conversely, almost all species found in the seed bank were retrieved in the above-ground vegetation (50 out of 54). *Trifolium hybridum* and *Lolium perenne*, which were found in the seed bank, but were absent in the surrounding vegetation, were contained in the seed mixture used at the time of the trial set up by the ski company for mending small turf damages arisen during the construction of the water pipeline. They are therefore likely to have been introduced by man into the research site. Concerning *Lolium perenne*, the hypothesis is corroborated by the fact that it is reported to be a species with a transient or short-term persistent seed bank (Douglas, 1965; Thompson and Grime, 1979; Bakker et al., 1990; Thompson et al., 1997). It has been often observed that only a part of the species of the above-ground vegetation are retrieved in the seed bank, while it is relatively rare that in the seed bank species occur, which are not found in the above-ground vegetation (Ryser and Gigon, 1985; Hatt, 1991; Ingersoll and Wilson, 1992; Diemer and Prock, 1993; Gugerli, 1993; Erschbamer and Scherer, 1999). Differences can be either due to a real absence of these species from the seed bank or to the unsuitability of the methodology used for the investigation of the seed bank. Concerning the first issue, a number of species with a transient seed bank (persisting in soil less than one year) is likely to have failed in the investigated samples. Most of the seeds of such species germinate usually at the beginning of the growing season. There is indeed evidence that seed densities tend to peak between end of the summer and early spring (Thompson and Grime, 1979; Niederfriniger-Schlag and Erschbamer, 1995; Erschbamer and Scherer, 1999), following dissemination of most species and achievement of peaks in the seed rain (Urbanska et al., 1998; Urbanska et al., 1999), while it declines rapidly during the growing season (Bartolome, 1979 rev. in Roberts, 1981), as seeds of species with a transient seed bank germinate in short time if exposed at climatic conditions favourable to the germination. The main possible cause for the failed germination of seeds occurring in the soil samples is that the experimental conditions chosen for the experiment did not match the germination requirements of all species, considering that seedling recruitment is environmentally controlled mainly by soil temperature and photoperiod (van der Valk and Pederson, 1989) and that each species shows an own optimum for such parameters.

The share of grasses, forbs and legumes in the seed bank is in accordance with the results of other authors, who found generally in grassland soils more extensive seed banks of forbs than grasses (Roberts, 1981; McGraw and Vavrek, 1989; Rice, 1989). The limited densities found for legumes, that are otherwise known to produce large seed banks (rev. in Rice, 1989), could be due to a lack of germination of hard seeds. The large amount of forbs found in the seed bank of the location investigated shows that in such situations the conservation of the topsoil as substrate is important for an enrichment in indigenous forbs of the plant stands deriving from restoration, as their seed is difficult to obtain on the market or it is very expensive.

Striking differences between above- and below-ground grassland vegetation, concerning species abundance, are relatively frequent (Thompson and Grime, 1979; Ryser and Gigon, 1985; Ingersoll and Wilson, 1992; Chambers, 1993; Prock et al., 1998; Erschbamer and Scherer, 1999). The attitude to form large seed banks is, to a certain extent, a feature of the single species, rather than a function of the abundance in the above-ground vegetation (Urbanska, 1992). Some of the species dominating in the seed bank at the Sudelfeld were found indeed in large amount also in different habitats (see i.e. *Alchemilla vulgaris* agg. in Prock et al., 1998; *Plantago media* in Ryser and Gigon, 1985; *Juncus articulatus* and *Deschampsia cespitosa* in Thompson and Grime, 1979; *Poa trivialis* in Roberts, 1981). The absence or the very low seed density of some of the major species determining the vegetation structure in the surrounding vegetation, such as *Carex flacca*, *Carex panicea*, *Leontodon hispidus*, that are among those leading the re-colonization processes of the plots together with the sown species, suggests that their spreading occurred mainly vegetatively through clonal growth, starting from plant residuals in the disrupted soil (i.e. root rests or rhizomes). This was observed for example for both the above-mentioned *Carex*-species, that spread themselves very quickly through stolons in the vegetation gaps of the restored plots and for *Leontodon hispidus*, which is indeed known to form, similarly to most *Compositae*, a transient or short-term seed bank (Thompson et al., 1997), because of the short longevity of the seed (Schütz, 1990).

Referring to the classification proposed by Thompson et al. (1997), most of the species found in the seed bank belong to the short- and long-term persistent categories, which means species whit seeds persisting in the soil more than one year. As the trial establishment occurred at the beginning of July, most species were not able to complete the seed ripening before the mother plants were destroyed. Therefore, among the species with a transient seed bank (seeds persisting in the soil for less than one year), here poorly represented, only those were found, whose seeds are ripening early in the growing season. Other species with seeds confined in the upper soil layer, and only for a short period after seed rain, were not found, even if they were very abundant in the above-ground vegetation.

3.4.3 Protection against erosion

The results obtained during four observation years indicate that under the relatively favourable conditions occurring in this experiment (climatic and edaphic conditions, partial conservation of the topsoil with the seed bank and the vegetative propagation units contained therein) a satisfactory protection against erosion could be short-term achieved, regardless of the kind of seed mixture used. The critical limit of a 70%-cover was exceeded in two growing seasons by almost all treatments. In the first phase of establishment of the vegetation, an important role was played by the application techniques. At this stage, lower vegetation covers in the mycorrhiza treatments may be explainable with the fact that development of the mycorrhizal infection and appearance of beneficial effects on plant growth were found to take 6 to 10 weeks to occur (Sutton, 1973 cit. in Galli, w.y.; Meyer, 1999), and the vegetation assessment were performed in the first growing season about two months after trial establishment. The use of chemical fertilisers resulted in higher vegetation cover in the first year, but later on the gap was quickly filled by the other techniques. Starting from the second growing season, differences ascribable to the application techniques were no longer significant. From the third observation year, the seed mixtures had instead a significant effect on vegetation cover, and those containing indigenous species performed better than that constituted by lowland species. The latter exhibited a negative trend, while the vegetation cover in plots with indigenous seed mixtures remained more or less constant. Such effects were repeatedly mid-term observed at high altitudes, if indigenous and commercial seed mixtures were compared in absence of maintenance fertilisation (Krautzer, 1996a; Krautzer, 1997b; Pröbstl et al., 1998; Krautzer et al., 2001). Also including indigenous species in commercial seed mixtures proved to have beneficial effects in this environment but, in contrast to the present findings, failed to result in higher cover scores in the montane vegetation belt (Holaus, 1997; Bozzo et al., 2000), probably because of the similar dynamics of the lowland species contained in both kinds of mixtures. The present findings indicate also that indigenous seed mixtures, under the given conditions, are suitable for the establishment of plant stands, whose biomass does not need to be periodically removed. Although the plots with the indigenous seed mixture without further utilisation were not mowed during the whole observation period, their cover remained mid-term satisfactory. In contrast, the periodical cut in areas revegetated with commercial seed mixtures is recommended by various authors (Brugger, 1981; Cernusca, 1986; Lichtenegger, 1994b), in order to reduce competition between the highly productive, fast growing forage species and in order to promote grass tillering, thus avoid thinning out of the plant stands.

The absence of differences between the cover scores of plots with the mycorrhiza-hydro-seed and those resulting from the use of the plain hydro-seed show that rejecting a chemical fertilisers input do not necessarily imply mid-term achievement of lower vegetation cover. Unfortunately, no definitive conclusion can be drawn about the efficacy of the mycorrhiza inoculum, because spores of mycorrhizal fungi, together with mycelia and infected roots, were most probably already present in the autochthonous topsoil. There is evidence that the infections can be effectively spread starting also through mycelial or root contact (Powell, 1976; Read et al., 1976). In field studies, in which the topsoil was left in place, mycorrhizal infection level was observed to return in few years, after an initial decrease following disturbance, to the values observed in the undisturbed surrounding vegetation. In such cases, however, a strong reduction of the number of mycorrhizal fungal species was found (Allen et al., 1987; Barni et al., 2002). However, even if changes to the chemical soil properties could be minimized through the return of the topsoil to the restored area, major changes of the physical structure of soil and different levels of organic matter may have affected the persistence and the effectiveness of mycorrhizal fungi (Jasper, 1994). Patchy distribution of propagules or low densities of mycorrhizal inoculum are considered possible limitations to the success of revegetation practices and in such situations it may be necessary to boost infectivity by inoculation (Jasper et al., 1989; Miller and Jastrow, 1992; Jasper et al., 1994).

As the straw cover was the only difference between the two treatments where mycorrhiza was employed, the higher vegetation cover achieved in the first growing season where the straw cover was used provides evidence for the short-term beneficial effect on seedling establishment, even below the timberline. Straw was found to be an effective mulching material for allowing satisfactory vegetation cover at high altitude (Pröbstl et al., 1998; Wild and Florineth, 1999; Florineth, 1999). At the Sudelfeld, positive effects of the straw mulch on the vegetation cover were observed in the sole phase of vegetation establishment. Similarly, long-term effects were not found at mine sites in Colorado (Berg et al., 1986). Organic mulches, such as straw, reduce turbulent air movements near the ground and thus decrease water evaporation from the soil surface; the dark colour of the bituminous emulsion increases soil temperatures during the day and shading can raise minimum soil temperatures at night reducing the net amount of outgoing long-wave radiation (Cochran, 1969). At high altitude, reduction of temperature extremes beneath mulch-protected aeras, small increases of water availability and reduction of seedling mortality were observed by Flüeler (1992). In general, mulches promote seedling establishment by improving the microclimate near the ground: water stress is prevented through promotion of condensation and increase of air moisture, and diurnal temperature excursions are reduced (Schiechtl, 1973; Schiechtl, 1974). Ciotti et al. (1994) assessed larger numbers of emerged seedlings under a straw mulch than in unprotected plots. Increase of survival due to surface mulching was also reported by Chambers et al. (1990) and by Meyer et al. (1971, cit. in Kay, 1984). Furthermore, mulches mitigate the detrimental effects of mechanical damages of hail and intense rain precipitation (Schiechtl, 1972; Schiechtl, 1980; Wild and Florineth, 1999). However, they have no beneficial influence on the soil properties. Experimental results of Chambers et al. (1990) did not show any effect of straw mulch on organic matter or total nitrogen two years after sowing. The shortness of the effect of mulch observed at the Sudelfeld may be also related to the rapid straw cover decrease (from 85% to 20% in two months) occurred already in the first growing season. It has to be remarked that this decrease is likely to have been partly overestimated, because of the methodic underestimation of straw after vegetation establishment (see 3.2.10), and may be also partially due to the action of wind, blowing away the straw material, as considerable wind speeds were measured at the experimental site. Berg et al. (1986) observed indeed that fastening of straw to the ground by use of plastic nets increased its persistence to about 7 years. The fast decrease of the straw cover in plots with application technique C was also reflected by the changes through time of the total cover. In the first growing season the straw cover allowed the achievement of the largest total cover and thus the best soil protection, although the greatest vegetation cover was achieved with the conventional hydro-seed. Later on, the proportions between the cover of the different treatments resembled those of the vegetation cover, as straw was no longer abundant, although it was still the major component of the necromass. Only in the fourth growing season a pattern differing from that of the vegetation cover was found, as within commercial and indigenous mixture for further utilisation differences due to the interaction of application technique and seed mixture were found. A different proportion of necromass among the treatments accounts for these differences. Concerning differences among seed mixtures, a lower cover of the necromass was produced by the indigenous seed mixture for further utilisation. Higher values were measured for the commercial seed mixtures probably because of plant decline, and for the indigenous mixture without further utilisation because the biomass was not periodically removed. Differences among application techniques could not be explained on the basis of analysis of variance of the necromass, but small, not significant differences in necromass abundance between application techniques may account for this, as the interaction between seed mixture and application technique was nearly significant for vegetation cover (p=0.69). The use of straw mulch is generally recommended for the revegetation of steep slopes and particularly above the timberline (Florineth and Gerstgraser, 1998; Krautzer et al., 2000). Despite of the relatively favourable experimental conditions (moderate slope, absence of extreme climatic events), the use of straw mulch at the Sudelfeld resulted in the highest soil protection in the first growing season. However, this practice seems to be on the whole superfluous under such conditions, as only short-term advantages were observed and mulching represents additional costs for the restoration.

Data concerning the below-ground biomass showed quite a large variability so that their interpretation was quite difficult. In general, the output of the experiment suggests that the use of indigenous seed mixtures results in hypogeal biomass values at least so high as those achieved with the commercial seed mixtures, and that straw mulch may reduce root growth in mown plots with indigenous seed mixtures. This latter effect is not easily interpretable, but may be related to shading of the vegetation provoked by the straw cover in the re-growth phase after cutting of plots with the indigenous seed mixture. Such effects were indeed not observed for the commercial seed mixture, which proved to have a faster growth than the indigenous ones, and were absent for the indigenous seed mixture which was not cut. Small differences in the composition of the indigenous seed mixtures should have played a minor role. However, straw cover was already very low at the time of the root biomass assessment so that this effect should have been already taken place in the first growing season. Further research would be required to provide evidence for this hypothesis. The re-establishment of intensive rooting at disturbed sites in mountain regions is known to be a long-term process. Root dry matter about 2 to 5 times larger than in Sudelfeld on the third year (between 3 and 7 g/100 cm^2) were measured in 10-year-old ski runs at similar altitudes (Schauer, 1981; Cernusca, 1984a; Schauer, 1988). Such values were still 1/10 to 1/5 of those founds for the undisturbed surrounding vegetation (Haid, 1982 rev. in Cernusca, 1986). In 5- and 10-year-old restored ski runs above the timberline in which the topsoil had been partially left in place, Barni et al. (2002) found a greater root length than in undisturbed reference areas, mainly because of a greater amount of fine roots ($\varnothing$<0.5 mm); nevertheless, soil stability of a 10-year-old restored ski run, measured as percentage of aggregates not affected by breakdown after wet sieving, was still approximately 8 times less than that of the nearby pastures, suggesting that also soil structure has a great effect on soil erosivity and that a long time is required for the normalization of this feature.

3.4.4 Vegetation dynamics

In general, according to the expectation, the competitive capacity of the species played an important role in the establishment phase, while later on specific ecological features became relevant. Most of the frequency changes through time occurred for the species of the commercial seed mixture were consistent with the abundant literature information about their use for revegetation at high altitude (most data referred to locations above the timberline). Fewer literature data were instead available for the alpine species included in the indigenous seed mixtures.

In the commercial seed mixture, the initial high cover scores of *Lolium perenne* were not surprising, as the seed amount per square metre used in the present experiment (2,3 g) was above the threshold (1.5 g/m²), beyond which increasing seed rates do not result in increasing plant density (Arens, 1973). High cover values are very frequent in the first phase of revegetation, as this species is highly competitive at this stage (Lichtenegger, 1985; Delarze, 1994; Ciotti et al., 1997) and, given a seed rate near the specific threshold, is able to rule other less competitive species out, regardless of their seed rate (Klapp, 1971; Arens, 1973; Voigtländer and Jacob, 1987). Also its rapid decline in revegetated areas in mountain regions was often observed (Bayfield, 1980; Stolz, 1984; Mehnert et al., 1985; Spatz, 1985; Florineth, 1988; Delarze, 1994), as this species has relatively high thermic requirements (6.5-9°C yearly mean temperature) and high demand for nutrients (Dietl et al., 1998). Nevertheless, literature data indicate that mid-term good persistence, but not dominance, can be achieved also at high altitude (Holaus, 1998; Pröbstl et al., 1998). Results of monospecific plot tests at 1,900 m a.s.l. in the Austrian Alps suggest that different responses can be expected depending on the varieties used (Holaus, 1998). A better persistence (around 20% frequency in the fourth growing season) was shown by *Phleum pratense*, whose seed rate (2.8 g/m²) laid above the specific threshold (1.0 g/m² according to Arens, 1973). The achievement of a frequency peak in the second growing season confirms that its initial competitive capacity is lower than that of *Lolium perenne* (Klapp, 1971). If adequate nutrient inputs are provided, *Phleum pratense* is one of the mid-term most persistent commercial species at high altitude (Spatz, 1978; Stolz, 1984; Thompson and Hutchinson, 1986; Delarze, 1994; Lichtenegger, 1994b). Nevertheless, abundance decreases through time in longer time periods were repeatedly observed (Bayfield, 1980; Spatz, 1985; Frain et al., 1986a; Delarze, 1994; Bayfield, 1996; Holaus, 1998). *Festuca rubra* confirmed to be little competitive in the first developmental stages, as juveniles develop slowly after germination (Florineth, 1982; Lichtenegger, 1985; Voigtländer and Jacob, 1987), but also to be the mid- and long-term most successful species among those currently used for commercial seed mixtures under a wide range of environmental conditions (Köck, 1975; Bayfield, 1980; Köck et al., 1982; Stolz, 1984; Spatz, 1985; Frain et al., 1986a; Guillaume et al., 1986; Thompson and

Hutchinson, 1986; Younkin and Martens, 1987; Florineth, 1988; Delarze, 1994; Bayfield, 1996; Gottardi, 1997; Holaus, 1998; Pröbstl et al., 1998; Barni et al., 2002). The relatively low frequency of *Trifolium hybridum*, which is usually quite competitive in the first stages of the revegetation (Lichtenegger, 1994b) and its decline through time may be due to the permeability of the substrate and to the deterioration of the water holding capacity of the soil, which is a very common consequence of machine-grading. Water availability seems to be decisive for the occurrence of this species at low altitudes, as Thompson and Hutchinson (1986) found its cover to be greater in flat sections of the ski run and to decrease with increasing slope angle and to be positively related to the soil depth. Also the steady increase of *Achillea millefolium* found at the Sudelfeld was repeatedly observed in revegetated areas (Stolz, 1984; Spatz, 1985; Frain et al., 1986a; Guillaume et al., 1986; Florineth, 1988; Delarze, 1994; Pröbstl et al., 1998). This species was indeed shown in monospecific plots to be well adapted to the climatic conditions of the high altitudes (Florineth, 1982). Contrasting results are reported in the literature about the performance of *Poa pratensis,* if used in seed mixtures for revegetation. Satisfactory cover can be indeed achieved only if an adequate nutrient supply is ensured in form of repeated fertilisation or high fertiliser input at sowing time (Park, 1984; Spatz, 1985; Guillaume et al., 1986; Lichtenegger, 1994b). Insufficient nutrient availability may account for the obtainment of unsatisfactory results (Park, 1984; Gottardi, 1997; Dietl et al., 1998), as in the case of the present experiment.

Concerning the indigenous seed mixtures, the abundance shifts observed are in accordance with literature data. *Poa alpina* was repeatedly found to be a successful colonist in revegetated ski slopes starting from the subalpine vegetation belt, regardless of the geological substrate (Spatz, 1978; Grabherr, 1982; Mosimann, 1983; Chambers et al., 1984; Stolz, 1984; Mehnert et al., 1985; Spatz, 1985; Neugirg, 1986; Spatz et al., 1987; Florineth, 1988; Meisterhans, 1988; Schütz, 1988; Urbanska, 1988; Bedecarrats, 1991; Gottardi, 1997; Barni et al., 2002). Evidence for the pioneer attitudes of this species was provided by Spatz (1978), who observed that high cover values of this species were found near the epicentrum of mechanical soil disturbances of ski runs, while the cover decreased with increasing distance from the epicentrum. Moreover, the present findings suggest that *Poa alpina* has a relatively scarce competition capacity, as a quantitative decline was observed with the abundance increase of *Festuca nigrescens* and of other autochthonous species. This hypothesis is corroborated by the fact that of *Poa alpina* was found to be mid- or long-term highly persistent at high altitudes, where competition of other species was reduced (Guillaume et al., 1986; Holaus, 1997; Pröbstl et al., 1998; Vescovo, 2000) or absent (Grabherr, 1995). An effect of the reduction of nutrient input cannot be excluded, as relatively high needs for nutrients are expected for this species, which is best represented in fat pastures of the subalpine vegetation belt (Lichtenegger, 1994b). *Phleum alpinum*, confirming positive results reported in the literature (Brown et al., 1984; Guillaume et al., 1984) achieved a more or less stable low frequency. Possible limitations to its establishment may have been represented by its low germination capacity (40%), resulting in an actual seed rate of 0.3 g/m², and also possibly by insufficient nutrient availability, as this species is bound in nature to the fat pastures of the subalpine region (Lichtenegger, 1994b). Lack of nutrient may also account for the frequency decrease of *Poa supina* through time (Lichtenegger, 1994b). *Festuca nigrescens* played in the indigenous seed mixture a role similar to that of *Festuca rubra* in the commercial one, exhibiting a slow start, but becoming mid-term dominant. Its pioneer attitude was indeed ascertained in ski runs (Grabherr, 1982), and successful use for revegetation was observed at low and high altitude (Lichtenegger, 1994b; Reyneri and Siniscalco, 1999). The decline exhibited by *Poa violacea* after a satisfactory establishment could be instead attributed to the fact that this species is considered to be calcifuge (Adler et al., 1994) and therefore not adapted to the conditions of the experimental site. The decrease of most alpine legumes in the plots was probably caused by their life form. *Anthyllis vulneraria* is known to be in most cases biennial, and rarely annual or pluriannual (Hegi, 1964), virtually disappearing after two years in monospecific test plots on ski runs (Pardini et al., 1997), while *Trifolium badium* is biennial or triennial (Hegi, 1964) and was observed to decline very rapidly after this time in the alpine environment (Urbanska, 1995; Vescovo, 2000). *Leontodon hispidus* confirmed to be a successful species for restoration of ski runs under different climatic conditions, as already shown by Pröbstl et al. (1998) above the timberline. However, it must be emphasized that this species was quite abundant also in plots with the commercial seed mixture (on average, 17.4% frequency against 31.7% in plots with the indigenous seed mixture for further utilisation), indicating that an important contribution was given by the spontaneous re-colonization of the local population. Such an attitude to the spontaneous immigration into and re-colonization of disturbed areas was repeatedly observed within a wide altitudinal range (Park, 1984; Neugirg, 1986; Spatz et al., 1987; Schauer, 1988; Spatz, 1988; Francescato and Scotton, 1999). In the indigenous seed mixture without further utilisation, *Silene vulgaris* demonstrated to be quite competitive, as shown by the depression of the abundance of *Leontodon hispidus* in the seed mixture without further utilisation, and to become very abundant, even if sown in small quantities.

Trifolium repens exhibited similar trends in all seed mixtures: the frequency of this species peaked in the second growing season and then declined. Frequencies in plots with the indigenous seed mixtures were almost double of those in plots with commercial seed mixture. Such differences were probably due to a lower seed rate in the commercial seed mixture (0.5 g/m² against 0.9 g/m² in the indigenous seed mixtures). The frequency decreases observed starting from the third year may be due to competition effects or, more likely, to insufficient P- and K-availability in soil, which were found to be a limiting factor for the achievement of high proportion of legumes under conditions partially resembling those of the Sudelfeld in pastures (Spatz et al., 1981) and recently sown ski runs (Park, 1984).

Shifts of species abundance and substitution effects through time do not necessarily represent a negative fact, if cover remains satisfactory and protection against erosion is ensured (Lichtenegger, 1985). The abundance of some species may decrease after they completed a certain task, such as in the case of pioneer species, which provide soil protection shortly after seeding and are able to colonize mineral or very stony substrates, and are later substituted by species growing slower or having higher requirements for soil conditions.

The marked vegetation dynamics taking place in all treatments was not surprising, as climate was relatively favourable, and sources for the re-colonization (vegetative organs in soil, seed bank, undisturbed surrounding vegetation in contact with the experimental trial) were present. Concerning the relationship between application techniques and seed mixtures, it has to be emphasized that the present results can be correctly interpreted only if account is taken of the fact that no fertilisation was performed after the establishment of the trial. The effect of the seed mixtures appeared only after those of the application techniques faded out, as many of the species contained in the commercial seed mixture are valuable fodder plants selected for high forage production and require an adequate nutrient input and intensive management, in order to achieve persistence in the plant stand. The analysis of the variation of the ecological indicators provided evidence that nutrients shortage was the most important factor influencing the vegetation dynamics in all plots, while climatic aspects affected the succession in the sole plots with the commercial seed mixture. The decrease of the indicator for nutrient must be indeed due either to a decrease in abundance of species depending on high nutrient availability or to an increase of species with opposite attitudes. Species with high temperature needs, such as the lowland forage species, tended to decline and to be substituted by less exigent, autochthonous species.

The results show that by using indigenous seed mixtures it was indeed possible to establish mid-term plant stands formed mainly by some of the species occurring in the surrounding semi-natural vegetation. Their share showed an increasing tendency also in plots with the commercial seed mixture, as indigenous species were colonising vegetation gaps left by the declining lowland cultivars. The results of the frequency analysis show that the decrease of some sown indigenous species was compensated by the increase of other sown and non-sown species. Such substitution effects may have been caused by competition, as most of the indigenous species included in the seed mixtures were at the lower limit of their altitudinal distribution or were missing at the Sudelfeld and may have been replaced by others able to exploit more efficiently the climatic favourableness of the experimental site. High altitude species are indeed known to have an inherently relative growth rate and to respond to a limited extent to improvements of the growth environment (Grime and Hunt, 1975; Woodward, 1979; Atkin et al., 1996a; Atkin et al., 1996b). In contrast, the compensation between decline of the sown species and increase of the native species did not take place in the commercial seed mixtures, despite of the equally increasing trend of the species from the surrounding vegetation. The ongoing enrichment in native species is also the cause of the converging trend of the different seed mixtures through time, represented by the ordination plot obtained through principal component analysis of the cover measurements.

In the Bavarian Alps, relatively low species numbers (20.1 on average) were assessed at the beginning of the 80's in ski runs in the montane vegetation belt (Schauer, 1981). In contrast, more than 50 species were found at the Sudelfeld in the fourth growing season, after increases of about 10 species between the second and the third observation year, indicating that considerable values were already obtained in the establishment year. The present findings show that a high species number is achievable in relatively short time, if potential sources of plant material (soil seed bank, vegetative propagation units in the topsoil) are not wasted. A further beneficial role may have been played at the margins of the trial by the surrounding vegetation, which was almost in contact with the experimental trial. Possible species immigration between plots sown with different mixtures could have taken place with increasing time; species enrichment of plots with commercial mixtures may have been enhanced, while the opposite did not, while commercial species were declining everywhere. Results of species number are therefore conservative for the hypothesis that a higher species richness can be long-term expected for plots with indigenous seed mixtures.

In the case of Sudelfeld the climatic conditions, which are not extreme, allowed virtually all species to attain the seed ripeness before the end of the growing season. Earlier attainment of seed ripeness in the third growing season than in the previous year (i.e. *Trifolium badium*, *Leontodon hispidus*, *Trifolium repens*), as well as start of the seed production in the third growing season (*Festuca nigrescens*, *Poa violacea*, *Phleum hirsutum*) may be due to the achievement of the size threshold for starting reproduction processes (see 1.4.1 for further discussion of such effects). The limiting factor for reproduction by seed under the given circumstances is instead represented by the management practices and particularly by the time of the biomass harvest. Only few species were able, and only with very low frequency, to get some plants flowering after the cut and ripening before the end of the growing season. For some species, dissemination would be important if their persistence is desired. For example, *Anthyllis vulneraria* is biennial (Hegi, 1964; Pardini et al., 1997) and *Trifolium badium* is at most triennial (Hegi, 1964; Urbanska, 1995) and their permanence in the composition of the plant stands is therefore bound to sexual reproduction and dissemination. For this reason, Pröbstl (1990) suggests cutting the plots in late summer. However, it has to be emphasized that especially in the first years following revegetation with commercial seed mixtures, priority should be given to the promotion of tillering and vegetative spreading of grasses able to form dense swards and to the reduction of competition effects between species with different growth forms. This is achievable through early and, if possible, repeated cuts, and results in a better vegetation cover (Lichtenegger, 1994b).

3.4.5 Dry matter yield and potential forage quality

The dry matter yield appeared to be mainly influenced by the composition of the seed mixture. As long as the productive forage species (*Lolium perenne*, *Phleum pratense*) were dominant in the plant stands obtained with the commercial seed mixture and the plant cover was similar in all treatments, a higher production was observed for the commercial seed mixture. Then, starting from the third growing season, the indigenous seed mixture achieved a forage production as good as that of the commercial seed mixture. It has to be emphasized that the biomass production does not refer to a stable plant composition, as substitution effects were very common and increasing with time, and a progressive increase of autochthonous species not included in the seed mixtures was observed in all treatments. The dry matter yield of all treatments in the third harvest year lies near the lower limit (3,000 kg/ha) indicated as typical for the *Festuco-Cynosuretum* by Bohner (1999).

Plant stands on ski runs, recently sown by using commercial seed mixtures, can exhibit an outstanding potential forage quality (Köck et al., 1989). In the present experiment, the commercial seed mixtures initially allowed to achieve a relatively high potential forage quality (around 6), which was only slightly better than that of the indigenous seed mixture. Differences in potential forage quality between the commercial and the indigenous seed mixtures for further utilisation became smaller through time and in the third growing season were no longer significant. This can be explained on one hand by the strong decline of valuable forage species (*Lolium perenne*, *Phleum pratense* and *Trifolium repens*) in the commercial seed mixture and by the ongoing enrichment in native species, whose quality is inferior to that of the sown species. The latter account also for the general potential quality decrease observed through time regardless of the seed mixture, as also in the indigenous seed mixture substitution effects of valuable species (i.e. *Poa alpina* and

Trifolium repens) were observed. On the other hand, also the increase of the proportion of sown species with lower value scores (i.e. *Festuca rubra*, *Leontodon hispidus*) had an effect on the declining forage quality. A similar decrease of the potential forage quality through time was observed by Park (1984) in revegetated ski runs in the montane vegetation belt of the calcareous Bavarian Alps in absence of maintenance fertilisation after sowing, while higher, slightly increasing values could be obtained following repeated application of P- and K-fertilisers. The potential forage quality in the fourth growing season was still comparable with that of a phosphorous- and potassium-fertilised *Crepido-Cynosuretum* investigated by Spatz et al. (1981) at slightly higher altitude (1,450 to 1,500 m a.s.l.), but more favourable exposition, under geological and edaphic conditions resembling those of the Sudelfeld. However, lower values (around 3) can be long-term expected for such plant communities in absence of external P- and K-supply.

A further aspect, which should be taken into consideration, is that the decline of the forage quality observed in the experimental plots may have been underestimated. Dietl (1982) assigned thresholds to the percentages of yield of some forage plants, above which decreases of the forage quality are expected. The specific contribution achieved by some successfully sown species laid above this limit; moreover, all of them showed an increasing trend, suggesting that the underestimation of the forage quality may increase through time. In particular, the specific contribution of *Festuca rubra* on the first harvest year (15.7%) was about the threshold for pastures (10%) and that of the following year (24.7%) trespassed also the threshold for forage and hay (20%). The specific contribution of *Achillea millefolium* in the commercial seed mixture (5.1% in 2001, 7.5% in 2002) slightly exceeded the limit for pastures, but laid below that for forage and hay. Concerning the indigenous seed mixture, no threshold is suggested for *Festuca nigrescens*, but if this species is assimilated to *Festuca rubra*, an analogous situation is found, as a specific contribution of 19.2% was measured in 2001 and of 25.6% in 2002. Furthermore, the specific contribution of *Leontodon hispidus* on the third harvest year (13.1%) exceeded the threshold for pastures (10%), but was still below that for forage and hay.

On the whole, from the point of view of the forage production, the advantages of employing commercial seed mixtures had a short duration and were coupled with negative mid-term effects on the soil protection against erosion.

Conclusions

The results of the present investigations confirm that the lowland seed propagation of alpine species according to the prescription of the organic farming is extremely problematic. One of the major obstacles is the low responsiveness of high altitude species, in terms of growth speed, to improvements of the growth environment, such as to temperature increases and nutrient availability. Their slow growth make them subsequently not capable to compete with the weed species of the lowland. As the mechanical weed control methods investigated in the present work were only able to control weeds located between the rows of the crop plant, the alpine species can be easily overgrown by weeds located within the rows. The second obstacle is the susceptibility of high altitude species against rusts and nematodes, which may be due to the absence of these pathogens in the natural alpine environment and, in turn, to the lack of development of defense mechanisms against them. *Sesleria albicans* and *Trifolium alpinum* are two typical representants for such problematic species. The seed production of slow growing and less competitive species is possible only if a lot of manual work is performed, but this implies an extreme cost increase for the seed. The organic seed production of such species under the investigated conditions seems therefore not to be economically advisable.

However, the investigations of *Trifolium alpinum* in a controlled environment suggest that improvements of the growth rate, which are important to reduce the competition gap between weeds and crop plant, may be achieved in the field if autochthonous, beneficial micro-organisms (rhizobia, mycorrhiza) would be introduced by use of suitable inoculants.

The organic seed propagation may be only possible for species having a relatively fast growth in the first developmental stages and being rather insensitive against pathogens, such as in the case of *Festuca nigrescens*. A reduction of the seed purity seems to be inevitable as a consequence of the use of mechanical weed control methods, and a higher proportion of weed seeds or greater costs for seed cleaning, can be expected in comparison to a conventional seed propagation of this species.

Concerning the restoration of ski runs, the topsoil conservation proved to be crucial for a successful restoration, because this allows to take advantage of important abiotic (nutrients bound to the organic matter) and biotic resources (autochthonous soil micro-organisms, seed bank and vegetative propagation units) naturally occurring in the topsoil. Topsoil conservation ensures therefore, after the construction of the ski run, the maintenance of minimum requirements of soil properties for allowing the establishment of the species sown and the re-establishment of nutrient cycles. Furthermore, it provides an adequate substrate for the autochthonous species, and the seed bank and other vegetative propagation units contained in the topsoil, contribute strongly to the re-colonization of the ski-run. If this first point is fulfilled, low fertilisation inputs (only an initial application) result in high species richness regardless of the seed mixtures employed for restoration. Moreover, renouncing to a chemical fertilisation does not lead to unsatisfactory protection against erosion. If the top soil is left in place, at least partially, also the addition of mycorrhizal inoculants may become superfluous, allowing a considerable decrease of the costs for restoration.

The use of indigenous seed mixtures seems to be of pivotal importance above the timberline because of the better adaptation of native species to the severe environment at high altitudes. However, the present results provide evidence that their use in the montane zone, under relatively favourable conditions, may bring about improvements of the results achievable with conventional revegetation techniques. Seed mixtures containing subalpine and alpine grasses, legumes and forbs lead mid-term to a more persistent vegetation cover, if compared with a commercially available seed mixture with lowland species. After an initial phase, in which the composition of the seed mixtures represents the main factor affecting the botanical composition of the plant stands and the proportion between species, quite a strong vegetation dynamics can be expected from year to year, and the re-establishment of native species occurs at a relatively high rate, regardless of the seed mixture used. The decline of the valuable forage varieties, which could be not compensed by the increase of the autochthonous species, seems to be mainly due to their high demands for nutrient availability and temperature, while that of the sown indigenous species may be primarily determined by competition effects with the non-sown and sown autochthonous species. Using indigenous seed mixtures, a higher cover of species in common with the surrounding vegetation can be mid-term achieved, and maintenance care can be eventually omitted. Concerning the agronomical aspects, the use of commercial seed mixtures results in short-term advantages (dry matter yield, forage quality), but later on, differences between mixtures may become negligible. Moreover, in case of further deterioration of the vegetation cover, differences in forage production may appear in favour of the indigenous seed mixtures.

On the whole, the results of the present research programme indicate that at least two kinds of important goals can be achieved by using indigenous seed mixtures. In first place, they proved to be more suitable than commercial seed mixtures for restoration in combination with low-impact, site-specific restoration techniques. Second, from the economical point of view, their use may allow a long-term decrease of the costs, if account of the reduction of fertiliser input and of post-restoration care is taken, despite of higher initial seed costs.

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Abstract

In the last 50 years there has been a strong increase of the summer- and winter tourism in the Alps. For this reason, numerous infrastructures have been built up, causing increasing soil erosion. The construction of ski runs contribute considerably to this problem. The replacement of the destroyed herbaceous cover after earth movements is a very important measure in order to prevent erosion. The commercial seed mixtures generally used for restoration contain varieties of lowland species, which are not adapted to the climatic and edaphic conditions of the mountain regions and require expensive maintenance works, such as periodical cut or fertilisation. In the opinion of numerous authors, the use of subalpine and alpine autochthonous species in seed mixtures could be a suitable approach for achieving a lasting revegetation in mountain regions, but with few exceptions their seed is not commercially available yet.

The present work focuses on two aspects of using indigenous seed mixtures for the restoration of ski runs in mountain areas. First, the organic seed propagation of three species was investigated. One of them (*Festuca nigrescens*) is already successfully propagated and its seed is currently available, while the cultivation and the seed propagation of the other species (*Sesleria albicans* and *Trifolium alpinum*) have been less studied until now. At the same time, two indigenous seed mixtures, containing alpine and subalpine species, and a commercial seed mixture were compared on a ski run in combination with environmentally sound practices (topsoil conservation, reduction of fertiliser inputs).

The seed propagation of *Sesleria albicans*, *Trifolium alpinum* and *Festuca nigrescens* was investigated in field trials in Hebenshausen (Hesse, 220 m a.s.l.). Four mechanical weed control methods (topping, hoeing, brush weeding and hand-roguing) and three harvesting methods (hand-harvesting, threshing, stripping/vacuum harvesting) were arranged in a two-factorial randomized complete block design. Cover and density of the crop plant and of weeds were assessed for *Sesleria albicans* at the time of the first two weed control interventions and for *Trifolium alpinum* at the time of the second interventions.

Sesleria albicans and *Trifolium alpinum* exhibited a very slow establishment- and initial growth phase, which made difficult a timely performance of the mechanical weed control. At the time of the first intervention, hoe and brush hoe could partially control weed density, while weed cover increase considerably thereafter. The second intervention left the weed density unchanged, a further increase of weed cover occurred. Weeds located within the rows could not be reached by the hoeing implements and were able to overgrow the crop plant. In the first harvest year, all exclusively mechanical treatments were overgrown by weeds to such an extent that no kind of mechanical harvest could be

performed. Hand roguing was the sole effective weed control method. In contrast, the establishment of *Festuca nigrescens* was relatively fast and this species showed higher growth rates and was therefore more competitive.

Trifolium alpinum was found to be extremely susceptible against phytophagous nematodes of the genus *Pratylenchus*. Plant mortality in the plots achieved such rates that no kind of mechanised harvest could be performed. *Sesleria albicans* exhibited high susceptibility against *Claviceps purpurea*. Despite of a continuous production of flowering stems throughout the whole growing season, only one harvest seems to be advisable, as late-harvested seed contained almost exclusively sclerotia. *Festuca nigrescens* did not show any noteworthy susceptibility against pathogens.

The germinative capacity of all species, for which wild provenances were used, exhibited a distinct increase after the seed propagation. For the grasses this is ascribable to the more favourable maternal plant environment, while for *Trifolium alpinum* the increase may be due to undesired mechanical scarification during seed cleaning.

For all species, the first harvest was possible in the second growing season. The hand collection of single plants of *Trifolium alpinum* yielded extremely low seed amounts (about 2.5 kg/ha). *Sesleria albicans* entered the reproductive phase only after achievement of a minimum size (tussock diameter of at least 10 cm). Low seed yields (about 5 kg/ha in the first harvest year and 35 kg/ha in the second one) were obtained. Considerable seed yields were instead achieved for *Festuca nigrescens* (more than 750 kg/ha in the first year and more than 600 kg/ha in the second year) in the mechanised variants (hoeing and brush weeding). Combine-harvesting was found to be a suitable harvesting method, while stripping/vacuum harvesting resulted in high seed loss but allowed higher seed purity.

On the whole, the results confirm that the organic seed propagation of high altitude species is an extremely difficult task. However, it is feasible for species with a relatively fast growth in the first developmental stages and little susceptibility against pathogens, such as in the case of *Festuca nigrescens*. Relatively high percentages of weed seeds or costs for seed cleaning can be expected following mechanical weed control.

Further pot experiments were carried out on *Trifolium alpinum* in order to elucidate the reasons for the failure of the cultivation in the field and work out possible cultivation strategies. Plants were grown in a controlled environment for up to four months and the influence on plant growth of diverse soil substrates, of inoculation with autochthonous micro-organisms, of phytophagous nematodes (*Pratylenchus* sp.) and of the P-level in soil was studied. Allochthonous soils (a similar grassland soil and a different arable soil), compared with the autochthonous soil, had lower contents of soil micro-organisms, but higher nutrient levels.

Plants grown in allochthonous soils achieved higher biomass, except where nematodes were detected. Here, plant decline occurred in 4-month-old plants. The addition of a thin layer of autochthonous soil was effective as rhizobial inoculum and proved to be advantageous for plant growth in allochthonous substrates. The other methods investigated for inoculation (mixing of a small amount of the original soil from the Alps, preparation of a soil extract) were less effective or completely ineffective. A detrimental effect of *Pratylenchus* sp. was detected on dry matter production. *Trifolium alpinum* appeared to be P-limited if grown on the autochthonous soil at temperatures higher than in the natural environment. It is concluded that a successful cultivation strategy of *Trifolium alpinum* in lowlands should include avoidance of soils potentially infested by *Pratylenchus* sp., supply of a suitable inoculum containing autochthonous micro-organisms and choice of soils with an adequate P-availability.

It is a common opinion that the use of indigenous species is a pre-requisite for the achievement of long-term successful restoration of erosion areas above the timberline. But also in locations at lower altitudes in the high montane vegetation belt they may represent an efficient alternative to seed mixtures with lowland-cultivars, particularly in case of limited nutrient availability.

A field test was established in the calcareous Bavarian Alps on a North-facing ski run at about 1,240 m of elevation, in order to test under practical conditions the use of alpine species in seed mixtures for the restoration of ski runs. A commercially available seed mixture (SM1) with lowland species was compared with two seed mixtures containing subalpine and alpine grasses, legumes and forbs (SM2 for further agronomic utilisation and SM3 without further utilisation). Three different restoration techniques were used in combination with the above-mentioned seed mixtures: a conventional hydroseed (ATA) including mineral fertilisers, a mycorrhiza-hydroseed (ATB) containing a mycorrhiza-inoculum and organic fertilisers and a mycorrhiza-hydroseed additionally protected with a layer of straw mulch (ATB). Treatments were arranged in a two-factorial split plot. Environmentally sound measures were applied to the whole experiment (partial topsoil conservation, a sole initial application of fertilisers). Priorities and criteria for the evaluation of the treatments were defined. Protection against erosion was regarded as main aim and was evaluated on the basis of vegetation cover and root biomass. The biotic interaction with the surrounding, autochthonous vegetation and succession towards site-specific vegetation types was appraised on the basis of the cover of species in common with the surrounding vegetation, number of species, vegetation dynamics and fertility of the species sown. The suitability for an agronomic use of the forage was evaluated on the basis of dry matter yield and potential forage quality.

Meteorological data collected at the experimental site showed a relatively favourable cours of temperature and precipitation, and very low frequency of extreme events. High wind speed was found as a factor adverse to plant growth. Five vegetation types were found in the surrounding vegetation, the high montane *Crepis aurea*-form of the *Festuco-Cynosuretum* being mainly represented. The comparison of soil samples from undisturbed soil profiles and from the experimental trial showed considerable reduction of organic matter content and nutrient levels following machine-grading.

The biotic resources naturally occurring within the experimental field were investigated. A mean cover of 6% and a mean species richness of 33 species/plot were assessed for residuals of the pre-existent vegetation. Investigations of the seed bank prior to application of the restoration treatments detected 55 species and a mean seed density of 1,929 seeds/m². The vegetative re-colonization of the plots starting from vegetative propagation units in the soil represented about one third of the total vegetation cover two months after trial establishment.

In the first growing season the factor application technique had a positive effect on the vegetation cover, and ATA achieved the best values. Also a short-term positive effect of the straw mulch in ATC was found. In the second year all treatments achieved the 70%-threshold. Starting from the third year, the factor seed mixture became significant and a decreasing trend of the vegetation cover was observed for SM1, while the indigenous seed mixtures had more or less constant values, even if no kind of maintenance care was carried out.

The use of indigenous seed mixtures resulted in higher cover of species in common with the surrounding vegetation, but differences with SM1 became smaller from year to year. Species number increased rapidly and was considerable in all treatments (56 species/plot in 2002). Frequency analysis showed that a marked vegetation dynamics took place in all treatments, and some of the sown species were substituted by species of the surrounding vegetation. Nutrient availability and temperature were presumably the factors determining vegetation dynamics in SM1, while only the first one seemed to affect SM2 and SM3. For species contained in the indigenous seed mixtures, frequency decrease seemed to be due also to competition effects. The relatively favourable climate of the experimental site allowed all species sown to attain seed ripeness.

SM1 achieved short-term higher dry matter yield than SM2, but differences were no longer found from the second harvest year. The potential forage quality decreased through time in all treatments and was initially higher for SM1.

The results indicate that the topsoil conservation during the construction of ski slopes in the montane vegetation belt is crucial for a successful restoration, in order to take advantage of important, naturally occurring

biotic resources. In case of a reduction of fertilisation inputs, a better protection against erosion can be mid-term achieved with indigenous seed mixtures. Their forage production and quality seem to be assimilable to those of the commercial seed mixtures.

Keywords:

seed propagation; *Sesleria albicans*; *Trifolium alpinum*; *Festuca nigrescens*; mechanical weed control; *Pratylenchus*; *Rhizobium*; *mycorrhiza*; ski runs; restoration; seed mixtures; protection against erosion; topsoil conservation; seed bank; vegetation dynamics

Zusammenfassung

In den letzten 50 Jahren hat der Sommer- und Wintertourismus in den Alpen stark zugenommen. Aus diesem Grund ist eine Vielzahl von Infrastrukturen aufgebaut worden, die eine zunehmende Bodenerosion zur Folge haben. Skipisten tragen in großem Umfang zu diesem Problem bei. Der Ersatz der zerstörten Vegetationsdecke nach Bodenbewegungen ist eine sehr wichtige Maßnahme, um Erosion zu verhindern. Die in der Begrünung praxisüblich angewandten kommerziellen Saatgutmischungen enthalten Flachland-Zuchtsorten, die den Klima- und Bodenverhältnissen der Bergregionen nicht angepasst sind und teure Maßnahmen (regelmäßige Düngung und Mahd) für ihre Erhaltung benötigen. Die Anwendung subalpiner und alpiner Arten in Saatgutmischungen könnte nach Ansicht mehrerer Autoren ein erfolgreicher Ansatz für eine langfristige Begrünung in Bergregionen sein. Bis auf wenige Ausnahmen ist solches Saatgut jedoch noch nicht kommerziell erhältlich.

Die vorliegende Arbeit konzentriert sich auf zwei Aspekte der Verwendung autochthonen Saatgutes für die Renaturierung von Skipisten im Gebirge. Zunächst wurde die Saatgutvermehrung von drei Arten nach den Richtlinien des ökologischen Landbaus untersucht. Eine davon (*Festuca nigrescens*) wird bereits erfolgreich konventionell vermehrt und ihr Saatgut ist kommerziell erhältlich, während Anbau und Saatgutvermehrung der anderen (*Sesleria albicans* und *Trifolium alpinum*) bis jetzt in geringerem Maß untersucht wurden. Parallel dazu wurden zwei autochthone Saatgutmischungen, die alpine und subalpine Arten enthielten, und eine kommerzielle Saatgutmischung wurden im Einsatz unter Praxisbedingungen auf einer Skipiste in Kombination mit umweltschonenden Maßnahmen (Erhaltung des Oberbodens, Verringerung der Düngung) verglichen.

Sesleria albicans, *Trifolium alpinum* und *Festuca nigrescens* wurden in Hebenshausen (Hessen, 220 m) in drei getrennten Versuchen angebaut. In jedem Versuch wurden vier Methoden der mechanischen Unkrautbekämpfung (Faktor 1) und drei Erntemethoden (Faktor 2) in einem Feldversuch in Kleinparzellen getestet (Drillsaat für Gräser und Saatband für *Trifolium alpinum* in 6 Reihen, 5 m lang für *Festuca nigrescens* und 3 m lang für die anderen Arten; 24 cm Reihenabstand; Ansaattermin Anfang Mai/Anfang Juni; Aussaatmenge 7,2 kg/ha für *Sesleria albicans*, 5 kg/ha für *Festuca nigrescens*, 4 cm Abstand in der Reihe für *Trifolium alpinum*). Versuchsdesign war eine vollständig randomisierte Blockanlage in drei Wiederholungen. Als Methoden der Unkrautbekämpfung kamen Jäten (Hacken zwischen den Reihen, Jäten in den Reihen), Hacken, Hackbürste und Schröpfen zum Einsatz. Für den Versuch wurden alpine Herkünfte verwendet, mit der Ausnahme von Sesleria albicans, von der Herkünfte aus den Alpen und der Schwäbischen Alb verwendet wurden. Das Wildsaatgut wurde

getrocknet, gereinigt und bei 6°C bis zum Versuchsbeginn aufbewahrt.

Deckungsgrad und Dichte der Kulturpflanzen und des Unkrauts wurden für *Sesleria albicans* bei den ersten beiden Unkrautbekämpfungen und für *Trifolium alpinum* bei der zweiten Unkrautbekämpfung untersucht. Sie wurden unmittelbar vor der Unkrautbekämpfung und zwei Wochen danach an 6 Dauerbeobachtungspunkten pro Parzelle mit dem Göttinger Schätzrahmen aufgenommen. Für jede Parzelle wurden die Ergebnisse gemittelt. Die Ernte wurde mit der Hand, mit einem Mähdrescher oder mit einem Bürst-Sauggerät (eigener Prototyp) durchgeführt. Die Handparzellen wurden mit Handscheren gemäht und das Mähgut wurde dann gedroschen. Randeffekte waren möglicherweise vorhanden und wurden nicht berücksichtigt. Das Erntegut wurde unter Luftzug bei Raumtemperatur eine Woche lang getrocknet und mittels Sieb und Steigsichter gereinigt. Reinheit und Fremdbesatz des Saatgutes wurden vor und nach der Vermehrung untersucht.

Die sehr langsame Feldetablierung- und Wachstumsphase von *Sesleria albicans* und *Trifolium alpinum* verspäteten und erschwerten erheblich den Einsatz der Methoden, bei denen die Erkennung der Reihen notwendig war (Jäten, Hacken und Hackbürsten). Beim ersten Einsatz konnten Hacken und Hackbürsten die Pflanzendichte des Unkrauts nur teilweise kontrollieren, während der Deckungsgrad nach dem Einsatz zunahm. Der zweite Einsatz von Hacke und Hackbürste ließ die Unkrautdichte unverändert, während der Deckungsgrad stark zunahm. Die Unkrautpflanzen, die in den Reihen vorhanden waren, wurden von den Hackelementen nicht mehr erreicht und waren in der Lage, die Kulturpflanze zu überwachsen. Langfristig waren alle ausschließlich mechanisch durchgeführten Varianten derart vom Unkraut überwachsen, dass keine mechanische Erntemethode durchgeführt werden konnte. Unter den getesteten Methoden war Jäten die einzige, die wirksam war. Die Unterschiede zwischen der geschröpften Behandlung und den Hackvarianten wurden mit der Zeit immer geringer. Festuca nigrescens etablierte sich hingegen wesentlich schneller und zeigte deutlich höhere Wachstumsraten und höhere Konkurrenzfähigkeit.

An *Trifolium alpinum* wurde eine extreme Anfälligkeit gegen pflanzenfressende Nematoden der Gattung *Pratylenchus* festgestellt. Der Versuchspflanzenbestand wurde derart dezimiert, dass keine maschinelle Saatguternte dieser Art möglich war. *Sesleria albicans* erwies sich als sehr anfällig gegen *Claviceps purpurea*. Trotz einer kontinuierlichen Produktion neuer Blüten durch die ganze Vegetationszeit erscheint deshalb nur eine einzige Ernte zu Beginn sinnvoll, da das Saatgut bei den späteren Ernteterminen fast ausschließlich Sklerotia enthielt. *Festuca nigrescens* zeigte im Gegensatz dazu keine nennenswerte Anfälligkeit gegen

Krankheitserreger.

Der erste Erntetermin war für alle Arten erst im zweiten Jahr möglich, mit einer wesentlichen Verkürzung der Zeit, die für das Erreichen der generative Phase unter natürlichen Bedingungen notwendig ist. Für *Sesleria albicans* wurde festgestellt, dass eine Mindestgröße der Pflanzenhorste (10 cm Durchmesser) erreicht werden musste, bevor fertile Halme von den Pflanzen produziert wurden. Nur eine Handernte von Einzelpflanzen war für *Trifolium alpinum* möglich und der Ertrag betrug 2.5 kg/ha. Niedrige Erträge wurden auch für die gejäteten Behandlungen von *Sesleria albicans* erhalten: im ersten Erntejahr konnten ungefähr 5 kg/ha, im zweiten Erntejahr höchstens 35 kg/ha erreicht werden. Da eine starke Zunahme des Deckungsgrades der Kulturpflanze während der ganzen Untersuchungszeit festgestellt wurde, scheint eine Verlängerung der Kultur um ein weiteres Jahr plausibel zu sein. Drusch erwies sich als geeignete Erntemethode, wenn das Saatgut zusammen mit der Blattmasse geerntet, getrocknet und anschließend gesiebt wurde. Das Bürstsauggerät zeigte im Gegensatz dazu hohe Saatgutverluste, die auf eine mangelnde Optimierung des Geräts zurückzuführen waren. Ein beträchtlicher Ertrag wurde für *Festuca nigrescens* erreicht. Die Hack- und Hackbürste-Varianten lieferten Erträge, die niedriger als diejenigen der Hand-Variante waren, aber durchschnittlich über 750 kg/ha im ersten Erntejahr und über 600 kg/ha im zweiten Erntejahr lagen. Drusch war genauso effizient wie die Handernte als Erntemethode, während das Saugbürstgerät Saatgutverluste von bis zu 50% zeigte. Die Berücksichtigung des Reinheitsgrades ergab einen sehr starken Fremdbesatz (13% im Durchschnitt) in den Druschvarianten, während wesentlich niedrigere Werte dieses Parameters (4%) für die Saugbürste-Behandlungen gemessen wurden.

Die Keimfähigkeit aller Wildsaatgut enthaltenden Arten zeigte eine deutliche Zunahme nach der Saatgutvermehrung, was als Effekt der Umweltbedingungen auf die Mutterpflanzen zu interpretieren ist. Es gibt den Verdacht, dass die Keimfähigkeitszunahme von Trifolium alpinum durch ungewollte Skarifizierung des Saatgutes während der Reinigung beeinflusst wurde.

Die Ergebnisse der zweijährigen Untersuchung bestätigen, dass die Saatgutvermehrung von Arten aus den alpinen Hochlagen nach den Richtlinien des ökologischen Landbaus extrem problematisch ist. Sie ist jedoch für Arten möglich, welche ein relativ rasches Wachstum in der ersten Entwicklungsphase und geringe oder keine Anfälligkeit gegen Krankheiten zeigen, wie im Fall von Festuca nigrescens. Ein Saatgutqualitätsverlust bei den mechanisierten Varianten ist allerdings unvermeidbar, und relativ hohe Fremdbesätze oder hohe Reinigungskosten sind zu erwarten. Eine Saatgutproduktion von langsamwüchsigen und weniger konkurrenzfähigen Arten ist nur möglich, wenn viel manuelle Arbeit eingesetzt wird, was eine extreme Verteuerung des Saatgutes zur Folge hat. Ihre ökologische Saatgutproduktion unter den untersuchten Bedingungen scheint deshalb wirtschaftlich nicht sinnvoll.

Zusätzliche Gefäßversuche wurden durchgeführt, um die Ursachen des Scheiterns des Feldanbaus von *Trifolium alpinum* zu klären, und Anbaustrategien zu erarbeiten. Pflanzen wurden bis zu 4 Monate lang unter kontrollierten Bedingungen angezogen, und der Effekt folgender Faktoren untersucht: unterschiedliche Substrate, Inokulation mit autochthonen Mikroorganismen, pflanzenfressende Nematoden (*Pratylenchus* sp.) und Phosphorversorgung im Boden. Allochthone Böden [ein ähnlicher Graslandboden aus dem Harz (Dammhaus, Clausthal-Zellerfeld, Niedersachsen, 550 m ü.N.N.) und der Feldboden des Vermehrungsstandortes] hatten einen niedrigeren Gehalt an Bodenmikroorganismen aber einen höheren Nährstoffgehalt im Vergleich zu dem autochthonen Boden aus den Alpen (Meran 2000, Hafling, Südtirol, 2.150 m ü.N.N.). In allochthonen Böden aufgezogene Pflanzen erreichten kurzfristig (48 Tage) höhere Trockenmassen. Es wurde aber ein reduziertes Wachstum und Pflanzenverfall nach vier Monaten in den Behandlungen beobachtet, in den Nematoden vorhanden waren. Die Zugabe einer dünnen Schicht autochthonen Bodens als Inokulum war effektiv und wirkte sich positiv auf das Pflanzenwachstum im allochthonen Substrat aus. Die übrigen getesteten Inokulationsmethoden (Beimischung einer kleinen Menge autochthonen Bodens aus den Alpen, Herstellung eines Bodenextraktes) waren weniger effektiv oder völlig uneffektiv für die Knöllchenbildung durch Rhizobien. Ein schädlicher Effekt von Pratylenchus sp. auf die Trockenmasseproduktion wurde festgestellt, da Pflanzen in unbehandeltem Boden aus dem Vermehrungsstandort Wachstumsstörungen und hohen Nematodenbefall zeigten, im Vergleich zu Pflanzen, die in autoklaviertem Boden aus dem Vermehrungsstandort aufgezogen wurden. Bei Anzucht auf autochthonem Boden bei höherer Temperatur als am natürlichen Standort schien das Wachstum von *Trifolium alpinum* P-limitiert zu sein, da die Zufuhr dieses Nährstoffelementes das Pflanzenwachstum erheblich förderte. Es wird geschlussfolgert, dass eine erfolgreiche Kultivierungsstrategie von Trifolium alpinum im Flachland die Vermeidung von Standorten, welche von Pratylenchus sp. potentiell befallen sind, die Anwendung geeigneter Inokula, die autochthonen Mikroorganismen enthalten, und die Wahl von Böden mit ausreichender Phosphorversorgung beeinhalten sollte.

Ein 4-jähriger Feldversuch wurde auf einer nordexponierten Skipiste in den Bayerischen Kalkalpen (Sudelfeld, Oberbayern, 1.250 m ü.N.N.) angelegt, um die Eignung von Saatgutmischungen (mit und ohne alpenländische Arten) und Anlagemethoden für die Renaturierung planierter Skipisten zu untersuchen. Das Versuchsdesign war eine zweifaktorielle Spaltanlage (split plot), in der die Anlagemethoden (ATA:

konventionelle Hydrosaat mit Mineraldüngern, ATB: Mykorrhiza-Hydrosaat mit einer Mykorrhiza-Inokulum und organischer Düngung, ATC: Mykorrhiza-Hydrosaat mit Mykorrhiza-Inokulum, organischer Düngung und Bitumen-Strohdecke) den Großteilstückfaktor, und die Saatgutmischung (SM1: kommerzielle, SM2: autochthone für weitere Nutzung, SM3: autochthone ohne weitere Nutzung, je mit einer Pflegemaßnahmen verbunden) den Kleinteilstückfaktor darstellten. Als kommerzielle Saatgutmischung wurde eine häufig verwendete Standardmischung mit Zuchtsorten von Futterpflanzen des Flachlands gewählt, während die autochthonen Saatgutmischungen vorwiegend alpine und subalpine Ökotypen enthielten. Für den ganzen Versuch wurden als Rahmenbedingungen umweltschonende Maßnahmen geplant und durchgeführt: der Oberboden blieb teilweise erhalten, die Düngung wurde auf eine einzige Gabe bei Anlage des Versuchs beschränkt und es wurde auf eine Erhaltungsdüngung verzichtet. Prioritäten und Kriterien für die Evaluierung der verschiedenen Behandlungen wurden festgelegt. Erosionsschutz wurde als Hauptziel betrachtet und anhand des Vegetationsdeckungsgrades (70%-Grenze) und der Wurzelmasse bewertet. Der Deckungsgrad autochthoner Arten, die Artenzahl, die Vegetationsdynamik und die Fertilität der Einzelarten wurden zur Beurteilung der biotischen Interaktion mit der autochthonen Vegetation und der Sukzession gegen standortgerechte Vegetationstype herangezogen. Die Eignung für eine landwirtschaftliche Nutzung des Futters wurde anhand der Trockenmasse und der potentiellen Futterqualität eingeschätzt.

Meteorologische Parameter wurden mittels einer Wetterstation am Standort detailliert erfasst und zeichneten einen relativ günstigen Verlauf der Temperatur (mindestens fünf Monate Vegetationszeit, relativ hohe monatliche Mitteltemperaturen und extrem seltene Frostereignisse während der Pflanzenwachstumsphase) und der Niederschläge (keine Trockenperiode, seltene Starkregenereignisse) auf. Hohe Windgeschwindigkeiten traten als ungünstiger klimatischer Faktor auf.

Die Kontaktvegetation wurde mittels Vegetationsaufnahmen nach Braun-Blanquet erfasst, wobei fünf Vegetationstypen identifiziert werden konnten. Die am weitesten in der Kontaktvegetation verbreitete Pflanzengesellschaft wurde der Rotschwingel-Kammgrasweide (*Festuco-Cynosuretum*) zugeordnet. Die *Crepis aurea* - hochmontane Form dieser Gesellschaft stellte die Haupteinheit dar, gefolgt von zwei weitere Ausbildungen derselben Pflanzengesellschaft (Ausbildung mit *Carex sempervirens* und Ausbildung mit *Poa trivialis*). Das *Caricetum davallianae* und kleine Waldfläche (*Fagion*-Verband) waren weniger repräsentiert.

Bodenproben aus repräsentativen Bodenprofilen in der Kontaktvegetation und aus der planierten Skipiste wurden vor Anlage des Versuchs auf ihre chemischen Eigenschaften untersucht und zeigten infolge der Planierung erhebliche Verminderungen der Humus- und Nährstoffgehalte, die jedoch dank der Erhaltung des Oberbodens auf niedrigem aber noch akzeptablem Niveau blieben.

Um die natürlich vorkommenden biotischen Ressourcen des Versuchsfeldes zu erfassen, wurden Untersuchungen über das Ausmaß der an der Bodenoberfläche verbliebenen Vegetationsreste, die Bodensamenbank und die vegetative Wiederbesiedlung der Versuchsfläche zwei Monate nach Anlage des Versuchs durchgeführt. Der Deckungsgrad wurde nach Augenmaß geschätzt und betrug durchschnittlich 6%, während die Untersuchung der botanischen Zusammensetzung durchschnittlich eine hohe Artenzahl (33 Arten) ergab. Die Samenbank wurde in einem 12-monatigen Gewächshausversuch an 27 1-dm³-Bodenproben untersucht. Keimlinge von 55 Arten wurden bestimmt und eine durchschnittliche Samendichte von 1.929 Samen/m² wurde in den Bodenproben festgestellt. Darunter waren numerisch vorwiegend Samen von Kräutern. Die Vegetation, die sich vegetativ nach der Anlage des Versuchs verbreitete, wurde zwei Monate nach Versuchsbeginn mit der "quadrat charting method" detailliert kartiert. Die so erfassten Vegetationseinheiten bildeten ungefähr ein Drittel des gesamten Deckungsdgrades der Vegetation und die rasche vegetative Verbreitung von Carex-Arten durch unterirdische Ausläufer wurde festgestellt. Anhand dieser Ergebnisse erscheint die Erhaltung des Oberbodens extrem wichtig für die Renaturierung von Skipisten. Die Vegetationsreste und die aus der vegetativen Wiederbesiedlung resultierenden Pflanzen üben mehrere positive Funktionen aus (direkter Beitrag zum Vegetationsdeckungsgrad, schützende Effekte während der Keimlingsetablierung, Darstellung einer Diasporenquelle). Die Samenbank erwies sich als wichtige Reserve von Verbreitungseinheiten krautartiger Arten, deren Saatgut kommerziell nicht verfügbar oder extrem teuer ist.

Der Vegetationsdeckungsgrad wurde jährlich zur Blütezeit von *Festuca nigrescens* (im Anlagejahr zwei Monate nach Versuchbeginn) an 100 Beobachtungspunkten pro Parzelle mit der "point quadrat method" gemessen, während die Frequenz der einzelnen Arten mittels linearer Analysen an denselben Beobachtungspunkten durchgeführt wurde. Ein Effekt der Anlagemethode war im ersten Jahr vorhanden, und die Mineraldüngung in ATA verursachte höhere Deckungsgrade. Ebenso konnte eine positive Wirkung der Strohdecke auf die Etablierung und die erste Wachstumsphase beobachtet werden. Ab der zweiten Vegetationsperiode waren diese Effekte nicht mehr sichtbar und alle Behandlungen erreichten die 70%-Grenze. Ein Effekt des Faktors Saatgutmischung war ab der dritten Vegetationsperiode vorhanden: für die autochthonen Saatgutmischungen wurden höhere und stabile Deckungsgrade gefunden, während SM1 eine negative Tendenz aufwies und im vierten Jahr im Durchschnitt leicht unter der 70%-Grenze lag. Unter Berücksichtigung des Schutzeffekts von Strohdecke und

Streu kam es zu ähnlichen Ergebnissen, mit Ausnahme der ersten Vegetationsperiode, in der ATC den besten Erosionsschutz ermöglichte. Da der Deckungsgrad vom Stroh im Laufe der Zeit schnell abnahm, war dieser Effekt später nicht mehr gegeben. Die autochthonen Saatgutmischungen erwiesen sich deshalb als besser geeignet für das mittelfristige Erreichen einer stabilen Vegetationsdecke. Befriedigende Deckungsgrade wurden von SM3 mittelfristig erreicht und erhalten, obwohl für diese Mischung keine Mahd und Biomassenabfuhr durchgeführt wurden. SM3 erwies sich deshalb für die Begrünung geeignet, wenn keine weitere landwirtschaftliche Nutzung oder Pflegemaßnahmen vorgesehen sind.

Der Deckungsgrad autochthoner Arten blieb während des ganzen Untersuchungszeitraums in den autochthonen Saatgutmischungen höher, da in diesen größere Prozentanteile von Arten enthalten waren, welche auch in der Kontaktvegetation vorkamen, allerdings wurden die Unterschiede zu SM1 mit der Zeit immer geringer. Die Artenanzahl war nur im dritten Beobachtungsjahr wegen der unterschiedlichen Anzahl von Arten in den Saatgutmischungen signifikant niedriger für SM1. Die Ergebnisse der Frequenzaufnahmen zeigten eine starke Vegetationsdynamik in allen Saatgutmischungen. In SM1 zeigten die Artenverschiebungen eine deutliche Abnahme der anspruchsvollen Futterpflanzen. *Festuca rubra* (dominant) und *Achillea millefolium* waren die einzigen erfolgreichen Arten. In SM2 und SM3 wies die Frequenz von *Festuca nigrescens* einen ähnlichen Verlauf wie *Festuca rubra* auf; auch *Leontodon hispidus* erwies sich als eine recht erfolgreiche Art. *Poa alpina* und alle Leguminosen nahmen allmählich ab, während einige Arten niedrige und mehr oder weniger konstante Werte zeigten. Die Frequenz zahlreicher standorteinheimischer Arten nahm in allen Behandlungen geichmäßig zu und wurde vor der Saatgutmischung nicht beeinflusst. Die gewichteten Mittelwerte der Zeigerwerte nach Ellenberg wurden auf der Basis des Deckungsgrades der Arten errechnet. Deutliche Abnahmen des Stickstoff-Zeigerwertes in allen Varianten deuteten auf einen allmählichen Rückgang von nährstoffliebenden Arten und auf eine Zunahme weniger anspruchsvoller Arten hin. Eine starke Reduzierung des Temperatur-Zeigerwertes fand nur für SM1 statt, während niedrigere und konstante Werte für SM2 und SM3 gefunden wurden. In SM2 und SM3 wurde der Rückgang einiger angesäter Arten durch die Zunahme einheimischer Arten kompensiert und könnte teilweise auch von Konkurrenzeffekten verursacht worden sein. Viele der Arten in diesen Mischungen waren an der unteren Grenze ihrer Höhenverteilung und das Wachstum von Arten aus den Hochlagen wird von klimatischen Verbesserungen weniger begünstigt als dasjenige von Arten, die in niedrigeren Höhenstufen den Schwerpunkt ihrer Höhenverteilung haben. Im Gegensatz dazu fand die Kompensierung in SM1 nicht statt. Der Rückgang der angesäten Arten war wahrscheinlich in erster Linie auf die fehlende Anpassung an die Standortbedingungen

zurückzuführen. Da die Persistenz einiger Arten in den Pflanzenbeständen von ihrer generativen Fortpflanzung abhängt, wurde die Fertilität der angesäten Arten untersucht, indem Zeitpunkt der Samenbildung und Keimfähigkeit der Samen untersucht wurden. Unter den nicht extremen klimatischen Verhältnissen des Versuchsstandortes waren alle untersuchten Arten in der Lage, vor Ende der Vegetationsperiode reife Samen zu bilden.

Für die Saatgutmischungen, bei denen eine weitere landwirtschaftliche Nutzung vorgesehen war, wurden ab der zweiten Vegetationsperiode die Biomassenproduktion und die potentielle Futterqualität mittels der Klapp´schen Wertzahlen ermittelt. Im ersten Erntejahr ergaben sich höhere Mengen an Trockensubstanz von SM1 im Vergleich zu SM2, während in den folgenden Jahren keine signifikante Unterschiede vorhanden waren, da eine Zunahme in SM2 und eine Abnahme in SM1 festgestellt wurden. Weiterhin wurde im ersten Erntejahr eine höhere potentielle Futterqualität von SM1 im Vergleich zu SM2 erreicht, während in den folgenden Erntejahren kein Unterschied vorlag. Insgesamt nahm die Futterqualität parallel zum Rückgang wertvoller Futterpflanzen in beiden Mischungen ab.

Die Ergebnisse der vierjährigen Untersuchung legen nahe, dass die Erhaltung des Oberbodens während des Aufbaus von Skipisten in der montanen Stufe von hochrangiger Bedeutung ist, um wichtige am Standort vorhandene Ressourcen ausnutzen zu können. Unter Berücksichtigung dieser Schlussfolgerung kann eine schnellere Renaturierung erzielt werden. Die Anwendung autochthoner Saatmischungen zeigte positive Effekte. Sie erzielten mittelfristig einen besseren Erosionsschutz und eine Futterproduktion und -qualität, die ähnlich derjenigen der untersuchten kommerziellen Saatmischung waren. Unter der Voraussetzung der Erhaltung des Oberbodens scheint eine Einschränkung des Düngereintrags keine Einbußen beim Erosionsschutz zu verursachen.

Schlagwörter:

Saatgutvermehrung; *Sesleria albicans*; *Trifolium alpinum*; *Festuca nigrescens*; mechanische Unkrautbekämpfung; *Pratylenchus*; *Rhizobium*; Mykorrhiza; Skipisten; Renaturierung; Saatgutmischungen; Erosionsschutz; Erhaltung des Oberbodens; Samenbank; Vegetationsdynamik

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