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## ***EFFECTS OF WOOD ESTABLISHMENT ON PLANT BIODIVERSITY AND HERBAGE PRODUCTION OF MOUNTAIN PASTURES***

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DATA CONSEGNA TESI

2 maggio 2012



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## Riassunto

Negli ultimi sessant'anni, nell'ambiente alpino ed in particolare nelle Alpi italiane, si è assistito ad un importante e senza precedenti cambiamento d'uso del suolo, dovuto all'abbandono delle zone montane. Questo fenomeno ha causato un avanzamento del bosco su prati e pascoli attraverso un processo naturale di riforestazione.

Per questo studio sono stati eseguiti rilievi floristici in otto pascoli montani delle Alpi italiane, soggetti ad avanzamento del bosco. Inoltre, in quattro di questi otto siti sono stati raccolti campioni di foraggio per la determinazione della produttività e della qualità della fitomassa. Infine, in un sito, caratterizzato dalla presenza di habitat a *Nardus stricta*, sono state raccolte carote di terreno per la caratterizzazione della componente ipogea. In tutti i casi i rilievi e i campionamenti sono stati eseguiti a percentuali crescenti di copertura arboreo-arbustiva. Sono stati considerati i seguenti parametri: numero di specie, composizione botanica, produzione e composizione chimica della sostanza secca nonché densità e diametro medio della radici a diversi livelli di profondità nel terreno, in modo da analizzare l'effetto della copertura arboreo-arbustiva su diversità vegetazionale, produzione e qualità del pascolo e stabilità del suolo.

L'effetto sul numero di specie causato dall'avanzamento del bosco è risultato diverso tra i siti a causa delle differenze che intercorrono nei fattori ambientali e nelle attività antropiche. In generale si è assistito ad una diminuzione del numero di specie per effetto dell'avanzamento del bosco, in alcuni siti però è stato osservato un leggero aumento per bassi valori di copertura arboreo-arbustiva. La diminuzione del numero di specie è stata più marcata nei siti ad altitudine inferiore. Usando un modello lineare misto generalizzato, è stato riscontrato che le temperature medie annue sono uno dei fattori che meglio spiegano la diversa relazione tra i siti. L'analisi della composizione floristica mediante l'indice di Bray, ha evidenziato un comportamento simile tra i siti.

Nella maggior parte dei siti analizzati si è riscontrata una diminuzione della produzione in sostanza secca per effetto della copertura arborea. Inoltre, già a partire da basse percentuali di copertura arboreo-arbustiva, è stata osservata una diminuzione della qualità della fitomassa prodotta dal pascolo, per effetto di una diminuzione del contenuto di proteina grezza ed un aumento delle fibre.

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Relativamente alle radici, si è osservato come la densità radicale in lunghezza e il diametro medio diminuissero drasticamente a percentuali di copertura arboreo-arbustiva del 75%, aumentando verosimilmente il rischio di erosione del suolo.

Sulla base di questi risultati possiamo dire che nelle regioni montane, laddove la vocazione produttiva (in termini di produzione casearia) è poco rilevante, andrebbe incoraggiato il mantenimento di questi habitat a vantaggio della biodiversità e della stabilità del suolo. Come visto in questo studio, l'effetto della temperatura media annua sulla diminuzione del numero di specie, suggerisce che il rischio di perdita di biodiversità aumenta ad altitudine minore e in aree esposte a sud. Il mantenimento di basse percentuali di alberi o arbusti può essere utile per conservare il valore naturalistico di questo habitat. Al contrario, nelle zone dove la vocazione casearia del pascolo è prevalente, la gestione dovrebbe essere tale da mantenere le superfici a pascolo completamente libere da alberi e arbusti.

## Summary

In the past sixty years, the Southern Alps have undergone a tremendous and likely unprecedented change in land-use due to land abandonment in mountain regions. This phenomenon causes a turn of mountain grasslands to forests through the process of natural succession.

Vegetation relevés in eight pastures under forest succession in the Italian Alps were collected at different percentage of wood cover. Moreover, in four of the eight sites, herbage samples were collected at different wood cover levels. In addition, in one site (characterised by *Nardus* grassland habitat) core samples were collected in order to study root characteristics. Species richness, botanical composition, dry matter production and contents, and root characteristics were taken into account in order to analyze the effect of wood cover on plant diversity, herbage yield and quality, and soil stability.

The effect on specie richness due to this process is different among sites because of differences in environmental factors and human activities. In general species richness decreased with increasing wood cover, and the reduction was more relevant in sites at low altitude. In some sites the effect of reforestation on plant species richness showed a slight increase at low

percentage of wood cover followed by a gradual decrease, while in the others the effect displayed a monotonic decrease. Modelling with a generalized linear mixed model suggested that mean annual temperature was the primary determinant of the functional relationship. Differences among sites were not found when botanical composition, and in particular Bray dissimilarity index, was taken into account.

A reduction of dry matter yield was noted in most of the studied sites. Forest succession affected herbage quality decreasing it starting from low percentage of wood cover because of changes in crude protein and fibrous contents.

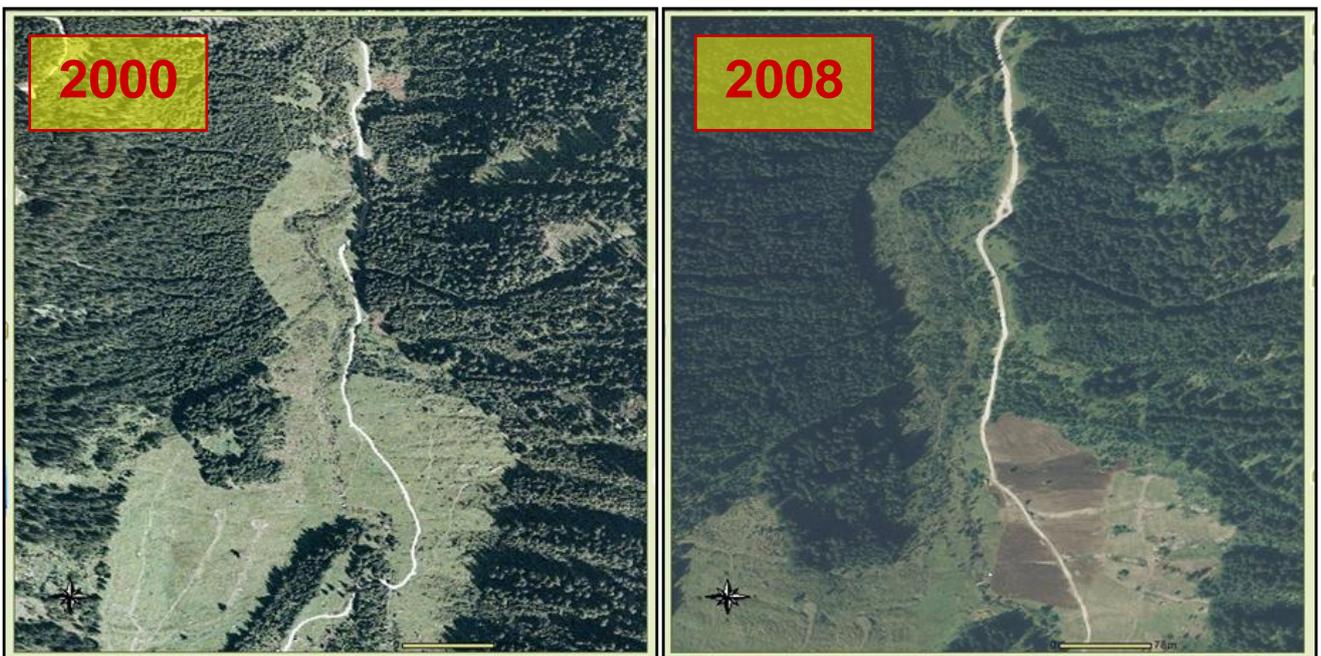
When root characteristics were analyzed, root length density and average diameter declined at 75% of wood cover increasing the risk of soil erosion.

Based on this results, mountain areas with low relevance for dairy production grazing should be encouraged for preserving both biodiversity and the stability of habitat. The strong effect of temperature on the loss of species richness suggests that the risk increases with lower altitude and stronger exposition towards South. The maintenance of low percentage of wood cover is useful for the habitat ecological value. On the contrary, in mountain areas where dairy production grazing is relevant, the management should be assessed for excluding wood establishment on the grazing surface.

## **Chapter I**

### **General introduction**

From the middle of last century, the Southern Alps have undergone a tremendous and likely unprecedented change in land use. Since 1960, about 800,000 hectares of grassland have been abandoned throughout the Italian Alpine arch, which correspond to 45% of the surface covered by pastures and meadows (Chemini and Gianelle, 1999; Bovolenta, 2004). As a consequence of land abandonment processes, mountain regions have been experiencing radical landscape changes, as once cultivated areas are turning to forests through the process of natural succession (Conti and Fagarazzi, 2006).



**Figure I.1.** Land-use changes on an Alpine pasture (Sadole summer farm, Trento, Italy) due to wood establishment on grazing surface. Comparison between 2000 and 2008 orthophoto.

The reduction and cessation of cutting or grazing favor the establishment of persistent, competitive species, mostly represented by woody shrubs or trees. In pastures, woody species establish first in close association with plants or vegetation patches that facilitate their establishment (Uytvanck, 2008). These associations can either be along forest edges or around forest patches, while individual trees establish within the grassland. The process of reforestation is non-linear in time. After the cessation of grazing, a transition phase of 4–11 years occurs in which few woody species establish in a scattered pattern across the entire originally grazed surface. A stable woody vegetation establishes only after 10–20 years from the grazing cessation (Finagan, 1984). However, it is known that on productive and nutrient

rich sites (such as pastures), it takes 30–45 years before woody species on former open vegetation types become dominant (Smit and Olff, 1998). The speed of establishment of new woodlands in grasslands is strongly influenced by vegetation structure of both habitats (Uytvanck, 2008). It depends on the proximity of seed sources, on density of wood cover, and on the abundance of perennials species that may cause competition with woody species (Finagan, 1984). The speed of wood establishment in grassland also depends on dominant woody species, in fact, every woody species has a specific response to environmental factors (Van Gils et al., 2008; De Gasperis and Motzkin, 2007; Tasser and Tappeiner, 2002). Van Gils et al. (2008) found that, at local scale, the establishment of *Fagus sylvatica* on abandoned farmland depends on the distance from seed sources and on the presence of protective forest or shrubby shade but not on the geological substrate. Moreover it depends on graze intensity or wind exposure of saplings. Del Favero et al. (1998), instead, observed that *Fagus sylvatica* established at different speed on various substrates because of the competition with other shrubby and woody species. Whereas grassland management is maintained, forest establishment is strongly affected by grazing (Uytvanck, 2008). Forest management influences the natural competition between different woody species (Del Favero, 1995).



**Figure I.2.** Natural forest succession process on an Alpine pasture (Marcesina di Sopra summer farm, Vicenza, Italy). Changes at landscape and pasture level.

Several studies reported a different influence of natural reforestation on pastures species richness and vegetational composition (see Anthelme et Al., 2001; Fischer and Wipf, 2002; Kesting, 2009). Authors analyzed forest succession as change on botanical composition and number of species. Some of those showed a reduction on species richness as consequence of reforestation, however the magnitude of species loss varied depending on local environmental conditions. In fact, most of those studies investigate the phenomenon at local scale including only the dominant woody species in the study area.

In the present study we investigated the effects of reforestation on plant composition on eight sites in the Southern Italian Alps. All the selected sites were not permanently inhabited and grazed by cows from May or June (depending on the altitude and annual weather conditions) to September. In all sites, grazing persisted up to now, but it was not sufficient to prevent the natural forest succession. This should be explained by grazing management practices such as the high use of concentrates in cow diet, or the release of saplings on less grazed surfaces due to time constraints.

In particular, the aim of the study was to investigate the effect of wood establishment on Alpine pastures, giving a global view on the pasture ecosystem under forest succession. We took into account changes on biodiversity, analyzing species richness and effective diversity, changes on pasture botanical composition. Furthermore we focused on the effects of wood establishment on herbage production and quality. In order to provide more information of Alpine pasture ecosystem under forest succession we investigated changes of root characteristics and their effect on soil stability. The four insights are reported in four different chapters as summarized below.

## **Chapter 2 - Plant species loss due to forest succession in Alpine pastures depends on site condition and observation scale**

Changes in land use of Alpine grassland influence biodiversity reducing the number of species (see for example Dirnböck et al., 2003; McDonald et al., 2000). Some studies underlined a slightly linear decrease on species richness in consequence of the increase of wood cover (for example Anthelme et al., 2001). Differently, Kesting (2009) demonstrated that woody cover, at low percentage, is favorable to species richness.

Variation in plant community composition was the result of several factors related to the competitive ability of individual plant in a given environment. The first investigated hypotheses of this chapter was that plant species richness decreases with the increase of wood cover is the consequence of competitive exclusion, because tree and shrub establishment leads to a decrease of essential elements in open ground habitats, and environmental heterogeneity reduction.

The second hypothesis was that the effect of wood cover on plant species richness depends on the environmental site conditions. These environmental conditions have direct effects on the existing grassland vegetation before abandonment as well as on the dynamics occurring thereafter (e.g. the wood species becoming dominant). The last hypothesis to be tested was that the observed patterns are not an artifact of changing species-area relationship with increasing wood cover, i.e. that reforestation would increase or decrease heterogeneity in species composition and thus make any results based on equal-area sampling questionable.



**Figure I.3.** Landscape view of pastures on Asiago highland (Vicenza, Italy)

### **Chapter 3 - Plant composition changes due to forest succession in eight Alpine pastures**

As a consequence of land abandonment processes and grasslands turning to forests through the process of natural succession, the grassland and the typical species rich vegetation disappears (Dierschke, 2006; Galvanek & Leps, 2008) under competition with grasses and forbs for light, water and nutrients (Kesting, 2009).

The investigated hypotheses was that botanical composition changes with the increase of wood cover due to competitive exclusion because the establishment of trees and shrubs leads to a change on specie richness, affecting primarily the low abundance species.

#### **Chapter 4 – Effect of forest succession on herbage quantity and quality of Alpine pasture**

There are few information available concerning the effects of wood establishment on herbage quality of pastures (Kesting, 2009) analyzed the effect of shrubs establishment on a calcareous grassland on Swiss Alps, and found an increase of herbage quality. Zweifel-Schielly et al. (2011) analyzed the differences on forage quality between forested slopes and farmed meadows on the Swiss Alps and they found significant differences on forage contents. These differences could be due to the tree shadow effect or to differences in botanical composition due to forest succession. Both herbage yield and quality are strongly affected by shade environment. However, the influence of shade on herbage chemical composition depends on single species.



**Figure I.4.** Exclusion cage used for collecting herbage samples from pastures.

In general, crude protein increases in most shade-grown forages, however, shade had less effect on crude protein of legumes than of grasses, moreover, under low light intensity, the internode length and leaf area increase while specific leaf dry weight decreases (Lin et al., 2001). Kephart et al. (1992) compared forage contents of C3 and C4 forage species under different percentage of sunlight reduction. Forage contents were affected by C3 and C4 species but not by shade. Our hypothesis was that herbage yield and quality could be significantly affected by

forest succession on Alpine pasture. However, understanding variations in forage quality in a complex habitats as a pasture evolving on forest succession is very difficult because it is the consequence of multiple factors, the combination of several factors that play together such as soil conditions, climate, grazing management and botanical composition.

### **Chapter 5 - Roots characteristics and botanical composition changes through *Rhododendron ferrugineum* succession on a Alpine pasture**

Grasslands are increasingly being considered valuable not only for livestock production but also for their socio-economic function, the regulation of physical and chemical fluxes in ecosystems, mitigation of pollution and maintenance of landscapes (Gibon, 2005; Lemaire et al., 2005). The effects of land use change may influence soil stability. Land cover, and therefore land use also play an important role on soil formation and erosion (Wischmeier and Smith, 1978). The importance of vegetation cover, including the belowground part, in reducing soil erosion by water has been widely studied (De Baets et al., 2006; Klima, 2007), however no information are available regarding the impact of grazing abandonment on pasture root system. The purpose of this study is to contribute to filling knowledge on the effects of shrubby establishment on root traits of the pasture plant community by studying changes in the main root characteristics of a mountain pasture under different levels of natural regrowth of shrubs in the Italian Alps.



**Figure I.5.** Core samples collector for analyzing root characteristics.



## **Chapter II**

**Plant species loss due to forest succession in Alpine pastures depends on site condition and observation scale**

## **II.1. Introduction**

Changes on land use of grassland on Alpine region influence biodiversity decreasing number of species (see for example Dirnböck et al., 2003; McDonald et al., 2000). Some studies underlines a slightly linear decrease on specie richness (for example Anthelme et al., 2001). Differently, Kesting (2009) suggest that woody cover is favorable to specie richness at lower percentage.

In our study we address three basic questions. The first asks how plant species richness is affected by natural forest succession. Our hypothesis is that a small number of established woody species outcompetes a comparatively larger number of shade-sensitive grassland species and hence species richness decreases with forest succession.

The second question is on how the effect of wood cover on plant species richness depends on the environmental site conditions. We hypothesize that these environmental conditions have direct effects on the grassland vegetation present before abandonment as well as on the dynamics occurring thereafter (e.g. the wood species becoming dominant). In order to test this second hypothesis we use generalized linear mixed models (GLMM). In extension to standard linear models, GLMM allow to analyze effects of environmental site descriptors in a hierarchical manner.

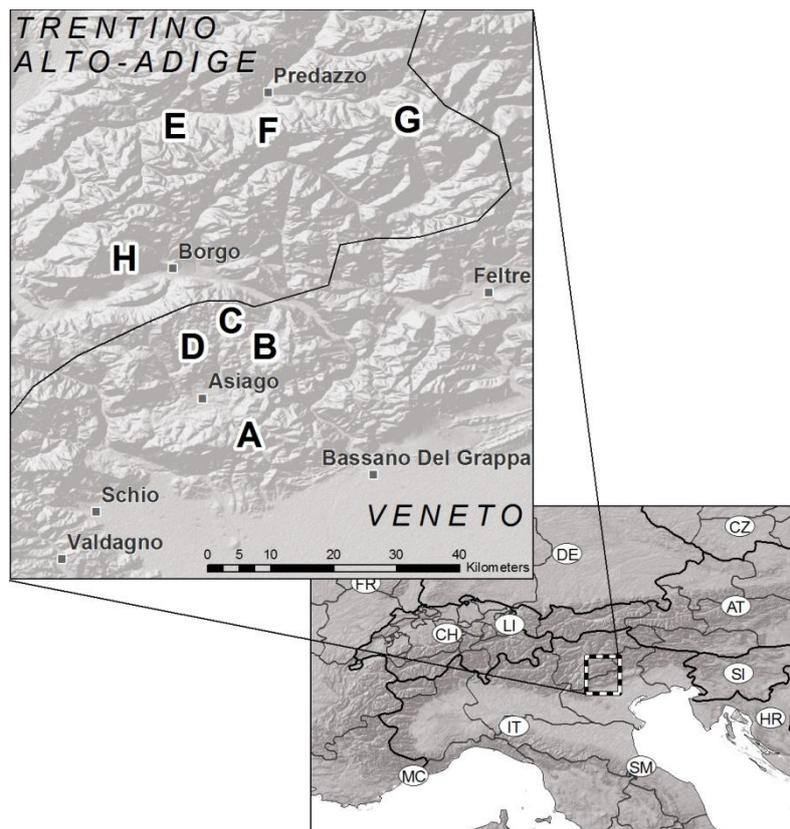
The last question to address is whether the observed patterns were not an artifact of changing species-area relationship with increasing wood cover. We therefore investigate to what extent forest succession alters heterogeneity in species composition and thus make any results based on equal-area sampling questionable.

## **II.2. Materials and Methods**

### **II.2.1. Survey sites**

All investigations were carried out on eight sites located in the Eastern Alps of Italy, between 2006 and 2010 (Figure II.1). Each site was dominated by a distinct woody species. The sites were selected in order that the primary establishing woody species in the region were present.

These species were *Fagus sylvatica* L., *Alnus viridis* (Chaix) DC. (with *Frangula alnus* Miller and *Berberis vulgaris* L.), *Picea excelsa* (Lam.) Link (on calcareous and silicate substrate), *Larix decidua* Miller (on calcareous and silicate substrate), *Pinus mugo* Turra, *Rhododendron ferrugineo* L. (Table II.1). In order to identify the different stages of woody species establishment, we used orthophotos and so-called forest settlement plans developed for the forest management. These plans delineate forest, agricultural and grasslands based on surveys of past management. For the purpose of this study, we considered areas delineated as forest outside the pasture lot to be totally established woodland. Inside the area delineated as pasture land, we identified different stages of forest succession based on the analysis of orthophotos. The resulting map was used in the field to identify sampling locations with different percentage of wood cover.



**Figure II.1.** Eight study sites located in the Eastern Alps of Italy

**Table II.1.** Main characteristic and environmental factors of the eight study sites.

ID site	A	B	C	D	E	F	G	H
Dominant wood species	Fagus sylvatica	Picea abies	Larix decidua	Pinus mugo	Alnus viridis	Picea abies	Larix decidua	Rodhodendron ferrugineum
Substrate	carbonate	carbonate	carbonate	carbonate	silicate	silicate	silicate	Silicate
X coordinates	5078100	5092270	5096180	5091965	5127085	5126725	5128975	5105810
Y coordinates	699850	701070	697195	693095	690120	700305	715490	685140
Altitude [m a.s.l.]	1150	1350	1700	1650	1050	1550	1750	1700
Annual mean temperature [°C]	6.68	5.38	3.66	3.48	6.75	4.00	3.51	4.36
Annual precipitation [mm]	1463	1546	1546	1546	800	1050	1050	917
pH	3.63	5.16	5.08	6.55	4.83	4.44	5.96	3.82
N content [g kg <sup>-1</sup> ]	2.6	6.9	14.3	5.4	4.9	5.3	7.3	7.8
Organic matter [g kg <sup>-1</sup> ]	128.6	212.2	309.5	295.0	156.7	148.7	245.5	226.0
Gamma diversity [N° species]	190	147	128	81	141	128	116	106

All study sites are still partly communally grazed by livestock during the summer. Their vegetation is heterogeneously structured, with grazed pastures without any woody species, sparse naturally established shrubland and completely established woodland.

The climate on all sites is temperate with mean annual precipitation between 800 mm and 1550 mm and a mean annual temperature between 4.0°C and 6.8°C (Table II.1). The rainfall shows a pronounced interannual pattern with May and November being the periods where most of the rainfall occurs. Four sites are situated on calcareous substrate and four sites are situated on siliceous substrate. Additional site characteristics are reported in Table II.1.

## II.2.2. Species sampling

At each study site, three different sampling methods were applied:

### *A. Small-area sampling along transects (1m<sup>2</sup>)*

At each site, six transects were established perpendicular to the edge of the forest, in order to have different stages of wood establishment evolution. Each transect started in a area pure grassland without woody species and finished in a completely established woodland. The lengths of the transects were different between sites depending on the wood type. Along the transect line, five quadrates of 1 m<sup>2</sup> were placed in order to represent one of five wood cover classes: 0%, 25%, 50%, 75%, 100%. In each quadrate all species present were recorded.

### *B. Large-area sampling (100m<sup>2</sup>) in wood-cover strata*

Along transects, it was frequently impossible to find areas of suitable size with a homogenous wood cover. Therefore, 30 additional squares of 10 x 10 m were established at each site. In a first step, five strata with different stages of shrubs and trees were delineated using orthophotos. In each strata, six locations were assigned randomly. However, the exact locations had to be frequently refined directly in the field because the orthophoto provided insufficient information to assess the real state of reforestation. It was taken care that the selected locations had homogenous wood cover, well distributed between 0 to 100 %. In this way we analyzed all the stages on woody species establishment evolution. For each plot, a complete floristic survey was carried out recording all herbaceous species and their relative abundance values were visually assessed. Besides, total wood and shrubby cover values were estimated using a 0 - 100 scale for each of the two layers. For every plot, the abundance of all vascular plants were recorded three time per year during two years. Prior to analysis the abundance of all 6 surveys was averaged.

### *C. Nested areas sampling*

Despite the difficulty of finding homogenous wood cover across larger areas a series of nested plots of 1, 4, 10, 25, 50 and 100 m<sup>2</sup> were established along three carefully selected transects in each site. In each nested plot, the presence of all vascular plants was recorded. The location of

each quadrat was chosen to have the same wood cover classes used on 1m<sup>2</sup> samples; taking care to have homogenous wood cover within quadrats.

### II.2.3. Additional plot and site properties

In order to validate wood cover estimates, PAR measurements were carried out using a Li-Cor PAR meter (Li-Cor Inc., 1996). In each small-area plot, readings were taken at 8 different positions. The average reduction of PAR was highly correlated ( $R^2 = -0.95$ ) with estimated wood cover, indicating that cover estimates are a reliable surrogate for shading. Therefore cover estimates were used in all subsequent analyses.

In small area plot, soil was sampled at two depths: depth 1 from 0 to 5 cm, depth 2 from 5 to 20 cm. Soil pH (1:5 soil:water solution), N content (Kjeldahl total nitrogen [g kg<sup>-1</sup>]) and soil organic matter (Springer–Klee method [g kg<sup>-1</sup>]) were measured using the Italian standard soil analysis techniques (G.U., 1999). For this study only samples collected at 0% of wood cover were used. They were averaged and used to indicate potential soil characteristic of each site.

For each large-area plot, slope and a wetness index were calculated using SAGA GIS 2.0.3 based on a 20m dtm (Ministero dell'ambiente, 2011). For slope the algorithm by Zevenbergen and Thorne (1987) was used, the wetness index calculation was based on the  $D_{\infty}$  algorithm (Böhner et al. 2001).

Mean annual temperature at each site was derived from the high-resolution alpine temperature interpolation by Hiebl et al. (2009). The interpolated mean monthly temperature were summed. Mean annual precipitation was derived from the climate station nearest to each site.

### II.2.4. Statistical analysis

Number of species ( $S$ ) was analyzed for the data from all three sampling schemes in order to describe specific biodiversity.  $S$  was calculated summing all species recorded into every single plot.

Based on the data of the 100m<sup>2</sup> samples, the influence of species rarity on the response of species diversity to forest succession was investigated. This was done by calculating effective species numbers  $D$  (Hill, 1973, Jost, 2007) of orders  $q=1$  and  $q=2$  as

$${}^qD = \left( \sum_{i=1}^n p_i^q \right)^{1/(1-q)} \quad (1),$$

where  $p_i$  is the relative abundance of species  $i$  out of  $n$  species (Jost, 2007). The order  $q$  determines a diversity measure's sensitivity to rare or common species (Keylock 2005) and increasing the order  $q$  means a successive downweighing of the species with low abundance. Effective species numbers of order  $q=1$  and  $q=2$  are equivalent to the exp(Shannon) and 1/Simpson respectively, but still have the properties of being Poisson-distributed counts. Calculations of effective species numbers (but not species richness) were restricted to herbaceous species only in order to avoid that the higher abundance of dominant arboreal or shrubby species systematically affects results at higher levels of forest succession.

Generalized linear mixed models (GLMM) were built to explain observed variation in species diversity (richness and effective species numbers) depending on wood cover and environmental conditions. GLMMs have the advantage of allowing the incorporation of random terms that control for non-independence in the data, arising from grouped observations (Pinheiro and Bates, 2000). Separate GLMMs were built for both sampling datasets with site as the . Wood cover was centered in order to have a more stable model. A full GLMM including all measured factors was reduced by subsequent likelihood ratio tests. The evaluated environmental descriptors were soil mean annual temperature, pH, soil organic matter content, soil nitrogen content, rainfall, altitude, slope and wetness index.

The effects of wood cover on the species-area relationship were analysed by fitting a number of non-linear models suggested in the review of Tjørve, 2003. The most promising were

the exponential model 
$$S = a \times A^b \quad (2)$$

the Monod or Clench model 
$$S = a \times (A/(b + A)) \quad (3)$$

and the cumulative Weibull model 
$$S = a \times (1 - e^{(-b \times A^c)}) \quad (4)$$

where  $a$ ,  $b$  and  $c$  are coefficients and  $A$  is the area.

In the models, the parameters have a biological interpretation. Parameter  $a$  is the number of species at very small areas in the exponential model and the asymptote of species richness in the cumulative Weibull model. Parameter  $b$  describes the increase of species richness with area in both models. Parameter  $c$  is an additional factor of modulation of the richness increase in the cumulative Weibull model.

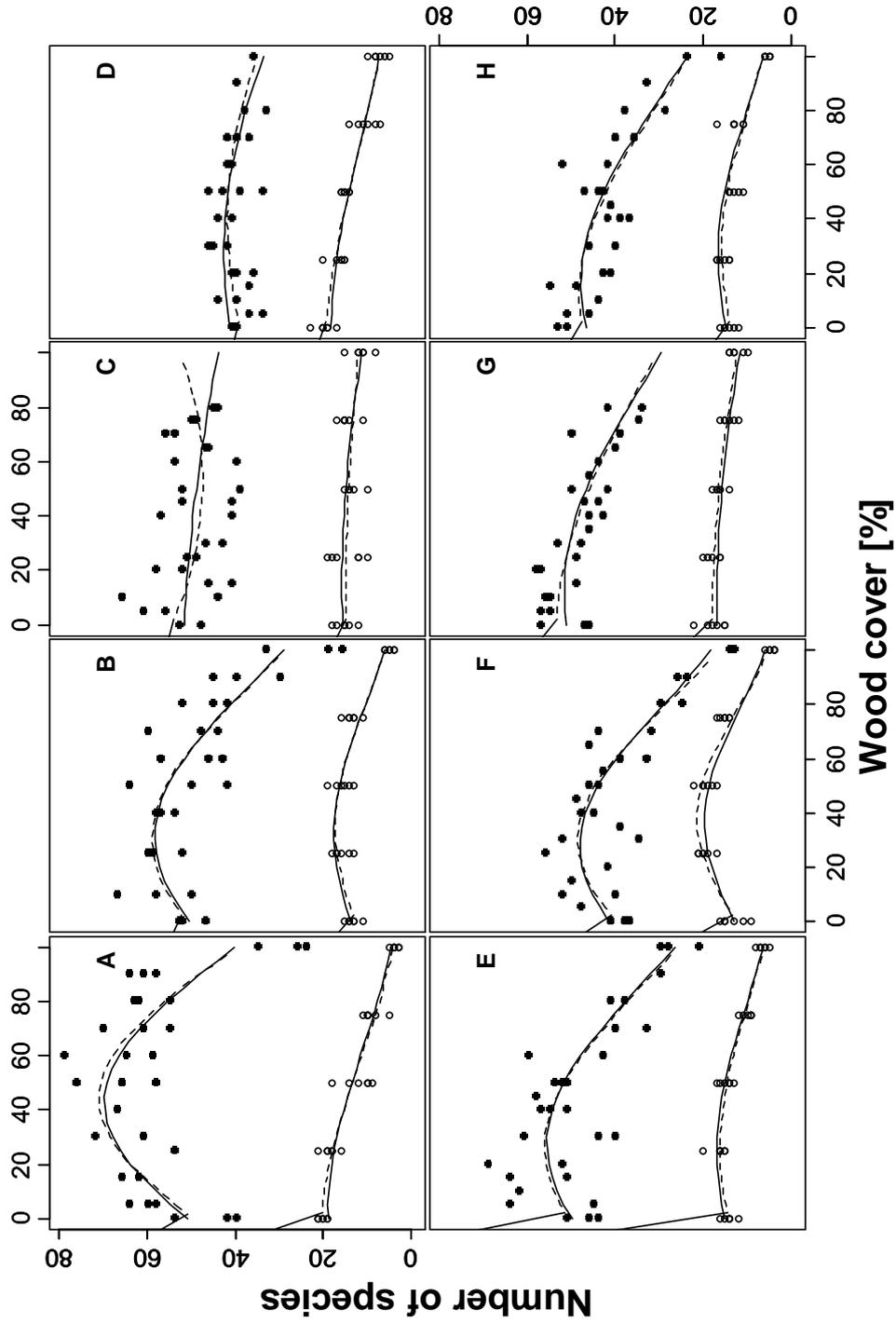
The fitting was performed as a non-linear mixed model with transect and site as nested random variables. Effects of wood cover on each parameter were assessed by including wood cover and its square as fixed effects (Pinheiro and Bates, 2000). Significances were determined by subsequent likelihood-ratio tests of reduced against full models. All calculations were performed in R 2.13.1 (R Core Team, 2011) using libraries *vegan*, *nlme* and *lme4*.

## II.3. Results

### II.3.1. Temperature had a significant influence on the effect of reforestation on species richness

The total number of 177 vascular plant were found in the 1 m<sup>2</sup> samples. 155 herbaceous species, 6 shrubby and 16 arboreal (we used *Nanophanerophita* life-form for shrubby species and *Phanerophita* life-form for arboreal species; following Pignatti,1982). The mean species richness per 1m<sup>2</sup> plot ranged from 2 to 23 (Figure II.2). The total number of plant species in the 100m<sup>2</sup> samples in the 8 investigated sites, was 348, of which 324 were herbaceous, 7 shrubby and 16 arboreal. The mean species richness ranged from 12 to 57 per 100 m<sup>2</sup>. The relationship between wood cover and the number of species was hump-shaped and negative overall (Figure II.2). The hump was well captured by including the square term of wood cover, which was significant at both sampling scales (LRT:  $P_{\chi^2}= 0.0018$  for the 1m<sup>2</sup> samples and  $P_{\chi^2}= 0.0004$  for the 100m<sup>2</sup> samples).

There were marked differences at the site level (Figure II.2). Differences between sites were found both in 1m<sup>2</sup> and 100m<sup>2</sup> samples, but the variation between sites was more pronounced than between scales. In some sites, the maximum number of species was found at intermediate, in others, at low levels of wood cover. In sites A, D, E and G, the relationship between species richness and wood cover depended on the sampling scale (1 m<sup>2</sup> or 100 m<sup>2</sup>). While in some sites ( as B, F and H) relationship down for 1m<sup>2</sup> sampling reflected relationship down for 100m<sup>2</sup> samples, with the higher number of species at intermediate wood cover in both scale. Site C had a linear slightly decrease of species richness at both scale levels.



**Figure II.2.** Effect of wood cover on number of species for the 8 sites. Dashed lines are GLM regressions and solid lines are GLMM regressions. Empty symbols represent 1 m<sup>2</sup> samples dataset, while full symbols represent 100 m<sup>2</sup> samples dataset.

Including covariates into the GLMM showed that a number of environmental variables have significant influence on species richness alone or on the effects of wood cover on richness (Table II.2). The most important environmental factors were mean annual temperature (1m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.001; 100m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.01), soil pH (1m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.001; 100m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = n.s.) as well as organic matter (1m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.01; 100m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.001) and N content of the soil (1m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.001; 100m<sup>2</sup> sampling LRT:  $P_{\chi^2}$ = 0.01). These four covariates had significant effects on richness alone as well as on the effect of wood cover. Precipitation, altitude, slope and wetness index had no significant effects on species richness at both scales.

**Table II.2.** Results of generalized linear mixed models 1m<sup>2</sup> and 100m<sup>2</sup> datasets with the explanatory variables of plant species richness.

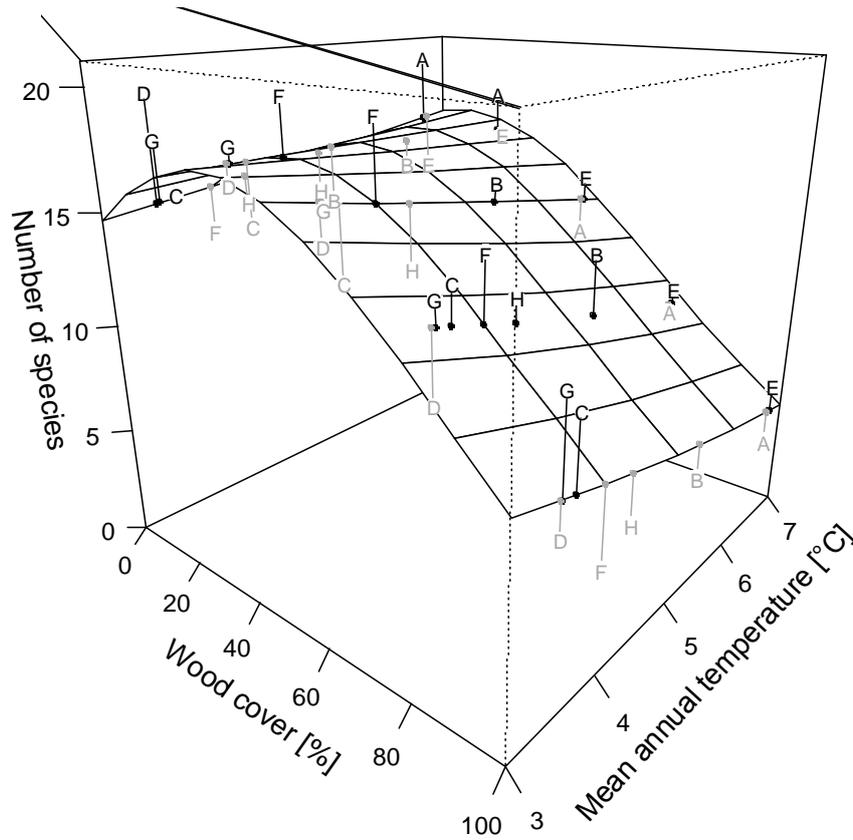
	1m <sup>2</sup> sampling		100m <sup>2</sup> sampling	
	Estimate	$P_{\chi^2}$	Estimate	$P_{\chi^2}$
Intercept	2.86		3.64	
cover	-1.95	<0.001	-1.71	<0.001
cover <sup>2</sup>	-4.04	<0.001	-3.53	<0.001
mean annual temperature	-0.08	<0.001	0.08	<0.01
pH	0.09	<0.001	-	n.s.
organic matter	-0.04	<0.01	-0.01	<0.001
nitrogen content	0.25	<0.001	-0.05	<0.01
rain	-	n.s.	0.35	<0.001
slope	-	-	-	n.s.
swi	-	-	-	n.s.
altitude	-	n.s.	-	n.s.
cover x mean annual temperature	-0.11	n.s.	0.09	n.s.
cover x pH	0.28	<0.01	-	n.s.
cover x organic matter	-0.06	<0.01	0.05	<0.001
cover x nitrogen content	1.45	<0.001	-0.53	<0.01
cover x rain	-	n.s.	0.52	<0.001
cover <sup>2</sup> x mean annual temperature	-	n.s.	0.10	n.s.
cover <sup>2</sup> x organic matter	0.12	<0.01	0.11	<0.01

Subsequent model reduction showed that the most parsimonious GLMM (i.e. having the lowest AIC) was a model with cover, its square and mean annual temperature as fixed effects (Table II.3). In this model, mean annual temperature serves as the only explanatory site variable along which the regression curves from Figure II.2 are placed. This can be visualized as a surface plot (Figure II.3).

**Table II.3.** Results of generalized linear mixed models for number of species with wood cover and mean annual temperature as fixed effects for 1m<sup>2</sup> and 100m<sup>2</sup> datasets.

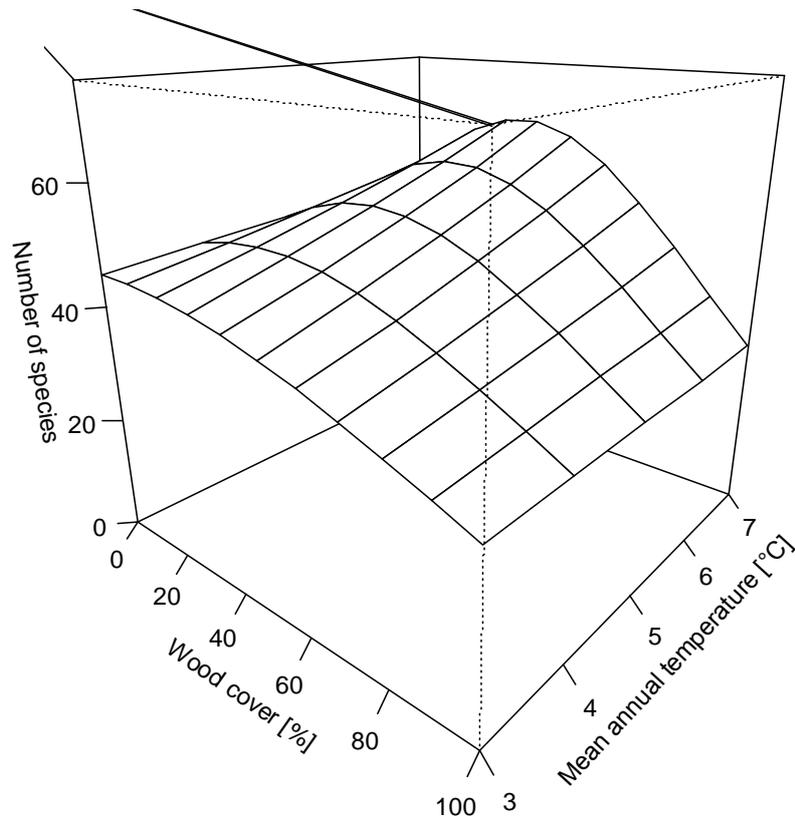
	1m <sup>2</sup> sampling			100m <sup>2</sup> sampling		
	Df	Estimate	P $\chi^2$	Df	Estimate	P $\chi^2$
Intercept		2.71			3.94	
cover	5	-0.85	<0.001	6	-0.48	<0.001
cover <sup>2</sup>	3	-1.45	<0.001	4	-1.27	<0.001
mean annual temperature	2	0.06	<0.001	3	0.10	<0.05
cover x mean annual temperature	1	-0.19	<0.001	2	0.01	n.s.
cover <sup>2</sup> x mean annual temperature		-	-	1	-0.26	<0.05

At the 1m<sup>2</sup> scale, the effect of wood cover on species richness was more linear at higher mean annual temperature, while at lower temperature the effect of wood cover had a maximum value at intermediate percentage. For higher temperature this initial increase on number of species was lower. The mean annual temperature and the wood cover had a significant effect on species richness (both with P  $\chi^2$ <0.001). The interaction between wood cover and mean annual temperature was also significant (P  $\chi^2$ <0.001). Figure II.3 illustrates this interaction. At 0% wood cover, the model predicts that mean annual temperature increases the number of species from 15 to 17, while at 100% wood cover it predicts a decrease from 9 to 5 species. Due to this effect, the reduction of species richness by forest succession is higher at higher mean annual temperature.

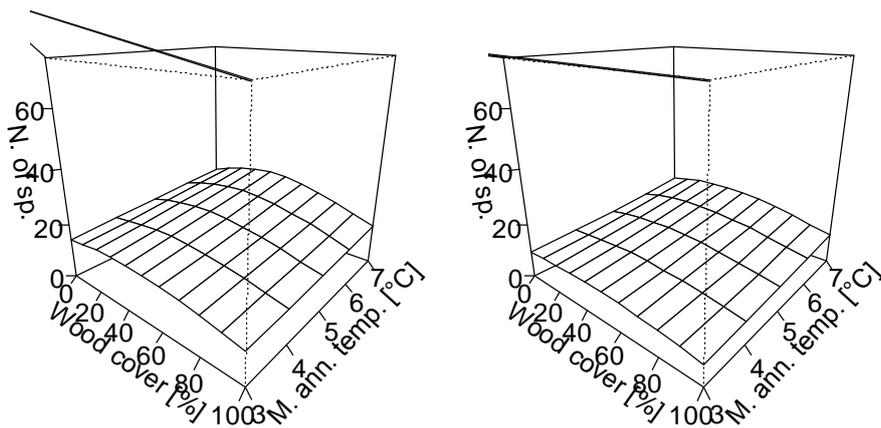


**Figure II.3.** Effects of wood cover on species richness of 1m<sup>2</sup> sampling, depending on mean annual temperature of sites: surface resulted from generalized linear mixed model. Letters are mean point of the five areas for each wood cover level for each site.

At 100m<sup>2</sup> scale, we found again that the effect of wood cover and its squared in species richness were significant (both  $P_{\chi^2} < 0.001$ ) (Table II.3). At lowest mean annual temperature the surface had a slightly influence depending on wood cover, while at higher temperature the number of species had a maximum value corresponding to intermediate wood cover (with a maximum value of 64 species) and a decrease from intermediate wood cover to 100% of wood cover (Figure II.4). We found also a significant effect of mean annual temperature ( $P_{\chi^2} < 0.05$ ) and a significant interaction between square of wood cover and temperature ( $P_{\chi^2} < 0.05$ ). It means that the wood cover and the model change significantly at different value of mean annual temperature. At 0% of wood cover, mean annual temperature had an increasing effect on number of species from 46 to 51, while at 100% wood cover this effect was null with 30 species for all temperature values.



**Figure II.4.** Effects of wood cover on species richness of 100m<sup>2</sup> sampling, depending on mean annual temperature of sites: surface resulted from generalized linear mixed model



**Figure II.5.** Effect of wood cover on effective diversity of order  $q=1$  and  $q=2$  of 100m<sup>2</sup> sampling, depending on mean annual temperature of sites: surface resulted from generalized linear mixed model

### II.3.2. Temperature effect depends on the weighting of species with low abundance

Differences in model surfaces between the two sampling methods can be explained with lack of the sampling of species with low abundance, which are more likely detected at 100m<sup>2</sup> than at 1 m<sup>2</sup>. This difference on sampling method was also underlined with the interaction between cover squared and mean annual temperature. This interaction was in fact significant in the 100 m<sup>2</sup> sampling GLMM, but not in the 1m<sup>2</sup> sampling GLMM. In fact, 1m<sup>2</sup> sampling species count hypothetically excluded the species with low abundance; species that we easily found on 100 m<sup>2</sup> sampling count. In order to test this point, GLMMs were fitted to effective diversities of orders q=1 and q=2. The GLMM to effective diversity of order q=1 identified no significant interaction between cover and mean annual temperature. Furthermore the mean annual temperature was not significant (Table II.4). The wood cover and its square value remained highly significant in this model. Figure II.5 shows that number of dominant species was higher at lowest and highest wood cover percentage than at intermediate wood cover. In fact the predicted effective diversity of order 1 value was between 14 and 20 at 0% wood cover and between 11 and 15 at 100% wood cover, while it was between 16 and 25 at 50% wood cover. The GLMM to effective diversity of order q=2 was similar to the results for effective diversity of order q=1. Mean annual temperature was not significant, while wood cover and its squared were highly significant (both  $P_{\chi^2} < 0.001$ )

**Table II.4.** Results of generalized linear mixed models for effective diversity of order q=1 and q=2 with wood cover and mean annual temperature as fixed effects for 1m<sup>2</sup> and 100m<sup>2</sup> datasets.

	Effective diversity (q=1)			Effective diversity (q=2)		
	Df	Estimate	P $\chi^2$	Df	Estimate	P $\chi^2$
Intercept		3.01			2.64	
cover	7	-0.28	<0.001	7	-0.27	<0.001
cover <sup>2</sup>	4	-1.26	<0.001	4	-1.18	<0.001
mean annual temperature	1	0.10	n.s.	1	0.13	n.s.

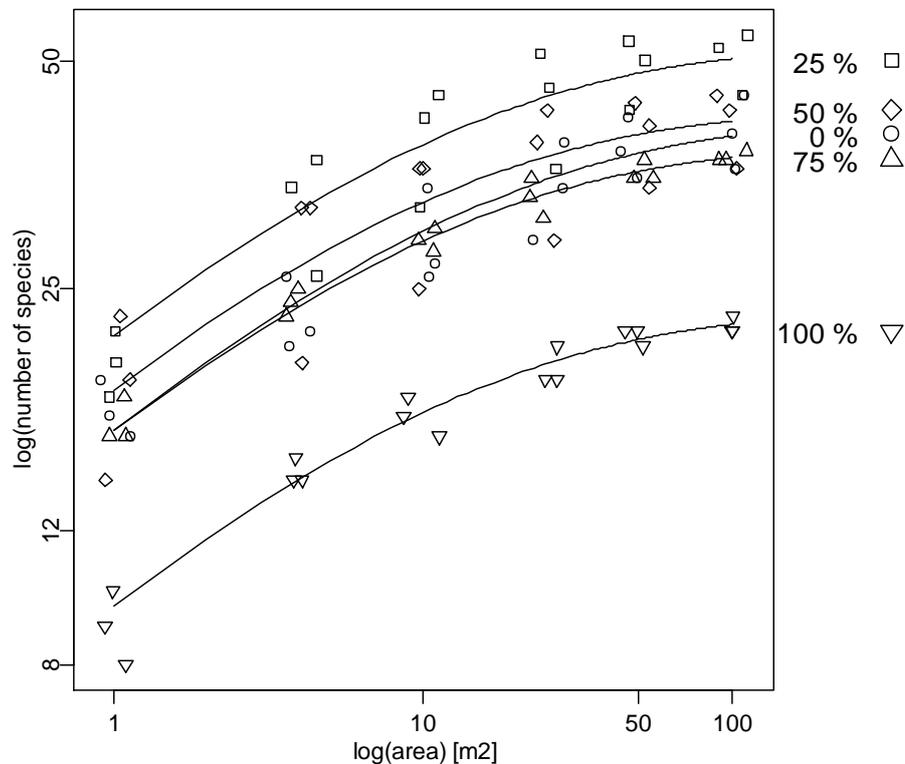
The flattening of effective diversity passing from  $q=0$  (species richness) and  $q=1$  was due to a loss on species with low abundance. At lowest mean annual temperature the loss on species with low abundance is less pronounced than at higher temperature and the relationship against wood cover is similar for the two orders. Moreover this loss on species with low abundance is more evident for highest mean annual temperature and intermediate wood cover.

### II.3.3. Species-area relationship is not log-linear and unaffected by wood cover

A non-linear mixed cumulative Weibull model with wood cover and its square as fixed effects of parameter  $a$  only was found to be the most parsimonious of a range of evaluated possibilities to describe the species-area relationship in the investigated pastures (Table II.5). Correlations between parameters were relatively small, so a diagonal variance-covariance matrix (Pinheiro & Bates 2000:157) performed best. We also tested various autocorrelation structures, but none was able to substantially improve the fit.

**Table II.5.** Parameter estimates for mixed non-linear cumulative Weibull model effects of wood cover and its square on parameter  $a$ .

Parameter		Coefficient	Standard	P ( $t_{596}$ )	Standard	Standard
			error of estimate		deviation (site level)	deviation (plot level)
a	Intercept	44.694	3.153	0.0001	8.334	5.072
	cover	0.102	0.052	0.05		
	cover <sup>2</sup>	-0.003	0.001	0.0001		
b	Intercept	0.540	0.044	0.0001	0.119	0.119
c	Intercept	0.390	0.009	0.0001	8.06E-06	0.080

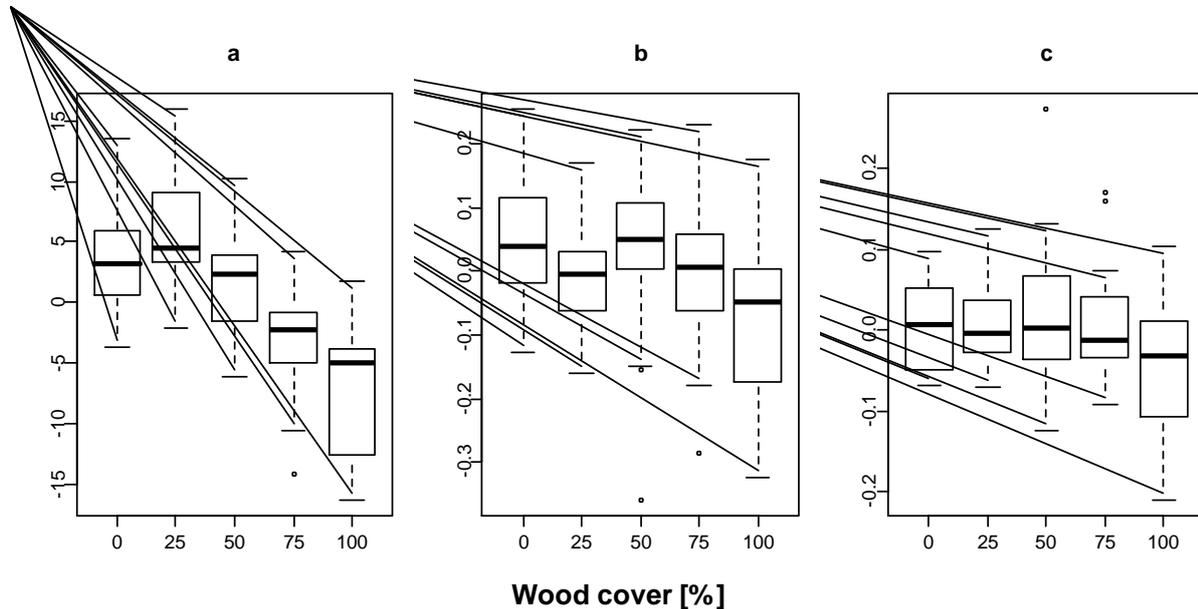


**Figure II.6.** Species-area relationship of site E on log-log scale. Symbols show richness estimates by nested-quadrat sampling, lines show predictions from a non-linear mixed cumulative Weibull model on the entire datasets from 8 sites in the Italian Alps (Table II.1).

Figure II.6 shows a log-log plot of the data from site E with cumulative Weibull models fitted for the five levels of wood cover by a non-linear mixed model. The data of site E was selected for plotting because there is least overlap between the curves for the five wood cover levels. The data clearly demonstrates that the species-area relationship in site E is not log-linear but sigmoid. The sigmoidal shape is captured by parameter  $c$  in the cumulative Weibull model which is estimated at 0.4 ( $P < 0.0001$ ). Despite using more degrees of freedom than the power or Monod model, the third parameter  $c$  allows the cumulative Weibull model to fit significantly better to the data than the power and Monod model ( $P\chi^2 < 0.0001$ ).

Figure II.7 illustrates the effect of wood cover on the random effects of the model described in Table II.5. The graph illustrates visually that wood cover has a strong effect on parameter  $a$  (i.e. the asymptote of the SAR curve (Table II.5)). Parameters  $b$  (the slope) and  $c$  (the sigmoidal

tempering) are only marginally changed at the highest levels of wood cover. This effect is not significant. The implication of this non-linear fitting exercise are that our analysis of species richness estimates using equal sampling areas across different wood cover levels is valid.



**Figure II.7.** Boxplots of random effects against wood cover on 8 sites in the Italian Alps. Random effects are estimate for parameters a, b and c of a cumulative Weibull model using non-linear mixed modeling.

## II.4. Discussion

### II.4.1 Plant species loss by forest succession

Our investigation show that species richness generally decreases with increasing cover of woody species. This is in line with most studies that investigated consequences of land use changes in the Alpine region (see for example Dullinger et al., 2003; McDonald et al., 2000). Despite this general acknowledgement, studies that explicitly investigated the relationship between wood cover and species richness is very limited. Anthelme et al., 2001 recorded species numbers in 108 plots with different cover of green alder (*Alnus viridis*) at a site in the French Alps at 1950 m asl. He found a no effect of the cover of green alder on species numbers

up to a cover of 10% but a constant decrease a higher values of green alder cover. Kesting (2009) found on a site in Central Germany that shrub cover was favorable to species richness at lower percentage, but decreased species richness at intermediate to higher percentages. Comparing the relationship at the eight investigated sites in the Italian Alps further revealed, that environmental conditions at least partly explain the differences between sites in the exact shape of the relationship between wood cover and species richness. Since plot size in the study of Anthelme et al. (2001) was 15m x 15m and in the study of Kesting was 10m x 10m they can be reasonably well compared to our data of the large-scale samples (Figure II.4). Whereas Kesting (2009) report a mean annual temperature of 8.7°C for his site, this is a bit more challenging for Anthelme et al. (2001). Again relying at the maps provided by Hiebl et al. (2009) we evaluated a range of possible locations at 1950 m. a.s.l. around the published coordinate and derived a mean annual temperature of around 2.5°C. Using these estimates we can then evaluate the consistency of our model with these published studies. Doing this we find that the monotonic decrease of plant species richness with increasing wood cover found by Anthelme et al. (2001) and the hump-shaped relationship reported by Kesting et al. (2009) agree very well with Figure II.4, which shows a less unimodal relationship at low mean annual temperature.

#### II.4.2. Integrating site and plot information into a mixed-effects model

To the best of our knowledge, this is the first study of plant species loss across a range of contrasting sites involving different dominant wood species. Using a multilevel model in our study permitted us to draw more general conclusion about the observed patterns than would have been possible from a study at a single site. Ecological studies, in fact, often involve variation among units of investigation, in our case among sites. Among the many possibility of formulating a multilevel model, the variation among units can be conveniently quantified as random effects in a mixed-effects model (Bolker et. al., 2009). A mixed-effects model is extension of regression in which data are structured in groups and coefficients can vary by groups. Within the model framework information can be available at site level and at plots level and in this way, different sites, which could not be truely replicated because of the high number of variables affecting it, can be analyzed together. The information available at site

level can then be used to investigate how site properties affect patterns observed at plot level (e.g. how site temperature affects species loss).

The second advantage of employing a mixed-effects model is that we obtain a much more parsimonious model representation. Considering sites as part of bigger entity of sites, reduced the degrees of freedom from 28 to 9 and improved the whole model substantially. A further advantage is that patterns fitted by a mixed model are much more realistic for site with weak data. In general, differences between GLM and GLMM on regressions were not very important (Figure II.2). However, where evidence was relatively weak such as at site C, the relationship obtained by GLMM was much more realistic than the GLM regression which had a polynomial pattern with the concave side up.

#### II.4.3. Plant species loss depends on observation scale

Ecological studies often just sample data at one scale of observation which is rather arbitrarily predefined based on experience from earlier studies. However, our study highlighted the importance of sampling scale for the observation of ecological patterns and demonstrates that interpretation based on just one scale of observation (be it 1 m<sup>2</sup> or 100 m<sup>2</sup>) could have been misleading. At the 1 m<sup>2</sup> observation scale sites with lower mean annual temperature had the highest species richness at intermediate wood cover, whereas at 100 m<sup>2</sup> highest species numbers were found at intermediate cover at sites with high mean annual temperature. This discrepancy can be explained by the presence of species with low abundance in warmer sites (and be downweighted using effective species numbers; Figures II.4 and II.5), a pattern that would not be detected if observation would just have been done at a single scale.

SARs can be used to test the appropriateness of the sampling area (Dengler and Oldeland, 2010). Our analysis of SAR showed that for pasture/forest transects the minimal area to capture species richness is about 25 m<sup>2</sup> (Figure II.6). When sampling areas smaller than 25 m<sup>2</sup>, we may underestimate richness considerable. We further found that the percentage of wood cover (i.e. the transition from grasslands into forests) changed the asymptotic level of the SAR but not the its increase. This is reassuring for a whole body of literature (e.g. Anthelme et al. 2001, Fischer and Wipf, 2002, Kesting et al. 2009) who investigated effects of forest succession with equal-

area sampling without even considering the observed effects could just be an artifact of changing SAR.

Finally, our analysis of SAR demonstrated that the power law is not a particularly good model to explain SAR in grasslands of reforested pasture. There is consistent and statistically significant evidence that the rate of increase of species numbers with area is not constant as assumed by the power model. The more flexible cumulative Weibull model allows for a rate decreasing with area and fits significantly better. Because the power model is a special case of the cumulative Weibull model with parameter  $c=1$  we advocate the more frequent evaluation of the model for SAR in grasslands and its subsequent reduction to the power model if parameter  $c$  is not significantly different from 1.

#### II.4.4. Mitigation of plant species loss in pastures has to take into account site conditions

Changes on species richness are different between sites because of the big number of factors that influence this phenomenon. In fact, in each site there are a lot of different factors (natural or anthropological) that influence forest succession and the associated loss of species richness. A multi-site study as the one presented is prerequisite to understand complex phenomena such as the forest succession on mountain pastures.

As show on Moser et al. (2005) and Ziliotto et al. (2004) altitude has a negative effect on species richness of habitats in general. We found that mean annual temperature had a significant reducing effect on species richness: sites with higher gamma diversity had higher mean annual temperature (Table II.1). It is well known that the initial evolution of reforestation is faster at warmer sites, while wood establishment on abandoned grasslands is slower for higher altitude (Tasser et al., 2007). We found that soil properties also had an effect on species richness and the species loss through reforestation, especially soil organic matter content and soil fertility.

Our investigations show that sites with higher mean annual temperature, low soil organic matter content and low soil fertility are the most vulnerable to the loss of species by forest succession, most likely because of the high number of species with low abundance. These sites

should receive primary attention of land managers and conservation bodies with the aim of halting biodiversity loss.

On the other hand, highest species numbers were frequently found at low to intermediate percentages of wood cover. Hence, the aim should not be to have no trees at all but to find management strategies by which low percentage of shrubs or trees on pastures can be maintained. Because of the higher heterogeneity created by grazing than by mechanical operations, a combination of animals with mechanical interventions to regulate shrub cover may be the way to go.

## **II.5.Conclusions**

Wood establishment and both natural and human variables have a complex relationship. The connection between natural evolution within site level and changes in the landscape structure at sites level is of crucial importance for a better understanding of cultural landscape transformation and their ecological impacts (Tasser et al., 2007). With this study we found that sites with lower altitude are more vulnerable and the influence of wood establishment have a stronger effect on species richness. However a low percentage of shrubs and trees on grassland improve biodiversity of pastures.

Now the question is: a low percentage of shrubs and trees on pasture improve biodiversity, but what does it happens to the botanical composition? And are these changes good for herbage quality?



## **Chapter III**

### **Plant composition changes due to forest succession in eight Alpine pastures**

### III.1 Introduction

As a consequence of land abandonment processes, mountain regions have been experiencing radical landscape changes, as once cultivated areas are turning to forests through the process of natural succession (Conti and Fagarazzi, 2004). In shrub invaded grasslands, shrub species compete with grasses and forbs for light, water and nutrients (Kesting, 2009). Therefore, pasture species richness and botanical composition are strongly affected by natural reforestation, with different response depending on site condition and especially on woody dominant species (see Anthelme et al., 2001; Fischer and Wipf, 2002; Kesting, 2009). It has been demonstrated that when woody plants invade grasslands the typical species rich vegetation disappears (Dierschke, 2006; Galvanek and Leps, 2008).

Based on results obtained in Chapter 2, that displayed different behaviors in species richness trend due to forest succession between sites, changes in botanical composition was also investigated. In this Chapter results of Bray index (Bray et al., 1957) and detrended correspondence analysis (DCA) are reported.

### III.2 Materials and methods

#### III.2.1 Survey sites

We refer to the survey sites description in Chapter III.

#### III.2.2 Species sampling

At each study site, the two sampling methods described in Chapter III were applied:

*A. Small-area sampling along transects (1m<sup>2</sup>)*

*B. Large-area sampling (100m<sup>2</sup>) in wood-cover strata*

### III.2.3 Additional plot and site properties

In small area plot, soil was sampled at two depths: from 0 to 5 cm (depth 1), and from 5 to 20 cm (depth 2). Soil pH (1:5 soil:water solution) [pH1 and pH2], pH (1:5 soil:KCl solution), N content (Kjeldahl total nitrogen [ $\text{g kg}^{-1}$ ]) [N1 and N2] and soil organic matter (Springer–Klee method [ $\text{g kg}^{-1}$ ]) [OM1 and OM2] were measured using the Italian standard soil analysis techniques (G.U., 1999).

### III.2.4 Compositional dissimilarity by means of Bray index and comparison between GLM and GLMM models.

Bray index (1957) [1] for both sampling methods and for each site was calculated. This index is used to quantify the compositional dissimilarity between two different sampling areas, based on counts at each site. Only first order terms (sums and differences) are used to calculate the index, and all are relativized by site total and reach their maximum value (1) when there are no shared species between two compared plots.

The Bray index dissimilarity between plot  $j$  and  $k$  is:

$$d_{jk} = \frac{A + B - 2J}{A + B} \quad [1]$$

where  $A$  is the number of species on  $j$ ,  $B$  is the number of species on  $k$  and  $J$  is number of shared species.

We built a control plot averaging for each site the plots with 0% wood cover, and then each plot was compared to this control plot. Separate GLMMs were built for both sampling datasets in order to analyze effects of wood cover on plots dissimilarity (Pinheiro and Bates, 2000; Venables and Ripley, 2002). We started with a simple model of Bray index against wood cover with site as the grouping factor (the random effect). As shown in Chapter 2, GLMM is a good tool if sites had different behaviors respect to the considered index (number of species). On the contrary, Bray index gives no significant effect of site in the model. Generalized linear mixed

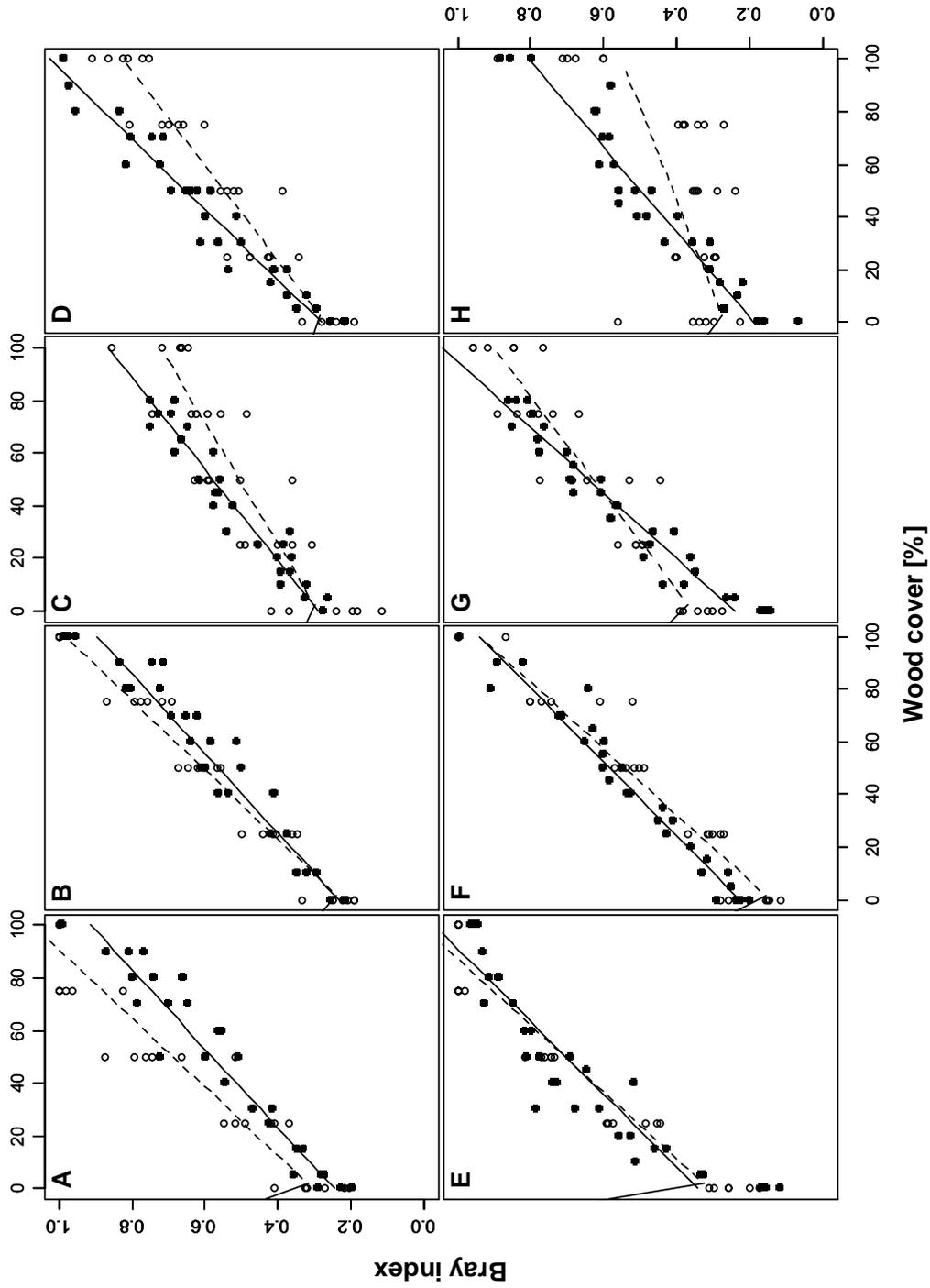
model was no better than GLM [i.e. having the smallest Akaike's Information Criterion (AIC)] for describing Bray index and wood cover relationship.

The matrices of sites botanical composition were subjected to detrended correspondence analysis (DCA), using R 2.13.1, that is considered a more robust method than principal components analysis for community ordination (Oksanen, 2010). The wood cover effect was analyzed using generalized additive models and model surfaces of wood cover was fitted to ordinations. Soil properties were also fitted as vectors onto ordination and only the significant soil properties were added in the DCA biplot. Using 100 m<sup>2</sup> samples dataset we calculated the weighted averages of Ellenberg's indicator values (Ellenberg, 1977) and the forage values for each plot.

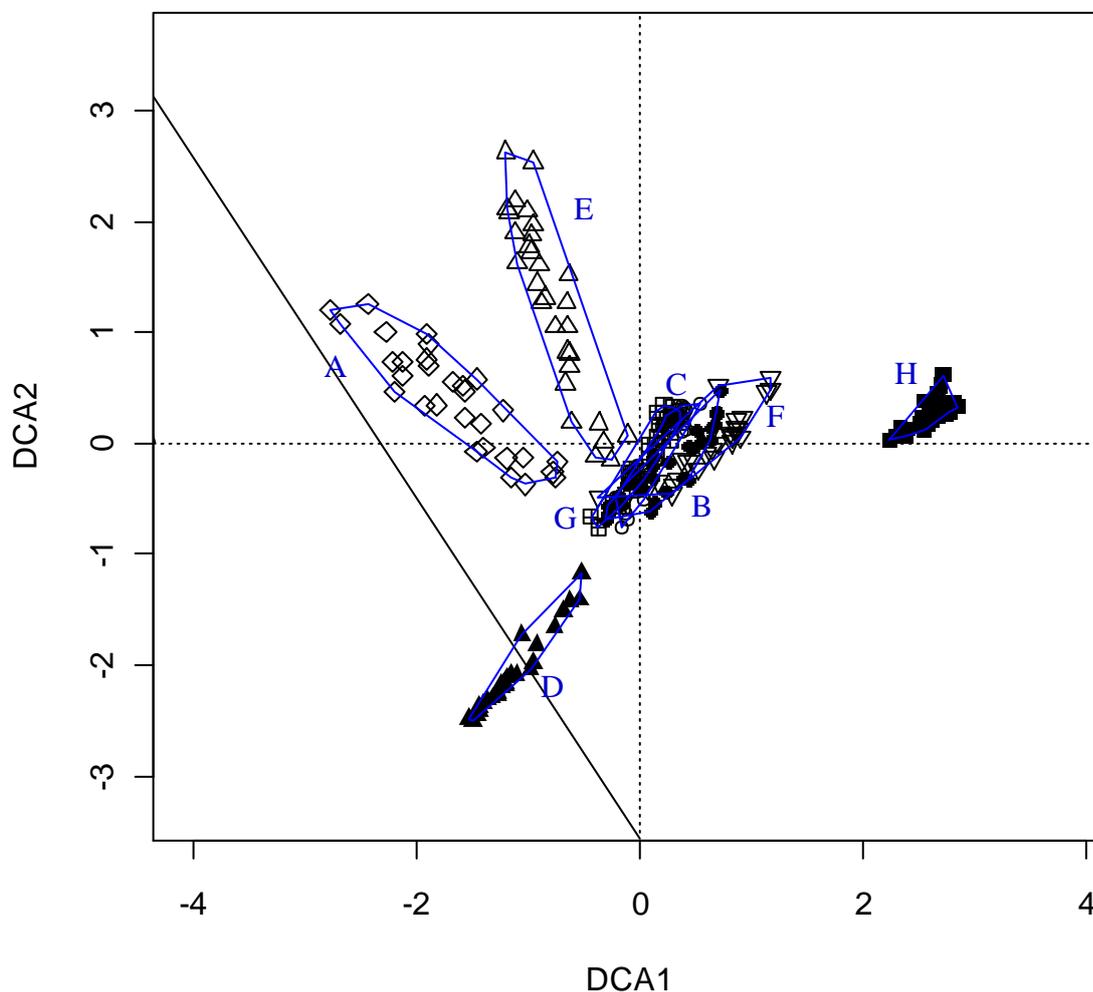
All calculations were performed in R 2.13.1 (R Core Team, 2011) using libraries Vegan, nlme and lme4.

### III.3 Results

The relationships between wood cover and Bray index for each site is displayed in Figure III.1. Both 1 m<sup>2</sup> and 100 m<sup>2</sup> samples GLM did not show a significant effect on curvature. The regression models were linear in both sampling methods, moreover the effect of wood cover and intercept were highly significant ( $P < 0.001$ ). In the 1 m<sup>2</sup> samples we found that the regression was steeper for sites A, B, E and F, while the slope was less pronounced for sites C, D, G and H. This difference within sites was not found when we focused on 100 m<sup>2</sup> samples. However, it is important to underline that in each site we have a highly significant ( $P < 0.001$ ) changing on floristic composition with the increase of wood cover.



**Figure III.1.** Effect of wood cover on Bray index for 8 sites. Dashed lines are GLM regression for 1 m<sup>2</sup> samples dataset. Solid lines are GLM regression for 100 m<sup>2</sup> samples dataset.



**Figure III.2.** Ordination plot of 100 m<sup>2</sup> samples botanical composition of the 8 sites along the first two axes of DCA. Blue polygons are the enclosing convex hull for the plots in each site.

The DCA performed on 1 m<sup>2</sup> samples dataset did not display a difference between sites, but only a gradient according to wood cover. While the DCA performed on 100 m<sup>2</sup> botanical survey displayed differences among sites, suggesting a separate analysis for each site in order to have a specific analysis of floristic variation. Indeed, figure III.2 pointed out the plots grouped for sites.

Based on this statement, results for each site with a botany board are reported in Appendix 1.

Each botany board includes mean annual temperature, altitude and annual precipitation of each site. Soil properties of samples collected in 1 m<sup>2</sup> plots are reported. A 100 m<sup>2</sup> samples matrices was used to analyze botanical composition. The original matrices were split in two matrices: one for dominant species (species with a cover percentage more than 3) and one for rare species (species with a cover percentage less than 1). Furthermore the average Ellenberg's indicators and forage values (Klapp, 1971) for 10 wood cover classes are also reported.

### III.3. Discussions and conclusion

With respect to the effect of wood cover on Bray index the different slope of 1 m<sup>2</sup> and 100 m<sup>2</sup> GLM regression can be due to the high presence of rare species on intermediate wood cover of sites at higher mean annual temperature. Higher values on regressions slope is due to the faster change of floristic composition together with the increase of wood cover. On sites with higher number of rare species, the change in the floristic composition is faster than others.

From these results we have come to the conclusion that wood cover affected the botanical composition of mountain pastures and this effect was common of all the sites studied even if each site responded to wood cover in a different way. The different response observed was mainly due to differences in species composing of pasture and underwood.



## **Chapter VI**

### **Effect of forest succession on herbage quantity and quality of Alpine pasture**

## **VI.1 Introduction**

There are few information available concerning the effects of wood establishment on forage quality of mountain pasture. Kesting (2009) analyzing the effect of shrubs establishment on a calcareous grassland on Swiss Alps, found an increase of crude protein and crude lipid together with a reduction on fiber content. Zweifel-Schielly et al. (2011) analyzed the differences on forage quality between forested slopes and farmed meadows on the Swiss Alps and they found significant differences on forage contents related to forest and meadow. These differences could be due to the tree shadow effect or to the botanical composition. Kephart et al. (1992) compared forage contents of C3 and C4 forage species under different percentage of ambient sunlight reduction. Forage content was affected by C3 and C4 species but not by shade. In a complex habitats as a pasture evolving on forest succession, some factors that could influence forage quality are shade, temperature, season and botanical composition together.

Our hypothesis was that, as a consequence of changes in botanical composition and light reduction due to forest succession on pasture, the herbage yield and quality could change. We also supposed that this effect depends on site conditions and on grazing season. Results of herbage yield and chemical components of samples collected in two times during the grazing season (July and August), in four of the eight sites investigated in the previous Chapters, are here reported.

## **VI.2 Materials and methods**

### **VI.2.1 Survey sites**

For the survey sites we refer to the survey sites description on Chapter 2. For this study we took into account only the sites on carbonate substrate: sites A, B, C and D.

### **VI.2.2 Surveys, sampling and chemical analysis**

Whereas the wood cover exceeded 50% (sites A and B) the height of herbaceous layer was too low to be considered as grazing surface; while on site D the herbaceous layer at wood cover

higher than 50% can't be reached by the herds because of the tree structure. In order to determine pasture production and herbage quality, samples were collected in plots with three stages of wood cover: 0% (C0), 25% (C25) and 50% (C50). In each site, three methods were used to collect samples: 1) herbage samples were collected with the exclusion cages on August, 2) forage samples were collected in grazed pasture on July, 3) forage samples were collected in grazed pasture on August.

With the first method 9 forage samples were collected at each wood cover stage using 1 m<sup>2</sup> cage placed on pasture in May, before the beginning of grazing season, to collect on August the potential yield of pasture. Because the heterogeneity of pastures in site A and B, we placed 3 cages at the 3 wood cover stages for each site. While in sites C and D only 2 cages and 1 cage were placed at each wood cover stage respectively.

With the second method 9 samples were collected on July at each wood cover stage using 1 m<sup>2</sup> plots placed close to the cages. With the third method 9 samples were collected on August at each wood cover stage using 1 m<sup>2</sup> plots placed close to the cages.

Each sample was cut at soil level and deadwood was removed. Samples were oven dried at 65° C for 36 h to determine dry matter yield (DM [t ha<sup>-1</sup>]) and subsequently they were subjected to conventional chemical analysis to determine: crude fiber (CF, Weende method [% of DM]), ash (ash [% of DM]), neutral detergent fiber (NDF, Van Soest method [% of DM]), acid detergent fiber (ADF, Van Soest method [% of DM]), acid detergent lignin (ADL, Van Soest method [% of DM]), dry matter crude protein (CP, Kjeldahl method [% of DM]) and crude lipid (CL, Soxtec method [% of DM]). From results of chemical analysis milk forage units (MFU) were calculated with two methods: 1) MFU1 according to INRA (Institut National de la Recherche Agronomique, 1981) equation [1]; 2) MFU2 according to Andrighetto and Ramanzin (1987) equation [2]. The MFU per hectare was calculated from DM and MFU (MFU1t and MFU2t respectively for the two methods).

$$MFU1 = 0.714 + 0.003786 * CP + 0.00000728 * CP^2 - 0.00000275 * CF^2 \quad [1]$$

$$MFU2 = 1.103 - 0.0119 * NDF + 0.0281 * ash + 0.0089 * CP \quad [2]$$

### VI.2.3 Statistical analysis

All chemical components (DM, CF, ash, NDF, ADF, ADL, CP and CL), both MFU and both MFU per hectare were subjected to the analysis of variance. Data were analyzed first comparing collecting method 1 (forage collected into exclusion cages) with collecting method 3 (forage collected on pasture in August) in order to determine differences between grazed and ungrazed herbage. Then method 2 (forage collected on pasture in July) were compared with method 3 (forage collected on pasture in August) to determine seasonal variations. Data were split in two groups and analyzed with a three way ANOVA using wood cover, site and method as factors. The three way interaction was not significant in both analysis, thus the results are not reported in tables. When necessary, data were previously transformed, to achieve normal distribution. Differences between means were separated using a Tukey's HSD (Honestly Significant Difference) test at 0.05 level of probability. All statistical analysis were performed with R 2.13.1 (R Core Team, 2011).

## VI.3 Results

### VI.3.1 Forage contents comparison between samples collected into cages and on grazed pasture

As reported in Table IV.1 wood cover and site had a significant effect on the most important herbage contents analyzed. The only significant interaction was between wood cover and site, for CF, DM, MFU2, MFU1t and MFU2t. MFU1t and MFU2t are strictly dependent from DM, so the ANOVA with these two parameters reflected the DM ANOVA results.

**Table IV.1.** Results of ANOVA for Crude protein (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), crude lipid (CP) and Ash; dry matter (DM) and milk forage units (MFU1, MFU2, MFU1t and MFU2t). Comparison between samples collected on August into cages and on grazed pasture.

Contents	Wood cover	Site	Method	Wood cover x Site	Wood cover x Method	Site x Method
CF [% of DM]	***	*	n.s.	*	n.s.	n.s.
NDF [% of DM]	**	***	n.s.	n.s.	n.s.	n.s.
ADF [% of DM]	***	***	n.s.	n.s.	n.s.	n.s.
ADL [% of DM]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CP [% of DM]	*	*	n.s.	n.s.	n.s.	n.s.
CL [% of DM]	*	***	*	n.s.	n.s.	n.s.
Ash [% of DM]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DM [t ha <sup>-1</sup> ]	***	***	**	*	n.s.	n.s.
MFU1 kg <sup>-1</sup> DM (INRA, 1981)	n.s.	*	n.s.	n.s.	n.s.	n.s.
MFU2 kg <sup>-1</sup> DM (Andrighetto and Ramanzin, 1987)	*	**	n.s.	*	n.s.	n.s.
MFU1t ha <sup>-1</sup> (INRA, 1981)	***	***	**	*	n.s.	n.s.
MFU2t ha <sup>-1</sup> (Andrighetto and Ramanzin, 1987)	***	***	**	*	n.s.	n.s.

Wood cover had a positive effect on NDF and ADF content (Table IV.2), while it had a negative effect on CL and CP. For CF content wood cover had a slightly influence in sites A and B and no significant influence in site C. Only in site D the CF value for C50 was significantly different from the two lower wood covers (C0 and C25).

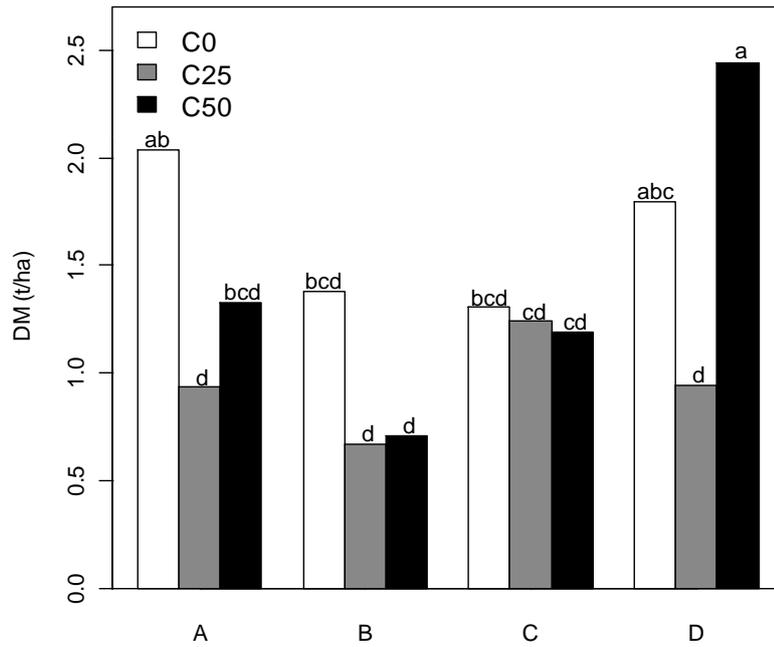
**Table IV.2.** Mean herbage contents for samples collected on August into cages and on grazed pasture. Main effects (wood cover, site and method) and interaction 'site x wood cover'.

Content [% of DM]	Wood cover			Site				Method				
	C0	C25	C50	A	B	C	D	Into cages	Out cages			
NDF	53.36 b	55.90 ab	58.15 a	55.38 ab	59.57 a	51.06 b	56.15 a	n.s.				
ADF	35.30 b	36.81 b	38.49 a	39.06 a	36.60 b	33.30 c	38.80 a	n.s.				
CP	13.27 a	11.38 b	12.32 ab	11.35 a	12.78 a	13.18 a	12.08 a	n.s.				
CL	3.88 a	3.57 b	3.35 c	3.19 d	3.41 c	3.97 b	4.52 a	3.81 a	3.39 b			
	Site X Wood cover											
	A			B			C			D		
	C0	C25	C50	C0	C25	C50	C0	C25	C50	C0	C25	C50
CF	22.72 b	25.61 ab	25.98 ab	23.82 b	23.65 b	25.95 ab	23.03 b	23.01 b	23.54 b	23.10 b	24.65 b	30.10 a

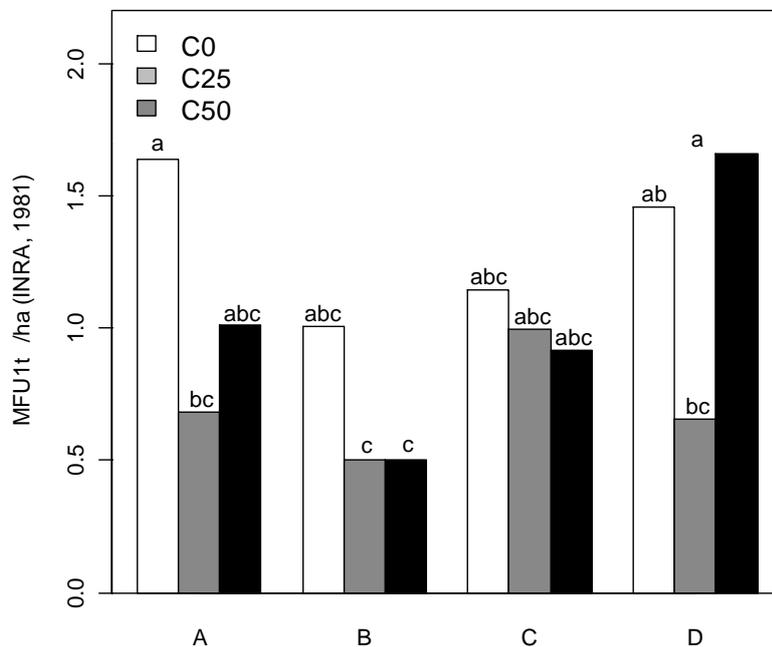
For each content, values followed by the same letters are not significantly different from one another (Tukey's HSD test,  $P < 0.05$ ).

When DM was analyzed, no significant interaction between wood cover and method or wood cover and site were found. The significant interaction between wood cover and site, as shown in Figure IV.1, displayed a different behavior of site C compared to the others. In fact, in site C no significant differences was found for DM. In site A the higher value of DM was found at C0, while a significant lower value was found at C25. In site B values of DM tend to be lower at C25 and C50 than at C0. In site D, C0 and C50 had the higher values, while C25 had the lower one. As aspected, DM of samples collected into the cages was significantly higher ( $p < 0.01$ ) than the DM of samples collected on grazed pasture (data not shown).

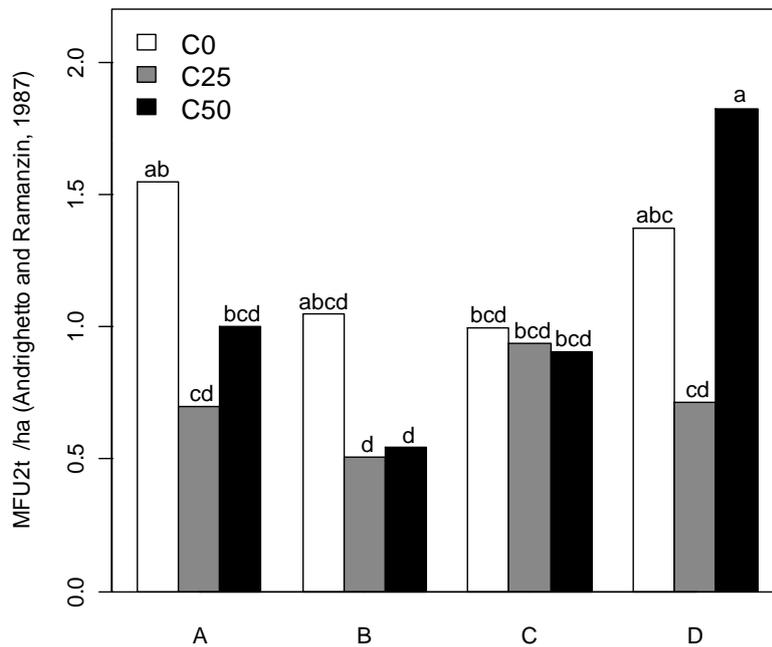
Concerning forage quality, we found that MFU1t and MFU2t reflected DM patterns (Figure IV.2 and IV.3), with the same significant interaction and the same site patterns. Site B was the site with the significant lowest MFUt (1 and 2) value. In addition, the difference between MFUt (1 and 2) of samples collected into the cages was significantly higher ( $p < 0.01$ ) than the MFUt (1 and 2) of samples collected on grazed pasture (data not shown).



**Figure IV.1.** Dry matter yield, interaction site x wood cover. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25 and C50 = 0%, 25% and 50% of wood cover respectively.



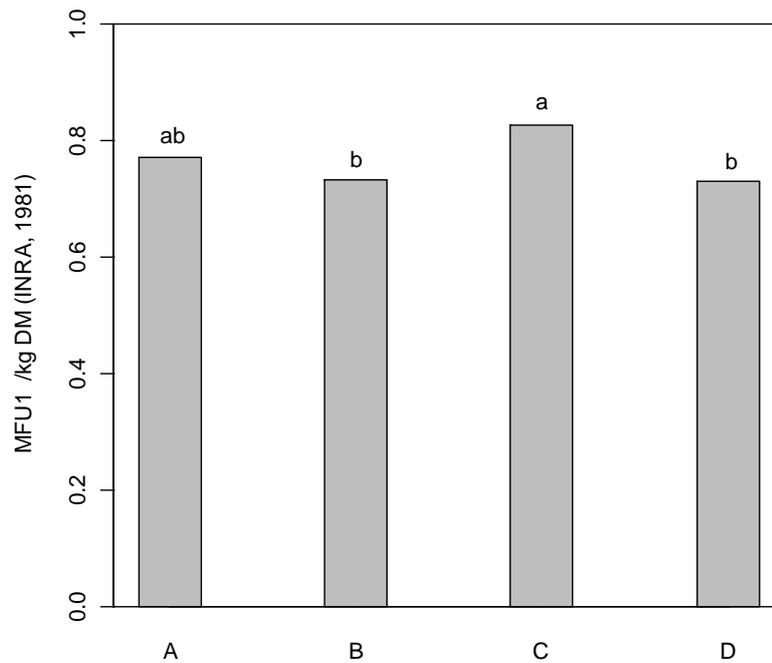
**Figure IV.2.** Milk forage units (MFU1t/ha, INRA 1981), interaction site x wood cover. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25 and C50 = 0%, 25% and 50% of wood cover respectively.



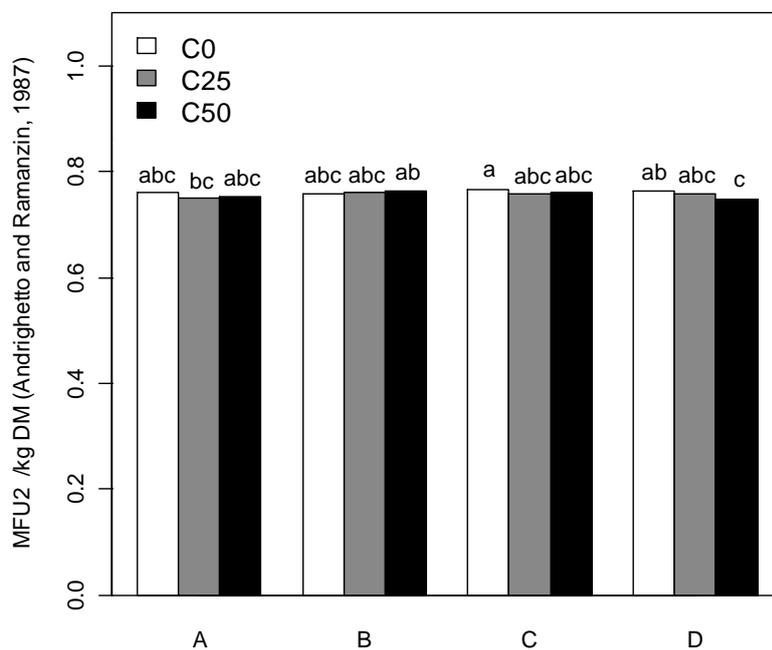
**Figure IV.3.** Milk forage units (MFU2t /ha, Andrighetto and Ramanzin 1987), interaction site x wood cover. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25 and C50 = 0%, 25% and 50% of wood cover respectively.

With respect to MFU1 no significant effects of wood cover and method were found. Only the effect of site was significant (Figure IV.4). Sites C showed higher values than site B and D, while no differences were found between site A and C..

For the MFU2 a significant interaction between wood cover and site was found. As shown on Figure IV.5, only site D showed a decrease of MFU2 from C0 to C50.



**Figure IV.4.** Site effect for milk forage units (MFU1/kg of DM, INRA 1981). Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.



**Figure IV.5.** Milk forage units (MFU2/kg of DM, Andrighetto and Ramanzin 1987), interaction site x wood cover. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25 and C50 = 0%, 25% and 50% of wood cover respectively.

### VI.3.2 Forage contents comparison of samples collected on grazed pasture, in July against in August

**Table IV.3.** Results of ANOVA for Crude protein (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), crude lipid (CP) and Ash; dry matter (DM) and milk forage units (MFU1, MFU2, MFU1t and MFU2t). Comparison between samples collected on grazed pasture, in July against in August.

Contents	Wood cover	Site	Method	Wood cover x Site	Wood cover x Method	Site x Method
CF [% of DM]	***	*	n.s.	n.s.	n.s.	n.s.
NDF [% of DM]	***	***	n.s.	n.s.	n.s.	*
ADF [% of DM]	***	***	n.s.	n.s.	n.s.	n.s.
ADL [% of DM]	*	*	n.s.	n.s.	n.s.	*
CP [% of DM]	*	n.s.	n.s.	n.s.	n.s.	n.s.
CL [% of DM]	***	*	**	n.s.	n.s.	n.s.
Ash [% of DM]	n.s.	*	n.s.	n.s.	n.s.	n.s.
DM [t ha <sup>-1</sup> ]	n.s.	***	n.s.	n.s.	n.s.	n.s.
MFU1 kg <sup>-1</sup> DM (INRA, 1981)	**	**	n.s.	n.s.	n.s.	n.s.
MFU2 kg <sup>-1</sup> DM (Andrighetto and Ramanzin, 1987)	*	n.s.	n.s.	n.s.	n.s.	n.s.
MFU1t ha <sup>-1</sup> (INRA, 1981)	*	***	n.s.	n.s.	n.s.	n.s.
MFU2t ha <sup>-1</sup> (Andrighetto and Ramanzin, 1987)	n.s.	***	n.s.	n.s.	n.s.	n.s.

As reported in Table IV.3 wood cover and site had a significant effect on the most important forage contents analyzed. The only significant interaction was between site and method, for NDF and ADL. It means that these parameters had a different interaction with method depending on sites.

Wood cover had a positive effect on NDF, ADF, ADL and CF, while it had a negative effect on CL and CP (Table IV.4). Method had a significant effect only for CL. The interaction between method and site displayed a decrease of NDF and ADL content passing from July to August on sites A, C and D. On site B the effect was the opposite, with an increase of NDF and ADL from July to August.

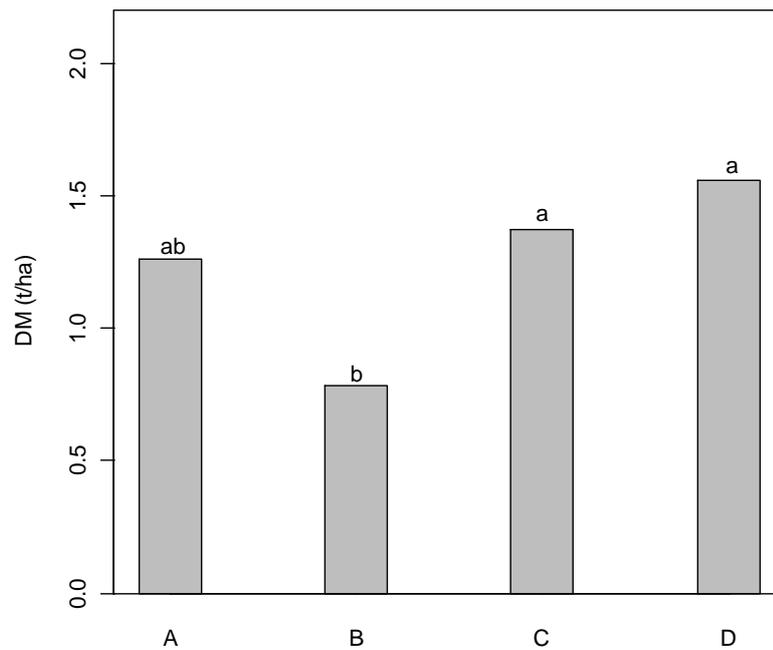
**Table IV.4.** Mean herbage contents for samples collected on grazed pasture, in July against in August. Main effects (wood cover, site and method) and interaction 'site x method'.

Contents [% of DM]	Wood cover			Site				Method	
	C0	C25	C50	A	B	C	D	July	August
CF	22.86 b	23.94 ab	25.38 a	24.70 ab	23.72 ab	22.99 b	25.27 a	n.s.	
NDF	52.06 b	54.01 b	57.25 a	Significant interaction Site x Method					
ADF	34.61 c	36.53 b	38.14 a	38.34 a	36.11 b	33.42 c	37.67 ab	n.s.	
ADL	6.44 b	7.66 ab	7.68 a	Significant interaction Site x Method					
CP	13.68 a	11.81 b	12.66 ab	n.s.				n.s.	
CL	4.16 a	3.58 b	3.23 c	3.38 b	3.49 b	4.17 a	3.97 a	3.92 a	3.39 b
Ash		n.s.		8.19 a	7.99 a	7.75 ab	6.61 b	n.s.	
	Site x Method								
	A		B		C		D		
	July	August	July	August	July	August	July	August	
NDF	56.11 b	55.07 bc	54.35 bc	60.04 a	49.57 de	48.41 e	52.40 cd	54.86 bc	
ADL	7.66 ab	7.39 ab	5.68 b	7.75 ab	7.20 ab	6.66 ab	9.37 a	8.19 ab	

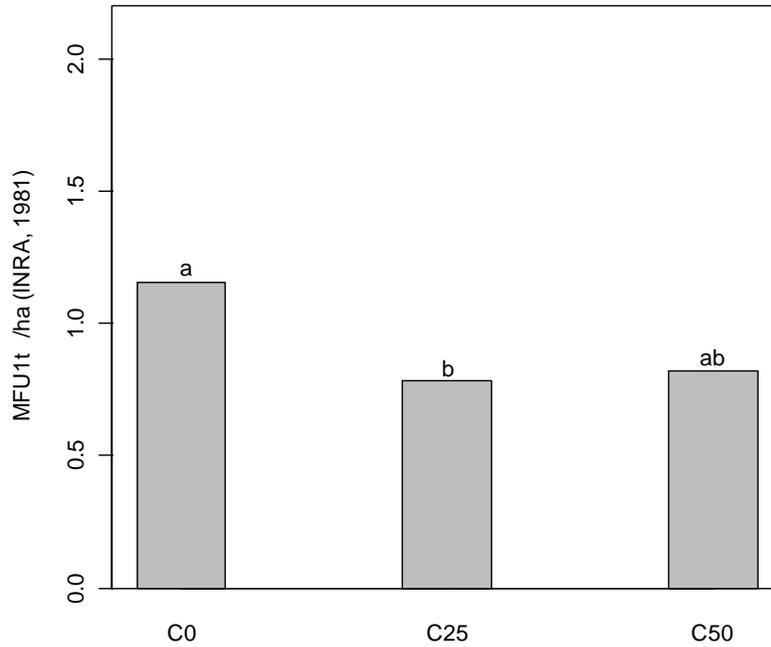
For each content, values followed by the same letters are not significantly different from one another (Tukey's HSD test,  $P < 0.05$ ).

Wood cover had no significant influence on DM yield, but a significant difference was found between sites D and C (higher values) and site B (lowest value) (Figure IV.6).

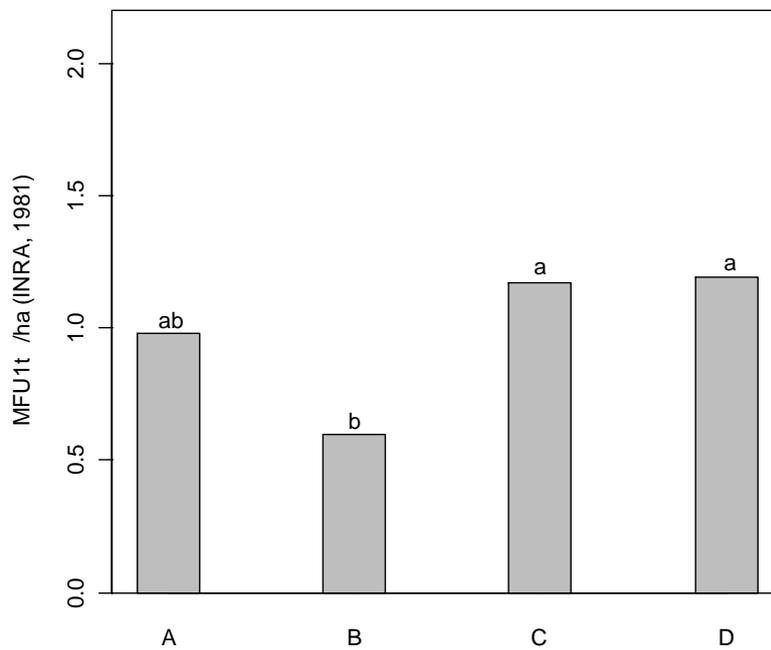
Wood cover had a significant effect only on MFU1. MFU1 decreased from C0 to C25, while no difference was observed between C25 and C50 (Figure IV.7). Sites C and D showed higher MFU1 values than site B. Besides, for MFU2 the highest value was found for site D, while the lowest for site B (Figure IV.8).



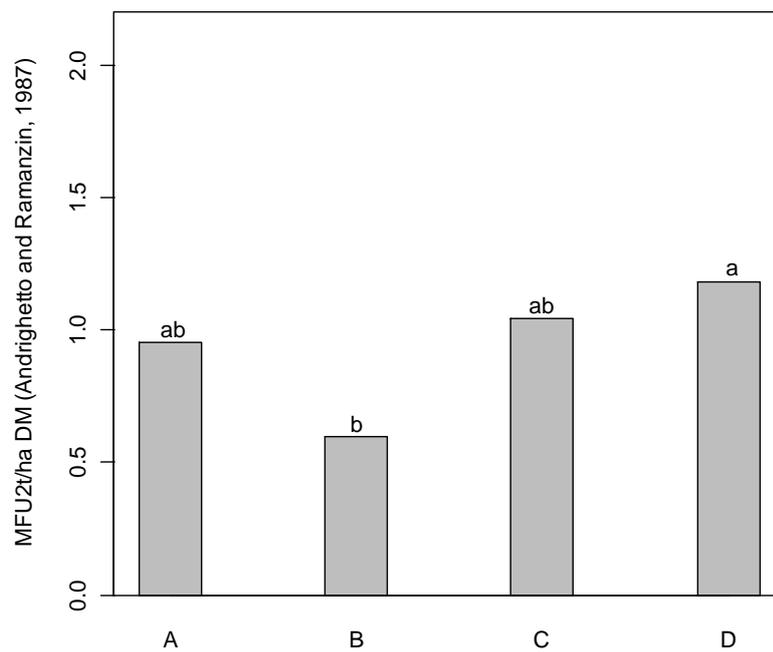
**Figure IV.6.** Dry matter yield, site effect Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.



**Figure IV.7.** Total milk forage units (MFU1t/ha, INRA 1981), wood cover effect. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.



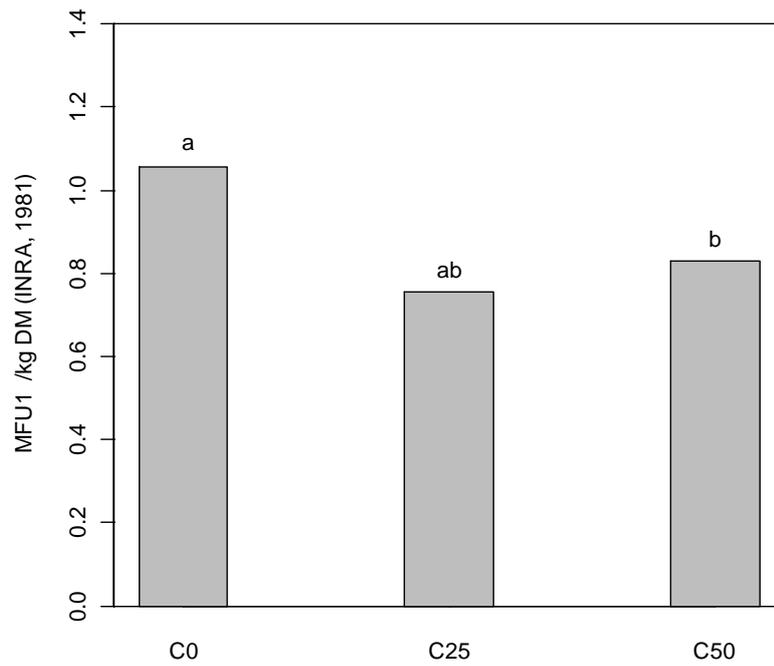
**Figure IV.8.** Total milk forage units (MFU1t/ha, INRA 1981), site effect. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.



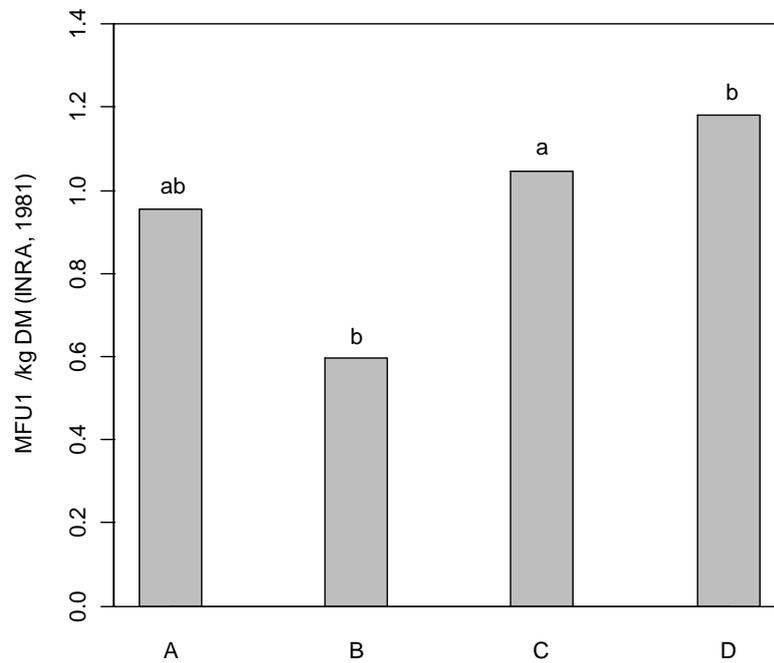
**Figure IV.9.** Milk forage units (MFU2t/ha, Andrighetto and Ramanzin 1987), site effect. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.

For MFU1 a significant effect of wood cover was found. A decrease of MFU1 from C0 to C50 was noted (Figure IV.10). Site effect was also significant. The site with highest MFU1 was site C, while sites with lower value were sites B and D (Figure IV.11). On the contrary the effect of collecting method was not significant.

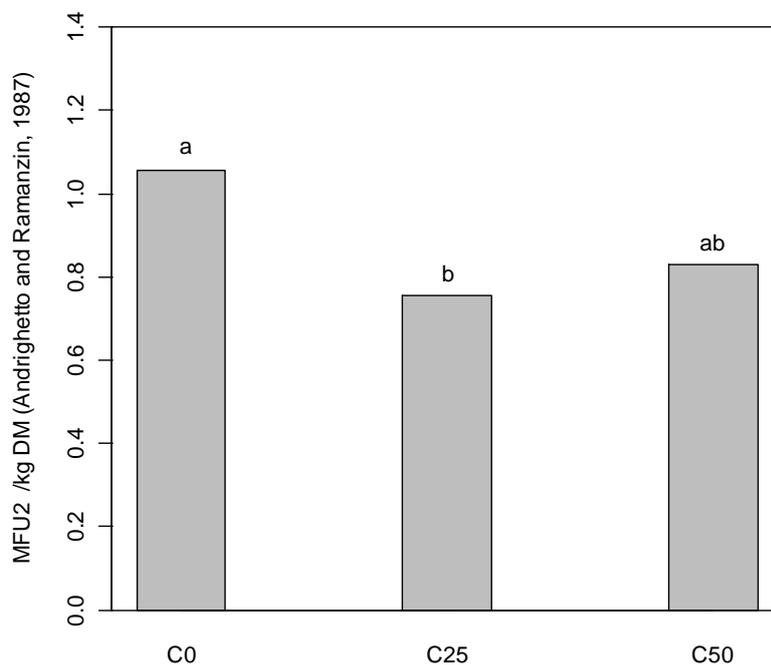
When MFU2 was taken into account, only wood cover effect was significant. As shown on Figure IV.12., C0 was significantly higher than C25.



**Figure IV.10.** Total milk forage units (MFU1 /kg of DM, INRA 1981), wood cover effect. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.



**Figure IV.11.** Total milk forage units (MFU1 /kg of DM, INRA 1981), site effect. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.



**Figure IV.12.** Milk forage units (MFU2 /kg of DM, Andrighetto and Ramanzin 1987), wood cover effect. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability.

## VI.4 Discussion

A reduction of DM yield was noted in sites A and B from C0 to C25. In site C wood cover effect was not significant, while in site D the highest value was observed for C50 and the lowest for C25. This different sites response was mainly due to the woody species composing the canopy (Table II.1).

Regarding herbage quality, results displayed an increase of CF, NDF and ADF as result of the increase of wood cover. Fiber values of site C tended to be lower than the other sites. According to parameters for pasture forages proposed by Zanatta and Bruni (2009), the herbage quality of all sites at wood cover 0% can be classified for ADF as a middle quality. Furthermore, ADF and ADL contents at 0% wood cover were higher than those obtained by

Bittante et al. (1990) in a similar study. The increase of NDF and ADF content due to forest succession downgrades the herbage to a lower quality. Since ADF content exceeded 33%, the herbage of studied pastures is classified by Zanatta and Bruni (2009) as “poor”. Changes in CF, NDF and ADL content could be due to a change in botanical composition, including the Monocotyledones and Dicotyledones ratio (Superchi et al., 2007), or to a decrease of light intensity that affect herbage fiber content at single species level (Zweifel-Schielly, 2011).

Observing variations between the two sampling periods, we expected an increase of CF, NDF, ADF and ADL from July to August due to advanced plant maturity stage (Andrighetto and Ramanzin, 1987). In more-mature forage leaves are usually lower in quantity than stems and this involves an increase of fibrous constituent (Buxton and Russel, 1988). Moreover, stem digestibility declines more rapidly than the leaves one with advancing maturity (Buxton, 1996). Our results did not confirm this expectation because the July values of CF, ADF and ADL were not significantly different of August values.

The CP content was affected by wood cover, it decreased from C0 to C25. According to Zanatta and Bruni (2009) classification, based on CP and ADF contents, the herbage quality of our sites ranged from high-middle (C0) to middle-poor (C25). The CP values found at C0 were in line with those reported by Andrighetto and Ramanzin (1987). Crude protein requirements in diets of livestock generally range from 7% to 19% of DM for high-producing dairy cows (NRC,1989). Degradable and undegradable protein ratio on pasture forages could ensure a milk production of 20 kg/day, if the crude protein content is 13-15% (Bittante et al., 1985). Season effect on CP content was not significant. Similar results were obtained for *N. stricta*, *Phleum alpinum* and *Alchemilla vulgaris* by Bovolenta et al. (2008).

Wood cover had a negative effect on CL content. A significant difference among studied sites was also observed. The lowest CL value was recorded in July (the youngest forage stage). Since for high milk production cows the lipid content in diet could be very high (up to 90% ), the decrease of CL observed in this study can be considered not relevant. Despite this, for animal health, it is recommended not to exceed in the diet the 5% of lipid content of DM (Piccioni, 1979).

Both MFU1t (INRA, 1981) and MFU2t (Andrighetto and Ramanzin, 1987) reflected the DM trends. The MFU1 displayed a significant reduction between C0 and C50. Moreover, a significant difference was found between site C and sites B and D, with site C that showed the

highest MFU1 values. The MFU2 showed a decrease from C0 to C25. Sites didn't display significant differences between each others.

## **VI.5 Conclusion**

Wood cover affect both herbage yield and quality. The decrease of digestibility due to wood cover because of fiber contents increasing, together with a reduction of protein content, downgrades herbage quality from high (middle) to middle (poor).

Sites A, B and D show a decrease of productivity and quality starting from 25% of wood cover. Site C has a good production level also for higher wood cover, but the forage quality decreases from 25% of wood cover as observed in the other sites.

## **Chapter V**

**Roots characteristics and botanical composition changes  
through *Rhododendron ferrugineum* succession on a  
mountain pasture**

## V.1. Introduction

Grasslands are increasingly being considered valuable not only for livestock production but also for their socio-economic function, their role in the conservation of biodiversity, the regulation of physical and chemical fluxes in ecosystems, mitigation of pollution and maintenance of landscapes (Gibon, 2005; Lemaire et al., 2005). In recent years many studies have recognized the effect of grasses on hydrological functions including water retention, runoff, soil erosion and nutrient leaching. Factors such as climate, topography and soil characteristics have important effects on soil formation and erosion (Gobin et al., 2004; Knapen et al., 2007). However land cover, and therefore land use also play an important role on these processes (Wischmeier & Smith, 1978). The abandoning of land in mountain regions is responsible for important changes in the once cultivated areas that are evolving through natural succession processes. The importance of vegetation cover, including the role played by the belowground parts, in reducing soil erosion by water has been widely studied (De Baets et al., 2006; Klima, 2007). However, most of these studies focused on agricultural crops, while little attention has been given to natural vegetation (De Baets et al., 2006). Only a few studies reported the effects of roots of natural vegetation on soil erosion processes. Li et al. (1991) studied the effect of roots of *Pinus tabulaeformis* and *Hippophae rhamnoides* on rill erodibility. De Baets et al. (2006) confirmed the effectiveness of roots in reducing soil erosion rates. In particular they indicated that grass roots are very effective in reducing soil detachment rates. Indeed, plant root systems contribute to soil water infiltration, soil structural stability and aggregate stability (Mamo & Bubenzer, 2001; Martens, 2002; Joseph et al., 2003; Màrquez et al., 2004). Root parameters such as depth and density are considered important soil stability factors and are included as input variables in most hydrological models (Beven & Kirby, 1979; Tasser et al., 2003).

Changes in land cover evolving from grassland to shrubland could affect the soil erosion due to variations in the belowground component. However, there is no information available concerning the effects of shrubby establishment on root traits of the pasture plant community. The purpose of this study was to contribute to filling this lack of knowledge by studying changes in the main root characteristics of a mountain pasture under different levels of natural regrowth of shrubs in the Italian Alps.

## V.2. Methods

### V.2.1. Site, location and climate

The research was conducted on the Lagorai massif, a mountain range of Eastern Alps, in the Trentino region of northern Italy. The study area was located on the Trenca summer farm (latitude 46°08'N; longitude 11°39'E; elev. 1700) in south-eastern Lagorai. The climate of the area is temperate–oceanic with an annual mean temperature [mean annual temperature derived from the high-resolution alpine temperature interpolation by Hiebl et al. (2009)] of 4.4°C and rainfall of 917 mm year<sup>-1</sup>. Monthly precipitations are higher than 80 mm between May and October. The soil at the site is an Umbric Leptosols (IUSS Working Group WRB, 2006).

### V.2.2. Site description

The pasture was grazed by 85 dairy cows, 50 goats and 70 sheep. The stocking rate was defined by the owner in order to achieve optimum performance. The grazed surface was characterized by a very steep sector, in the higher elevation areas, and a less steep one near the farmstead. The latter was grazed by cows, while the steeper surface was grazed by goats and sheep. The areas were not fenced, so it was difficult to establish the effective surface grazed by different herds. However, we assumed that the grazing was managed at low intensity because the animals were free to graze neighbouring pastures. Cows were kept outdoors during the entire grazing season (from June to September), they were milked twice per day and were accompanied after milking to areas further away from the stead.

The farm surface was heterogeneous in vegetation, three different vegetation areas were identified: 1. typical grazed pasture; 2. pasture with few sparse natural *Rhododendron ferrugineum*; 3. completely established shrubland (*R. ferrugineum*). The study site consisted of a surface of 20,000 m<sup>2</sup> (200 x 100 m) including the three vegetation areas.

### V.2.3. Surveys, sampling and analysis

Thirty square areas of 10 x 10 m were selected in the study site to determine botanical composition of pasture at different stages of *Rhododendron ferrugineum* establishment. Each square represented homogenous shrub cover that was visually estimated using a 0-100 scale. For each square, herbaceous species and their abundance (%) were assessed visually. The percentages of dominant and more characteristic species in squares with a shrub cover of 0, 25, 50, 75 and 100% are reported in Table V.2.

Six linear transects were placed across the three above-mentioned vegetation areas, and five plots (1 x 1 m) were set along the line. Plots were chosen according to one of the following shrub cover classes: 0% (C0), 25% (C25), 50% (C50), 75% (C75), 100% (C100). During summer 2009, botanical surveys were performed in each plot, and root and soil samples were collected. The botanical composition was investigated in July and all species in each 1 m<sup>2</sup> plot were recorded based on the La Flora d'Italia nomenclature (Pignatti, 1982). The life-form of vascular plants was also reported according to Raunkiaer (1907) and Pignatti (1982). One soil-core (47 mm diameter) was taken from each plot to determine main root parameters in different soil layers. Soil cores were divided in three sections: 0 - 5 cm; 5 - 10 cm; and 10 - 20 cm depths, and then frozen prior to being washed. Before washing a solution of oxalic acid at 2% (Heringa et al., 1980) was used to disperse soil. Roots were separated from soil and organic debris using sieves, samples were then hand-cleaned to remove all the remaining impurities. Samples were temporarily stored in a 12% ethanol solution. Length and diameter of root samples were measured by means of winRHIZO<sup>TM</sup> (Arsenault et al., 1995, Regent Instrument, Canada) analysis software. The software gives all measurements grouped for 10 diameter classes from 0 to 1 mm. Roots were then dried at 105 °C for 36 h to determine biomass dry weight. Root length density (RLD) and root weight density (RWD) of each soil section were calculated based on soil volume.

In each plot one soil sample was also collected, and split into two depths: 0-5 cm (SD1) and 5-20 cm (SD2) to perform soil analysis. Total N content (Kjeldahl total nitrogen [g kg<sup>-1</sup>]), soil organic matter (Springer–Klee method [g kg<sup>-1</sup>]) and pH (1:5 soil:water solution), were measured using the Italian standard soil analysis techniques (G.U., 1999). Main soil characteristics are reported in Table V.1.

**Table V.1.** Mean soil characteristics at two depths of a mountain pasture in eastern Italian Alps at different *Rhododendron ferrugineum* cover classes (C0, C25, C50, C75 and C100).

Depth	Soil properties	Shrub cover classes				
		C0 <sub>a</sub>	C25	C50	C75	C100
0 – 5 cm	pH	3.71	3.62	3.42	3.64	3.54
	N content (g kg <sup>-1</sup> )	11.53	11.39	11.94	9.96	12.73
	Organic matter (g kg <sup>-1</sup> )	318.57	326.94	359.64	341.65	455.77
5 – 20 cm	pH	3.93	3.86	3.78	4.00	3.64
	N content (g kg <sup>-1</sup> )	3.99	3.79	4.67	3.66	5.32
	Organic matter (g kg <sup>-1</sup> )	133.42	140.89	160.38	138.17	188.95

<sup>a</sup> C0, C25, C50, C75 and C100 = 0%, 25%, 50%, 75% and 100% of *R. ferrugineum* cover respectively.

#### V.2.4. Statistical analysis

Number of species per square meter and root parameters (RLD, RWD and average diameter) were subjected to analysis of variance, with the linear transects as replicates, by using SAS mixed procedure (SAS Institute Inc., Cary, NC). Root data were previously transformed to achieve normal distribution. Differences between means were separated using a Tukey's HSD (Honestly Significant Difference) test at 0.05 level of probability. In addition the distribution of roots into diameter classes, as grouped by the winRHIZO software, were correlated with the five shrub covers and also subjected to Partial Redundancy Analyses (partial RDAs) performed with R 2.13.1 (R Core Team, 2011) using vegan library. The RDA is an extension of Principal Component Analysis, in which the canonical vectors are linear combinations both of the response and explanatory variables (Legendre and Legendre, 1998).

### V.3. Results

From botanical surveys we found changes in the floral composition with the increase of *Rhododendron ferrugineum* cover. The most frequent species at C0 level were *Nardus stricta* and *Festuca airoides* followed by *Phleum alpinum*, *Anthoxantum odoratum* and *Carex sempervirens* (Table V.2). According to European Union habitats the pasture can be classified as *Nardus* grassland. A gradual decrease of *Nardus stricta* was observed with the increase of *R. ferrugineum* cover, while the percentage of *F. airoides* was substantially unchanged. Other species, such as *Luzula sieberi*, *Vaccinium myrtillus* and *Calluna vulgaris* increased with the increase of *R. ferrugineum*. More interesting was the analysis of the Raunkier life form. The most represented groups were Hemicryptophyta caespitosa, Hemicryptophyta rhizomatosa and Nano-Phanerophyta (Table V.2). The first group had a mean decrease in percentage with increasing *R. ferrugineum* cover. The percentage of Hemicryptophyta caespitosa species decreased from 36 (C0) to 20 (C100). Differently, Hemicryptophyta rhizomatosa species had maximum values at C100 and C0. We also noted an increase of Nano-Phanerophyta with the increase of *R. ferrugineum* cover.

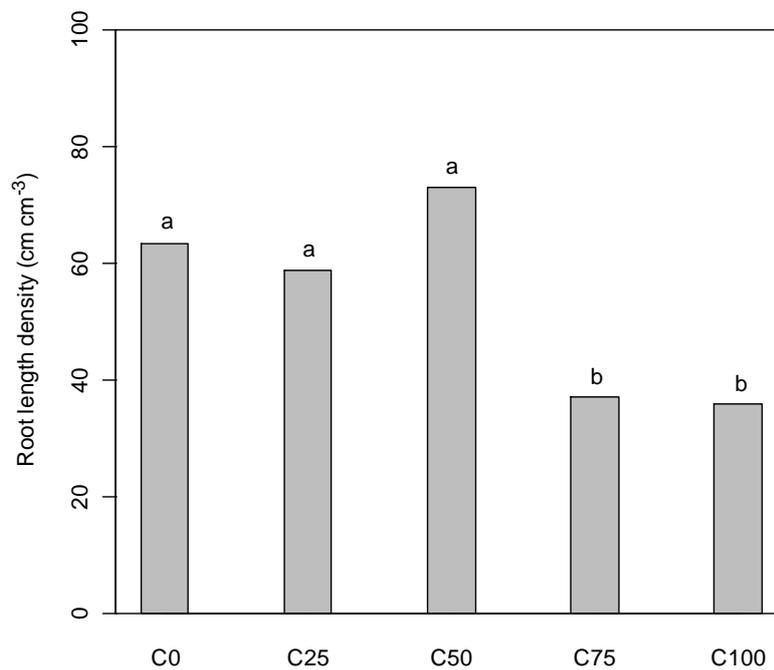
The percentage of shrub cover appeared to have a negative influence on the number of herbaceous species. The shrub cover influenced the number of species per square meter reducing it by 64% passing from C0 to C100. However, significant differences among shrub covers were observed only for C100 (Table V.2).

**Table V.2.** Dominant species, life form and total number of species of a mountain pasture in eastern Italian Alps at different *Rhododendron ferrugineum* cover classes.

Species and life form	Occurrence of species (%)					Occurrence of life form (%)				
	C0 <sub>a</sub>	C25	C50	C75	C100	C0	C25	C50	C75	C100
Geophyta bulbosa						<b>7</b>	<b>7</b>	<b>0</b>	<b>0</b>	<b>0</b>
<i>Crocus albiflorus</i> Kit.	0.2	0.1	-	0.5	-					
Geophyta rhizomatosa						<b>14</b>	<b>14</b>	<b>17</b>	<b>8</b>	<b>20</b>
<i>Lathyrus montanus</i> Bernh.	2.1	4.9	4.0	3.5	3.7					
<i>Oxalis acetosella</i> L.	-	-	-	0.3	3.6					
Chamephyta rhizomatosa						<b>7</b>	<b>7</b>	<b>0</b>	<b>8</b>	<b>0</b>
<i>Antennaria dioica</i> (L.) Gaertner	0.9	0.9	0.4	1.2	0.7					
Hemicryptophyta caespitosa						<b>36</b>	<b>36</b>	<b>33</b>	<b>33</b>	<b>20</b>
<i>Anthoxanthum odoratum</i> L.	2.9	0.2	3.9	3.6	1.7					
<i>Danthonia decumbens</i> (L.) DC.	0.4	1.3	1.1	1.5	-					
<i>Festuca airoides</i> Lam.	12.6	14.8	21.7	13.2	10.1					
<i>Luzula sieberi</i> Tausch	1.1	4.0	7.7	6.1	10.6					
<i>Nardus stricta</i> L.	44.5	38.9	26.6	26.1	11.4					
<i>Phleum alpinum</i> L.	4.6	1.4	1.4	0.6	-					
Hemicryptophyta rhizomatosa						<b>36</b>	<b>29</b>	<b>33</b>	<b>33</b>	<b>40</b>
<i>Leontodon helveticus</i> Merat	0.1	0.2	0.2	0.5	-					
<i>Trifolium pratense</i> L.	0.3	1.0	0.5	0.4	1.1					
<i>Trifolium repens</i> L.	0.3	-	-	-	-					
Nano-Phanerophyta						<b>14</b>	<b>21</b>	<b>25</b>	<b>25</b>	<b>40</b>
<i>Rhododendron ferrugineum</i> L.	-	25.0	50.0	75.0	100.0					
<i>Calluna vulgaris</i> (L.) Hull	2.5	2.3	2.4	5.9	3.3					
<i>Vaccinium myrtillus</i> L.	3.5	8.8	8.5	9.7	34.0					
Number of species						<b>14</b>	<b>15</b>	<b>13</b>	<b>13</b>	<b>5</b>

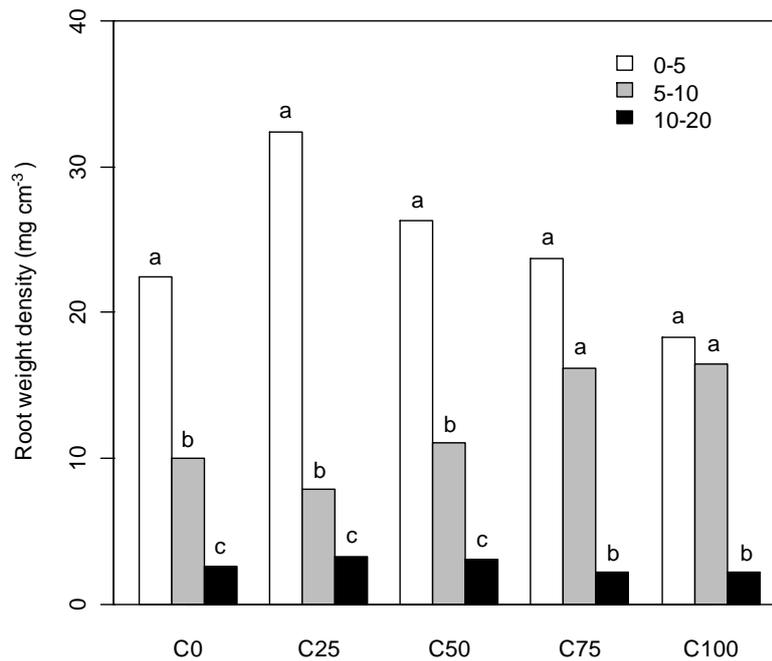
a C0, C25, C50, C75 and C100 = 0%, 25%, 50%, 75% and 100% of *R. ferrugineum* cover respectively.

Results of analysis of variance for RLD showed significant differences among shrub covers ( $P < 0.001$ ) and depth ( $P < 0.001$ ), while no significant interaction was found. Concerning all the shrub cover classes, the percentage of RLD decreased from 53 - 59% in the upper layer (0-5 cm) to 10 -15% in the lower (10-20 cm), with intermediate values (31 -34%) found in the 5-10 cm layer (data not shown). The RLD values of high shrub cover classes (C75 and C100) were significantly lower than those of C0, C25 and C50 (Figure V.1). A drastic reduction of 49% was observed from C50 to C75, while the difference between mean RLD of C0, C25, C50 and mean RLD of C75, C100 was less than 41%.



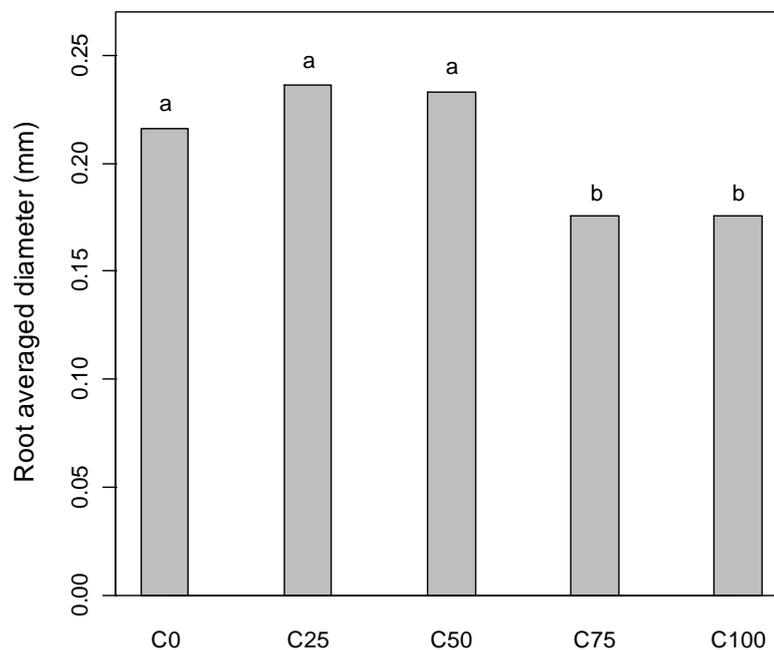
**Figure V.1.** Effect of *Rhododendron ferrugineum* cover on root length density, mean of three depth levels (0-5, 5-10 and 10-20 cm). Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25, C50, C75 and C100 = 0%, 25%, 50%, 75% and 100% of *R. ferrugineum* cover respectively.

The analysis of variance for RWD revealed a significant shrub cover  $\times$  depth interaction ( $P < 0.01$ ), the depth effect was also significant ( $P < 0.001$ ), while no significant differences were found for the shrub cover effect. Differences were found among all three depth layers for the cover classes C0, C25 and C50, while for C75 and C100 only the deepest layer (10-20 cm) was significantly different than the two upper layers (Figure V.2). Regarding the depth effect, a gradual decrease from the upper to the lower layer was observed (data not shown).



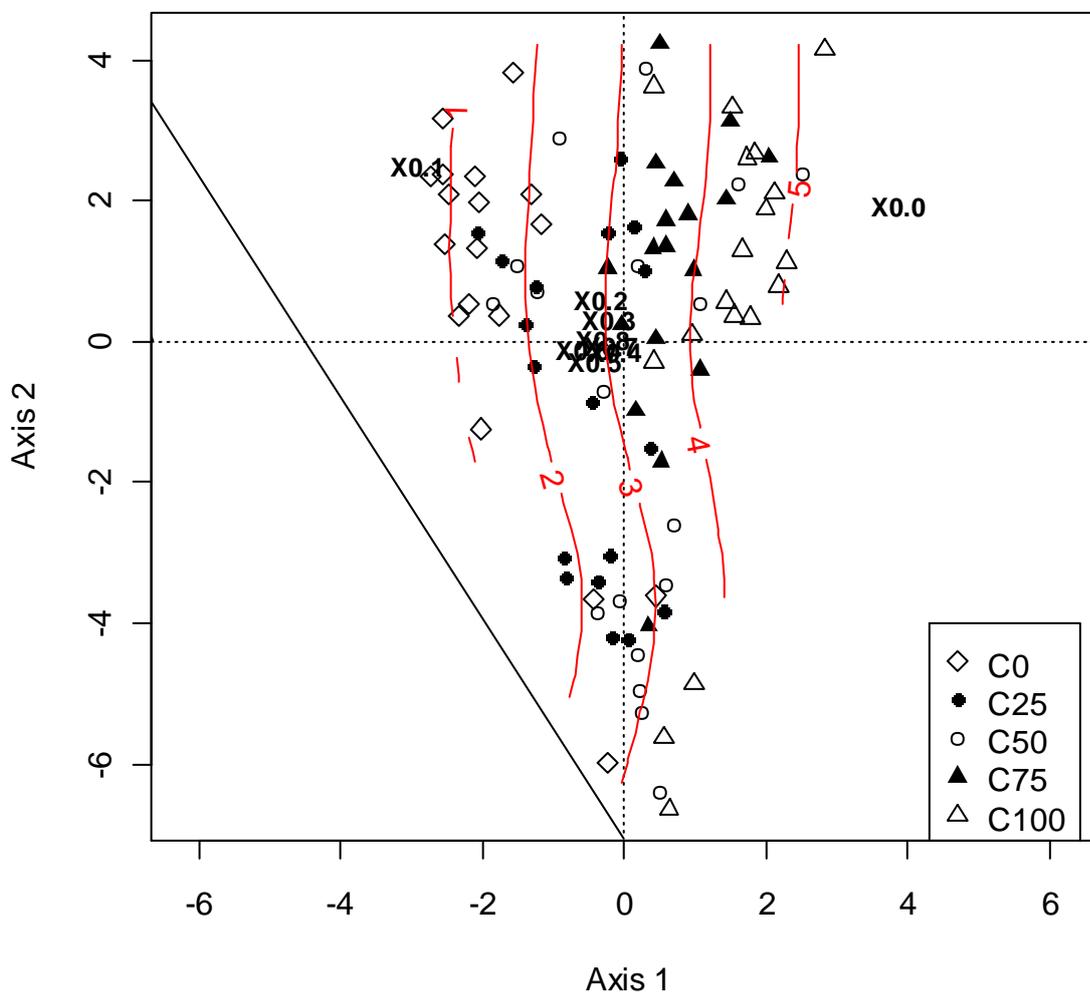
**Figure V.2.** Effect of *Rhododendron ferrugineum* cover on root weight density. Interaction depth x shrub cover. Within *R. ferrugineum* cover class bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25, C50, C75 and C100 = 0%, 25%, 50%, 75% and 100% of *R. ferrugineum* cover respectively.

The analysis of variance for root diameter revealed significant differences only for main effects [shrubs cover ( $P < 0.001$ ) and depth ( $P < 0.001$ )]. Average diameter, for all the shrub cover classes, decreased significantly from 0.23 mm in the upper layer (0-5 cm), to 0.19 mm in the lower (10-20 cm) (data not shown). Average root diameters of classes with high shrub cover (C75 and C100) were significantly lower than those of C0, C25 and C50 (Figure V.3), passing from 0.18 mm (average of C7, C100, ) to 0.23 mm (average of C0, C25 and C50).



**Figure V.3.** Effect of *Rhododendron ferrugineum* cover on root average diameter. Bars with the same letter are not significantly different according to Tukey's HSD test at 0.05 level of probability. C0, C25, C50, C75 and C100 = 0%, 25%, 50%, 75% and 100% of *R. ferrugineum* cover respectively.

The heterogeneity of vegetation suggested conducting an additional RDA analysis for the distribution of roots into diameter classes. A first RDA showed that three diameter classes could describe the samples distribution on the graph. The three classes were 0-0.1 mm, 0.1-0.2 mm and >1.0 mm. We expected that the class with higher diameter values had a positive correlation with the increase in shrub cover. But when we tested correlations between the three classes and the shrub cover, we found that this correlation was significant only for the two smaller classes [ $R^2=0.61^{***}$  (0-0.1 mm);  $R^2=0.33^{***}$  (0.1-0.2 mm)]. We found that the bigger class was not important for the description of samples similarity, as a consequence of vegetation heterogeneity. Therefore we decided to exclude it from RDA analysis. Results of RDA are reported in Figure V.4.



**Figure V.4.** Ordination plot of root diameter classes and core samples along the first two axes of RDA. Solid lines are the model surface of cover variable fitted to ordination. C0 = 0% of *Rhododendron ferrugineum* cover; C25 = 25% of *R. ferrugineum* cover; C0, C25, C50, C75 and C100 = 0%, 25%, 50%, 75% and 100% of *R. ferrugineum* cover respectively. X0.0 = 0-0.1 mm diameter class; X0.1 = 0.1-0.2 mm diameter class; X0.2 = 0.2-0.3 mm diameter class; X0.3 = 0.3-0.4 mm diameter class; X0.4 = 0.4-0.5 mm diameter class; X0.5 = 0.5-0.6 mm diameter class; X0.6 = 0.6-0.7 mm diameter class; X0.7 = 0.7-0.8 mm diameter class; X0.8 = 0.8-0.9 mm diameter class.

The first axis accounted for 49.9% of the total variation and separated most core samples based on a gradient of shrub cover. The correlation between ordination and shrub cover was significant ( $P < 0.05$ ). The figure shows that C0 and C100 were well separated from axis 1, while the intermediate classes were more blended. The second axis was less important, capturing only 11.0% of the total variation, and dividing the samples mainly according to the depth. Moreover, the depth was highly significant and perpendicular to the percentage of shrub cover. The ordination showed that the first two diameter classes (0.0-0.1 and 0.1-0.2 mm) were the most influenced by axis 1. In fact, these classes were the most represented with total value that ranged from 28% to 56%. Their percentage changed with increasing shrub cover. Class 0.0-0.1 mm had a mean percentage of 13 at C0, a value that increased to 24% at C100. Class 0.1-0.2 mm had a mean value of 33% at C0, while at C100 it was 23%.

#### **V.4. Discussion**

The under-grazing of *Nardus* grassland, due to the difficult topography (with steep and flatter areas on the same pasture), together with the addition of concentrates in the livestock diet, allows the establishment of *Rhododendron ferrugineum* in areas that receive very little grazing pressure. The establishment of this species involves a loss of canopy cover in the herbaceous layer together with a loss of species richness. The effectiveness of plant cover for reducing erosion by runoff depends on the height and continuity of the canopy, plant density and ground cover (Morgan, 2005). Therefore, a loss of herbaceous cover could influence the capacity of vegetation to reduce soil erosion. This was demonstrated in the study of Gysels and Poesen (2003), where the cross-sectional areas of gullies remained small for high shoot densities of grass, whatever the water flow intensity was. This loss of the herbaceous layer could be replaced by the cover of a shrub layer, but the question is: “have the two layers a similar effect on the soil erosion control?”. The present study showed important variations in the vegetation composition as well as in the occurrence of Raunkiaer life forms with increasing shrub cover. Rhizomatosea and Nano-Phanerophytae life forms increased with the increase of shrub cover replacing Caespitosea life form. This change in the life form composition involves changes, not only at canopy level, but also in the belowground layer.

Root length density is the best indicator of soil exploration capacity by roots. Grasses are characterized by many fine roots forming a very dense root system, on the contrary the root system of trees and shrubs consists of fewer thick roots (De Baets, 2006). Mapfumo et al. (2002) demonstrated that intraspecific competition causes a reduction in root mass. Our results confirmed the negative effect of intraspecific competition on RLD of the herbaceous layer. A marked reduction in RLD was observed at 75 and 100% of *R. ferrugineum* cover. Instead, the effect of *R. ferrugineum* cover on RWD was not clear. The three depths layers were clearly differentiated by the analysis of variance for C0, C25 and C50, while the difference between the two upper layers (0-5 cm and 5-10 cm) was not significant for C75 and C100. This could be the consequence of an increase of root biomass in the middle layer and a reduction of root mass in the upper layer. The deepening of RWD was due to the change of life forms together with the increase of *R. ferrugineum* cover. The Nano-Phanerophytæ, in fact, has a deeper root system than the Hemicryptophytæ or Chamephytæ. It is well documented that root reinforcement of the soil against erosion is due not only to the number but also the size of roots (Cammeraat et al., 2005; Reubens et al., 2007). Roots, especially the fine ones, are important factors that account for the reinforcement of the soil against erosion (Canadell et al., 1996; Jackson et al., 1997; Van Beek et al., 2005). The results of our study revealed that the belowground component of *Nardus* grassland is mainly composed of fine roots. The increase of *R. ferrugineum* cover caused a decrease in average diameter as demonstrated by analysis of variance. Moreover, observing the percentage of single classes on total samplings we found that the 0-0.1 mm class increased in percentage at the expense of the 0.1-0.2 mm class.

## V.5. Conclusion

The natural establishment of *Rhododendron ferrugineum* on *Nardus* grassland impacted on both biodiversity and soil stability. This study demonstrated a loss of herbaceous species and a decrease in root length density and average diameter because of the invasion of *R. ferrugineum*. Important changes were observed for the root mass distribution in the soil profile. The differences between shallower soil layers (0-5 cm and 5-10 cm) decreased with the increase in *R. ferrugineum*. At low shrub cover, root mass was mainly located in the upper soil layer (0-5 cm), while at high shrub cover, it was found either in the 0-5 or 5-10 cm depth. It is interesting

to underline that all root characteristics analyzed gave significant differences passing from 50 and 75% of *R. ferrugineum* cover. These results may have an important consequence on soil stability. Under high *R. ferrugineum* cover the soil of *Nardus* grassland could be more vulnerable to erosion.

## **Chapter VI**

### **Main conclusions**

The results deriving from the first insight denote a wood establishment effect on number of species. We found that sites with lower altitude are more vulnerable and the influence of wood establishment have a stronger effect on species richness. In addition, results suggests that a low percentage of shrubs and trees on grassland improves biodiversity of pastures.

However, as expected, from botanical composition analysis we have come to the conclusion that wood cover influences the presence and the abundance of mountain pastures species, and this effect is common of all the sites studied.

Together with the shift in plant biodiversity (loss of number of species) and botanical composition, wood establishment negatively affects also herbage yield and quality. A decrease that occurs starting from low percentage of wood cover.

Furthermore, based on root biomass reduction due to wood establishment observed on Alpine pastures the soil stability can be damaged, and especially at high cover percentage.

In a global view, preserving Alpine pasture habitats means to preserve a high ecological habitats. Wood establishment and both natural and human variables have a complex relationship. The connection between natural evolution within sites and changes in the landscape structure at site level is of crucial importance for a better understanding of cultural landscape transformation and their ecological impacts (Tasser et al., 2007). In order to prevent the decay of these habitats grassland management is required

Maintaining human activities on Alpine pastures is a good tool for preserving high ecological value of this habitat. It is necessary to identify the specific vocation of sites: productive or ecological. From results of this study, we suggest that in an Alpine pasture, where the dairy production is economically sustainable, the grazing surface should be preserve without shrubs or trees. In fact, a decrease of herbage quality due to low percentage of wood cover could affect dairy quality and in particular the cheese taste and moisture (Buchin et al 1999). Whereas productivity vocation is not so relevant, maintenance of low percentage of shrubs and trees on grazing surface could improve ecological value of this habitat.



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## Appendix 1

Botany board for each studied site.

Each botany board includes mean annual temperature, altitude and annual precipitation of each site. Soil properties of samples collected in 1 m<sup>2</sup> plots are reported. A 100 m<sup>2</sup> samples matrices was used to analyze botanical composition. The original matrices were split in two matrices: one for dominant species (species with a cover percentage more than 3) and one for rare species (species with a cover percentage less that 1). Furthermore the average Ellenberg's indicators and forage values (Klapp, 1971) for 10 wood cover classes are also reported.

## A.1. SITE A – Camporossignolo, Campomezzavia and Valforbice summer farms.



Altitude (m a.s.l.): 1050-1150

Mean annual temperature (°C): 6.68

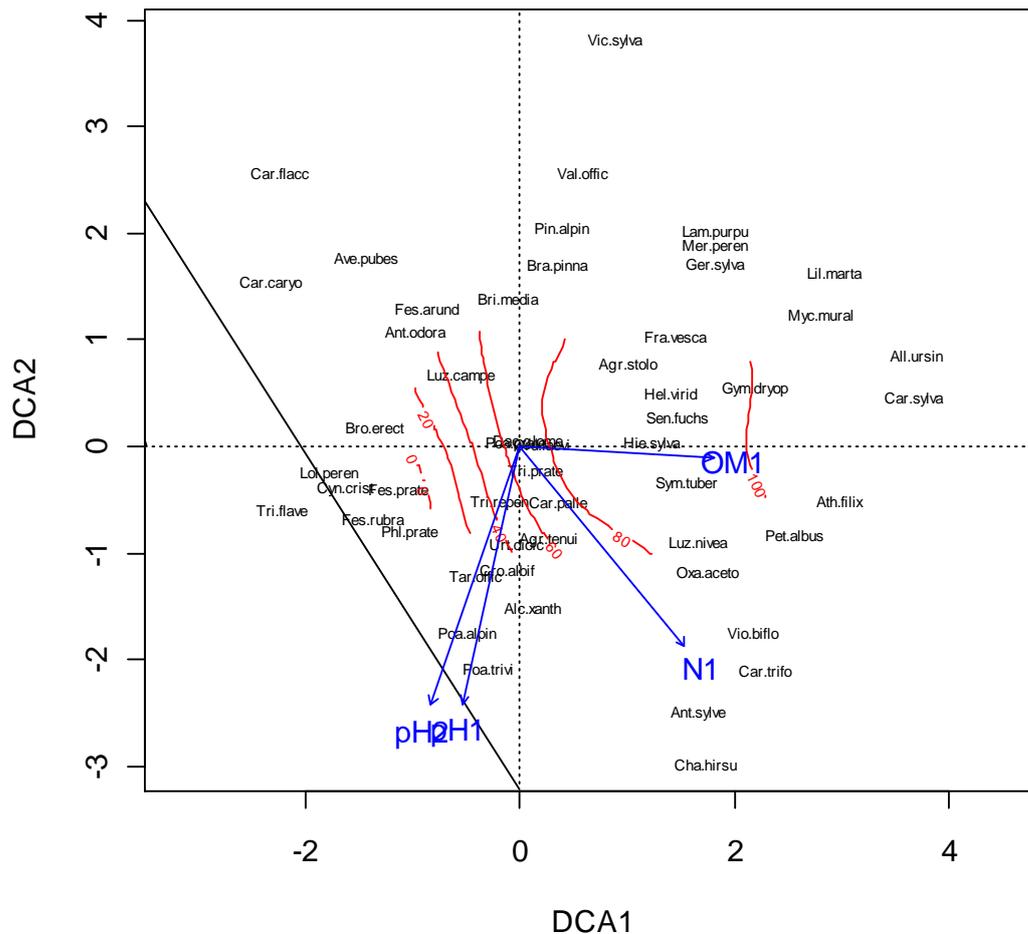
Precipitation (mm year<sup>-1</sup>): 1460

Main wood and shrub species:

*Fagus sylvatica* L. with *Corylus avellana* L., *Berberis vulgaris* L., *Rosa canina* L., *Crataegus monogyna* Jacq..

**Table A.1.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5-20 cm).

Depth	Soil properties	wood cover class				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	3.42±0.14	3.32±0.08	3.44±0.12	3.51±0.14	3.18±0.12
	pH (KCl)	3.26±0.18	3.20±0.11	3.24±0.16	3.30±0.25	3.00±0.13
	N content (g kg <sup>-1</sup> )	3.41±0.30	4.00±0.52	3.62±0.40	4.34±0.22	4.88±0.10
	Organic matter (g kg <sup>-1</sup> )	151.9±36.4	191.0±51.6	238.8±21.1	226.5±22.0	403.3±80.8
5 – 20 cm	pH (H <sub>2</sub> O)	3.84±0.17	3.72±0.19	3.93±0.13	3.85±0.23	3.62±0.24
	pH (KCl)	3.80±0.27	3.54±0.24	3.87±0.14	3.59±0.34	3.46±0.32
	N content (g kg <sup>-1</sup> )	1.84±0.30	2.42±0.52	2.39±0.20	2.64±0.43	2.00±0.32
	Organic matter (g kg <sup>-1</sup> )	101.7±33.9	169.8±33.3	187.6±7.5	164.4±26.5	146.5±20.8

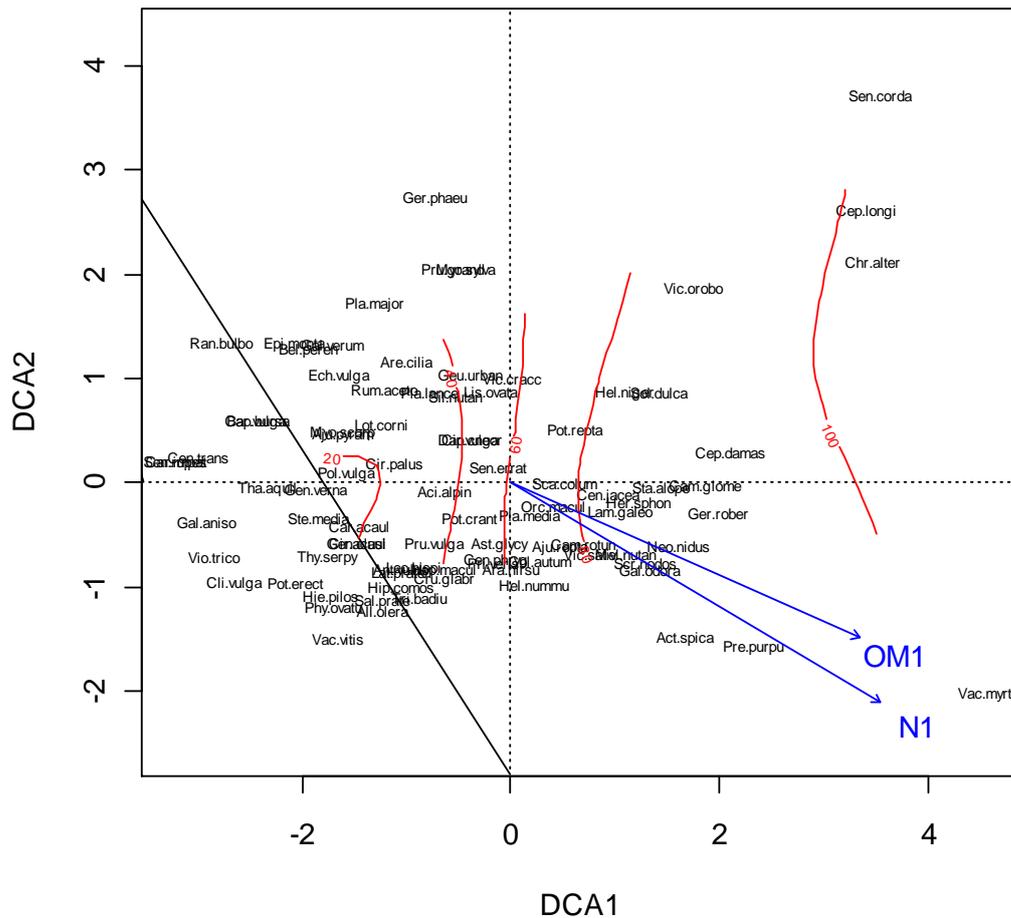


**Figure A.1.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vectors are significant ( $P < 0.05$ ) fitted soil proprieties vectors. With DCA and samples dataset we found three behaviors of dominant species:

Decreasing with wood cover: *Trisetum flavescens* (L.) Beauv., *Lolium perenne* L., *Cynosurus cristatus* L., *Festuca rubra* L., *Festuca pratensis* Hudson, *Phleum pratense* L., *Bromus erectus* Hudson, *Carex caryophyllea* La Tourr., *Poa alpina* L..

Maximum percentage at intermediate wood cover: *Avenula pubescens* (Hudson) Dumort., *Festuca arundinacea* Schreber, *Anthoxanthum odoratum* L., *Luzula campestris* (L.) DC., *Carex pallescens* L., *Brachypodium pinnatum* (L.) Beauv..

Increasing with wood cover: *Fragaria vesca* L., *Agrostis stolonifera* L., *Helleborus viridis* L., *Senecio fuchsii* Gmelin, *Symphytum tuberosum* L., *Luzula nivea* (L.) Lam. et DC., *Oxalis acetosella* L., *Gymnocarpium dryopteris* (L.) Newman, *Athyrium filix-foemina* (L.) Roth, *Carex sylvatica* Hudson, *Allium ursinum* L..



**Figure A.2.** Ordination plot of rare species (specie cover < 1%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vectors are significant ( $P < 0.05$ ) fitted soil proprieties vectors. With DCA and samples dataset we found three behaviors of rare species:

Decreasing with wood cover: *Galium anisophyllum* Vill., *Viola tricolor* L., *Centaurea transalpina* Schleicher, *Gentiana verna* L., *Polygala vulgaris* L., *Thymus serpyllum* L..

Maximum presence at intermediate wood cover: *Acinos alpinus* (L.) Moench, *Prunella vulgaris* L., *Rumex acetosa* L., *Lotus corniculatus* L., *Geum urbanum* L., *Listera ovata* (L.) R.Br., *Potentilla crantzii* (Crantz) Beck, *Senecio erraticus* Bertol., *Centaurea phrygia* L. (C. A. Meyer) Gugler, *Potentilla reptans* L., *Scabiosa columbaria* L., *Orchis maculata* L., *Ajuga reptans* L..

Increasing with wood cover: *Helleborus niger* L., *Solanum dulcamara* L., *Cephalanthera damasonium* (Miller) Druce, *Centaurea jacea* L., *Geranium robertianum* L., *Neottia nidus-avis* (L.) L. C. Rich..

**Table A.2.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	5	20	35	50	60	70	80	90	100
pH	1.79	2.47	2.16	2.24	2.59	2.61	2.84	2.79	3.54	4.51
	± 0.38	± 0.37	± 0.26	± 0.34	± 0.43	± 0.45	± 0.10	± 0.48	± 0.40	± 0.41
Nutrient	4.13	3.86	3.97	4.10	4.17	4.09	4.49	4.70	4.62	5.63
	± 0.45	± 0.33	± 0.06	± 0.40	± 0.51	± 0.46	± 0.25	± 0.33	± 0.13	± 0.04
Light	6.65	6.54	6.35	6.44	5.80	6.07	5.80	5.68	5.17	3.77
	± 0.20	± 0.10	± 0.21	± 0.16	± 0.24	± 0.15	± 0.25	± 0.20	± 0.31	± 0.06
Temperature	1.27	1.11	1.26	1.57	1.44	1.42	1.79	1.76	2.05	2.31
	± 0.18	± 0.05	± 0.24	± 0.22	± 0.21	± 0.18	± 0.08	± 0.10	± 0.33	± 0.33
Humidity	4.07	3.55	3.56	3.93	3.80	4.12	4.38	4.16	4.27	5.17
	± 0.29	± 0.20	± 0.06	± 0.51	± 0.61	± 0.16	± 0.40	± 0.28	± 0.16	± 0.19
Forage value	4.89	4.63	4.59	4.65	3.63	3.77	3.23	3.45	2.28	0.38
	± 0.17	± 0.30	± 0.17	± 0.11	± 0.22	± 0.33	± 0.68	± 0.57	± 0.45	± 0.03

## A.2. SITE B – Marcesina di Sopra and Ronchetto summer farms.

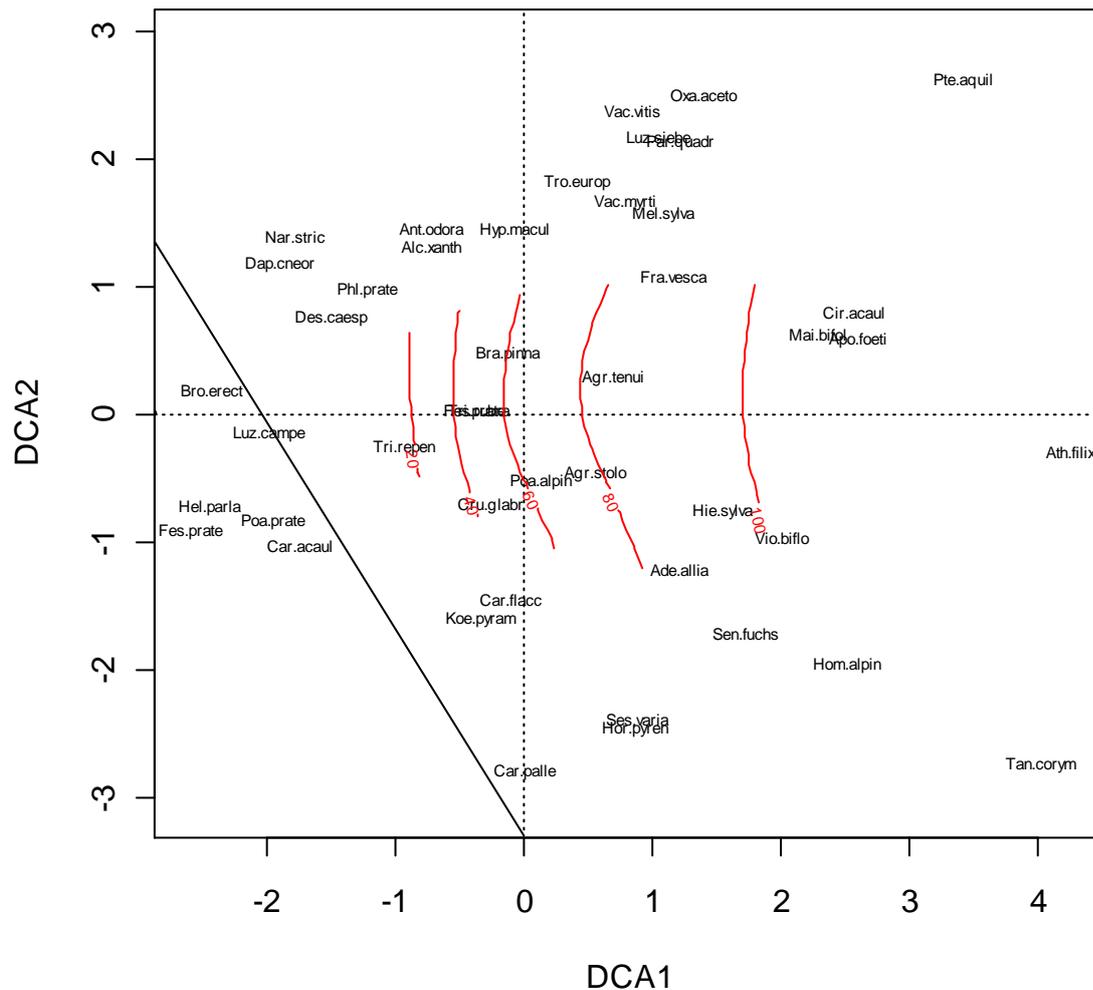


Altitude (m a.s.l.): 1300-1350

Mean annual temperature (°C): 5.38

Precipitation (mm year<sup>-1</sup>): 1550Main wood species: *Picea excelsa* (Lam.) Link**Table A.3.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5-20 cm).

Depth	Soil properties	wood cover class				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	4.84 ± 0.15	5.32 ± 0.19	5.05 ± 0.29	4.85 ± 0.09	4.52 ± 0.22
	pH (KCl)	4.15 ± 0.31	4.60 ± 0.15	4.53 ± 0.40	4.24 ± 0.08	3.90 ± 0.23
	N content (g kg <sup>-1</sup> )	8.50 ± 0.57	7.90 ± 0.70	8.60 ± 0.90	9.00 ± 1.20	10.60 ± 1.20
	Organic matter (g kg <sup>-1</sup> )	254.8 ± 14.4	231.8 ± 5.6	287.7 ± 24.9	355.6 ± 43.4	590.1 ± 48.8
5 – 20 cm	pH (H <sub>2</sub> O)	5.24 ± 0.13	6.11 ± 0.29	5.94 ± 0.31	5.72 ± 0.27	4.61 ± 0.24
	pH (KCl)	4.17 ± 0.21	5.66 ± 0.44	5.32 ± 0.46	5.19 ± 0.45	4.03 ± 0.26
	N content (g kg <sup>-1</sup> )	5.70 ± 0.90	4.80 ± 0.70	4.80 ± 0.90	5.90 ± 0.80	8.30 ± 1.20
	Organic matter (g kg <sup>-1</sup> )	172.9 ± 14.8	155.1 ± 14.7	153.8 ± 16.7	181.5 ± 14.9	311.0 ± 45.0



**Figure A.3.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination. With DCA and samples dataset we found three behaviors of dominant species:

Decreasing with wood cover: *Bromus erectus* Hudson, *Festuca pratensis* Hudson, *Helictotrichon parlatorei* (Woods) Pilger, *Luzula campestris* (L.) DC., *Poa pratensis* L., *Nardus stricta* L., *Deschampsia caespitosa* (L.) Beauv., *Trifolium repens* L..

Maximum percentage at intermediate wood cover: *Anthoxanthum odoratum* L., *Anthriscus sylvestris* (L.) Hoffm., *Cruciata glabra* (L.) Ehrend., *Koeleria pyramidata* (Lam.) Domin, *Poa alpina* L., *Brachypodium pinnatum* (L.) Beauv. *Agrostis tenuis* Sibth..

Increasing with wood cover: *Agrostis stolonifera* L., *Adenostyles alliariae* (Gouan) Kerner, *Fragaria vesca* L., *Melampyrum sylvaticum* L., *Hieracium sylvaticum* (L.) L., *Aposeris foetida* (L.) Less., *Maianthemum bifolium* (L.) Schmidt.



**Table A.4.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood.cover.

Ellenberg's indicators values	wood cover (%)									
	0	10	25	40	50	60	70	80	90	100
pH	2.80	3.01	3.08	3.49	3.84	3.70	3.49	3.84	3.73	3.82
	± 0.49	± 0.27	± 0.42	± 0.42	± 0.40	± 0.32	± 0.22	± 0.46	± 0.29	± 0.20
Nutrient	3.51	3.34	3.39	3.19	3.32	3.26	3.52	3.53	3.60	4.15
	± 0.42	± 0.30	± 0.46	± 0.21	± 0.18	± 0.13	± 0.29	± 0.35	± 0.01	± 0.23
Light	6.72	6.73	6.45	6.45	6.41	6.20	5.97	5.99	5.70	4.70
	± 0.32	± 0.21	± 0.26	± 0.18	± 0.27	± 0.21	± 0.29	± 0.45	± 0.23	± 0.22
Temperature	1.13	1.44	1.08	1.46	1.42	1.35	1.40	1.30	1.28	1.95
	± 0.29	± 0.29	± 0.35	± 0.22	± 0.27	± 0.21	± 0.09	± 0.15	± 0.11	± 0.20
Humidity	3.90	3.90	3.80	3.79	3.75	4.08	4.21	4.18	4.29	4.60
	± 0.29	± 0.27	± 0.36	± 0.05	± 0.14	± 0.06	± 0.06	± 0.07	± 0.40	± 0.24
Forage value	3.56	3.41	3.53	3.07	2.98	2.51	2.44	2.12	1.53	0.54
	± 0.26	± 0.32	± 0.24	± 0.04	± 0.08	± 0.20	± 0.23	± 0.35	± 0.22	± 0.12

## A.3. SITE C – Moline and Fossetta summer farms.

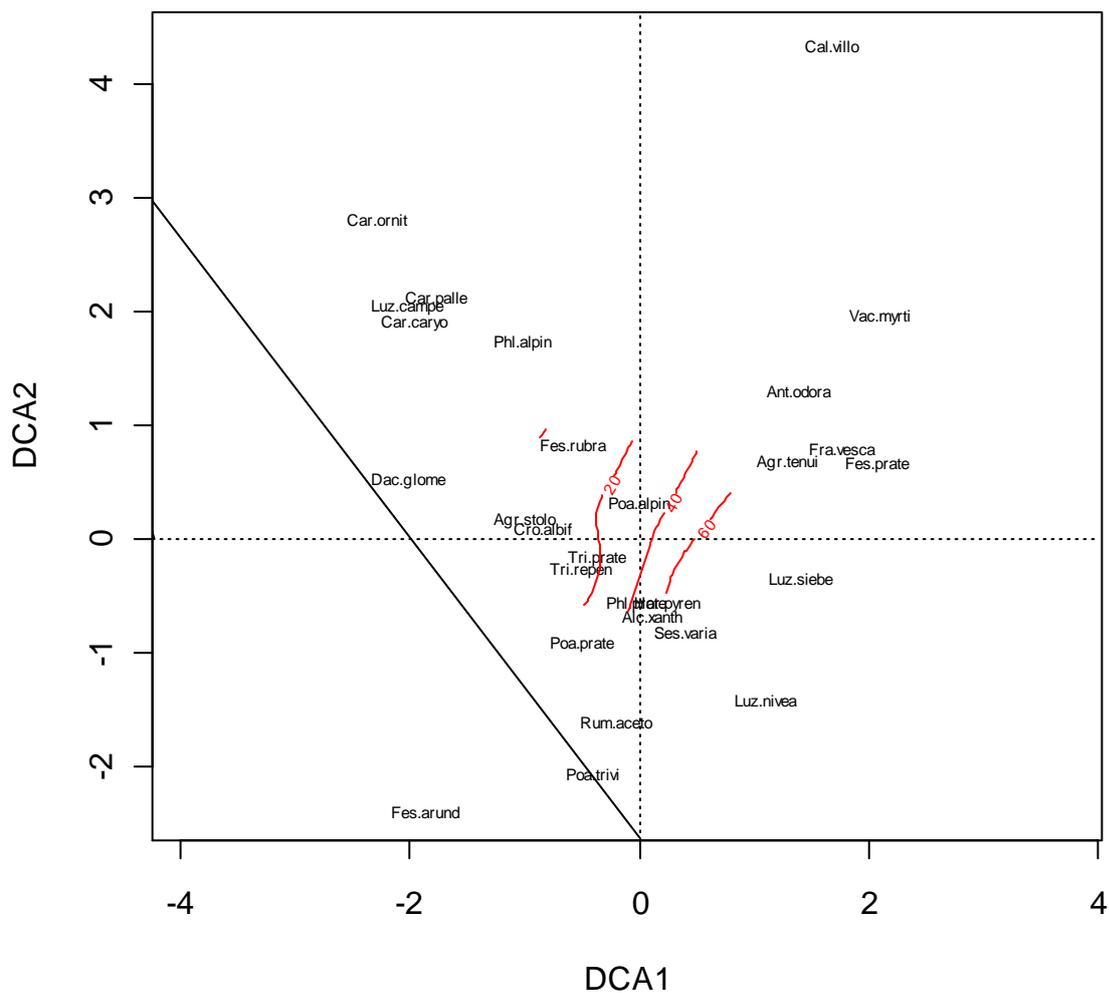


Altitude (m a.s.l.): 1650-1750

Mean annual temperature (°C): 3.66

Precipitation (mm year<sup>-1</sup>): 1550Main wood species: *Larix decidua* Miller**Table A.5.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5–20 cm).

Depth	Soil properties	wood cover classes				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	4.76 ± 0.19	4.45 ± 0.20	4.26 ± 0.24	4.36 ± 0.12	3.88 ± 0.19
	pH (KCl)	5.66 ± 0.18	5.25 ± 0.17	5.06 ± 0.16	5.03 ± 0.06	4.61 ± 0.18
	N content (g kg <sup>-1</sup> )	17.72±1.95	22.63±2.69	21.31±3.45	20.12±3.50	19.49±2.01
	Organic matter (g kg <sup>-1</sup> )	383.9±25.1	496.1±57.0	513.1±62.3	551.8±74.9	588.4±50.6
5 – 20 cm	pH (H <sub>2</sub> O)	5.39 ± 0.40	4.82 ± 0.32	4.67 ± 0.49	5.23 ± 0.28	4.90 ± 0.29
	pH (KCl)	6.11 ± 0.29	5.72 ± 0.27	5.71 ± 0.35	5.93 ± 0.22	5.44 ± 0.29
	N content (g kg <sup>-1</sup> )	10.89±0.58	13.80±2.19	14.94±3.49	17.02±2.63	16.39±2.20
	Organic matter (g kg <sup>-1</sup> )	235.1±31.2	290.0±58.6	311.6±86.4	382.2±49.8	387.9±66.1

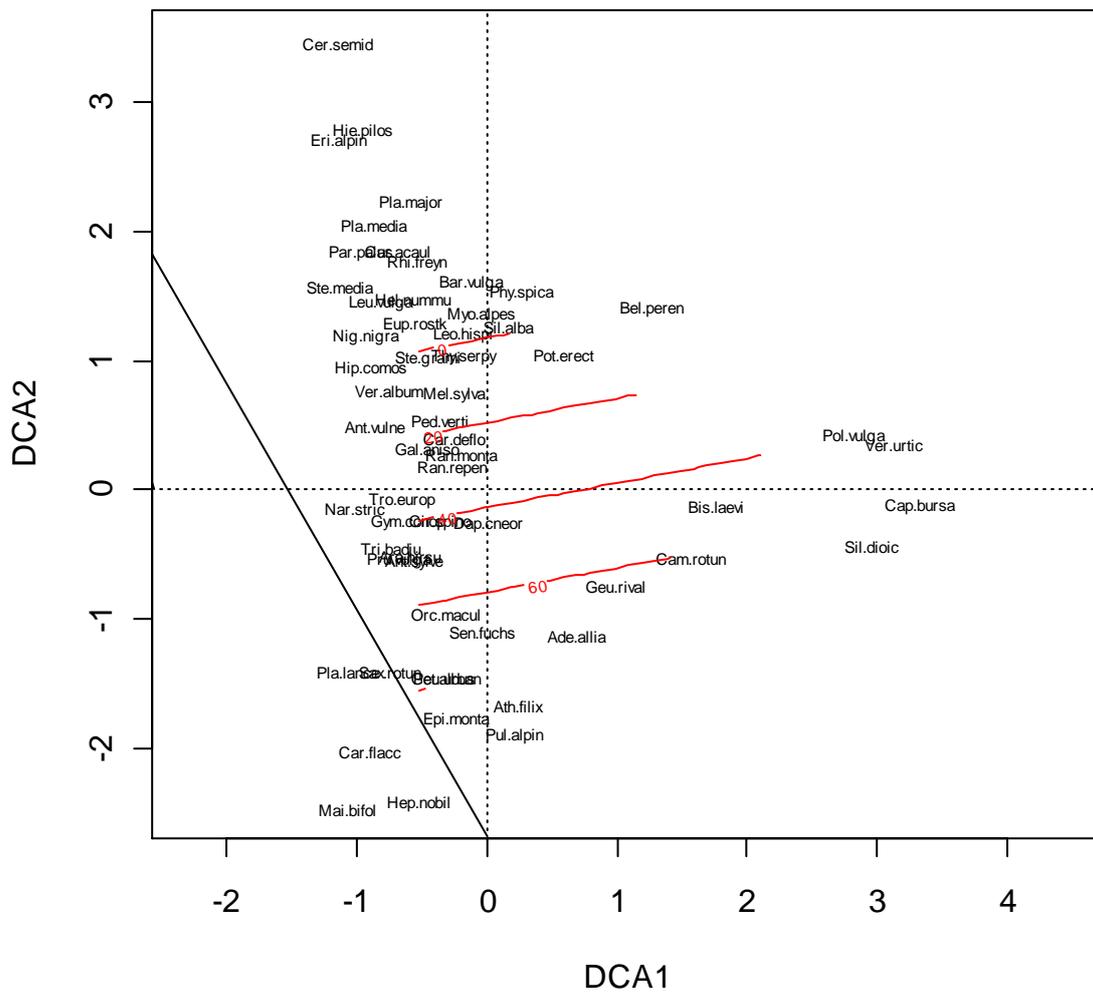


**Figure A.5.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination. With DCA and samples dataset we found three behaviors of dominant species:

Decreasing with wood cover: *Luzula campestris* (L.) DC., *Carex caryophyllea* La Tourr., *Carex pallescens* L., *Phleum alpinum* L., *Dactylis glomerata* L., *Festuca rubra* L., *Agrostis stolonifera* L..

High percentage at lower and intermediate wood cover: *Trifolium pratense* L., *Trifolium repens* L., *Poa alpina* L., *Poa pratensis* L., *Alchemilla xanthochlora* Rothm..

Increasing with wood cover: *Anthoxanthum odoratum* L., *Agrostis tenuis* Sibth., *Fragaria vesca* L., *Festuca pratensis* Hudson, *Luzula sieberi* Tausch, *Luzula nivea* (L.) Lam. et DC.



**Figure A.6.** Ordination plot of rare species (specie cover < 1%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination. With DCA and samples dataset we found three behaviors of rare species:

Decreasing with wood cover: *Hieracium pilosella* L., *Erigeron alpinus* L., *Euphrasia rostkoviana* Hayne, *Myosotis alpestris* F. W. Schmidt, *Leontodon hispidus* L., *Potentilla erecta* (L.) Rauschel, *Pedicularis verticillata* L., *Carduus defloratus* L., *Ranunculus repens* L..

High presence at intermediate wood cover: *Campanula rotundifolia* L., *Geum rivale* L., *Orchis maculata* L., *Senecio fuchsii* Gmelin.

Increasing with wood cover: *Athyrium filix-foemina* (L.) Roth, *Maianthemum bifolium* (L.) Schmidt, *Hepatica nobilis* Miller, *Carex flacca* Schreber,

**Table A.6.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	5	10	20	30	40	50	60	70	80
pH	2.61	2.86	2.36	2.25	2.33	2.28	1.98	2.10	2.12	2.16
	± 0.44	± 0.28	± 0.33	± 0.19	± 0.19	± 0.08	± 0.19	± 0.49	± 0.16	± 0.12
Nutrient	4.09	3.67	3.94	4.30	4.18	4.05	4.22	3.82	3.85	3.84
	± 0.55	± 0.16	± 0.43	± 0.17	± 0.25	± 0.27	± 0.28	± 0.56	± 0.23	± 0.21
Light	7.16	6.93	6.54	6.86	6.63	6.05	6.02	5.62	5.54	5.24
	± 0.06	± 0.16	± 0.09	± 0.08	± 0.19	± 0.10	± 0.08	± 0.23	± 0.05	± 0.11
Temperature	0.98	1.08	1.01	0.98	1.15	1.26	1.11	1.26	1.33	1.43
	± 0.10	± 0.21	± 0.14	± 0.10	± 0.19	± 0.16	± 0.11	± 0.11	± 0.09	± 0.03
Humidity	4.42	4.30	4.42	4.63	4.71	4.48	4.27	4.40	4.44	4.36
	± 0.42	± 0.34	± 0.15	± 0.14	± 0.27	± 0.39	± 0.17	± 0.42	± 0.11	± 0.11
Forage value	3.85	3.37	3.61	4.19	4.09	3.24	3.73	3.39	3.36	3.21
	± 1.16	± 0.18	± 0.25	± 0.34	± 0.13	± 0.62	± 0.22	± 0.35	± 0.13	± 0.15

## A.4. SITE D – Galmarara summer farms.

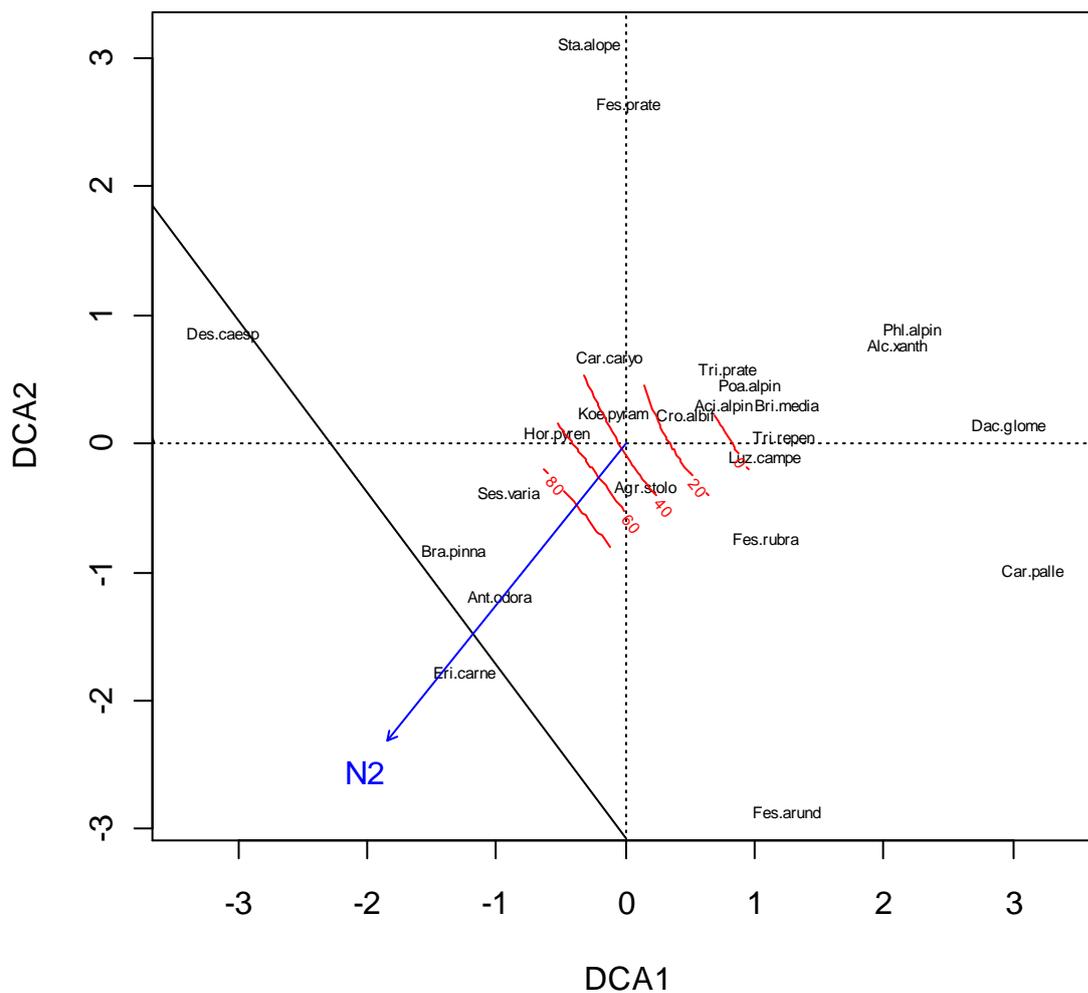


Altitude (m a.s.l.): 1600-1700

Mean annual temperature (°C): 3.48

Precipitation (mm year<sup>-1</sup>): 1550Main wood and shrub species: *Pinus mugo* Turra and *Juniperus communis* L..**Table A.7.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5-20 cm).

Depth	Soil properties	wood cover classes				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	6.46 ± 0.12	6.42 ± 0.10	6.02 ± 0.20	5.58 ± 0.22	4.95 ± 0.28
	pH (KCl)	6.06 ± 0.22	5.71 ± 0.34	5.66 ± 0.29	5.05 ± 0.20	4.76 ± 0.52
	N content (g kg <sup>-1</sup> )	6.75 ± 0.80	6.45 ± 0.61	5.44 ± 0.59	5.49 ± 1.20	5.45 ± 0.48
	Organic matter (g kg <sup>-1</sup> )	359.4±35.0	337.5±38.2	311.5±45.2	369.0±18.5	431.0±37.4
5 – 20 cm	pH (H <sub>2</sub> O)	6.64 ± 0.09	6.71 ± 0.10	6.55 ± 0.09	6.45 ± 0.09	6.09 ± 0.28
	pH (KCl)	6.77 ± 0.06	6.68 ± 0.05	6.60 ± 0.14	6.49 ± 0.10	5.89 ± 0.25
	N content (g kg <sup>-1</sup> )	4.07 ± 0.35	4.00 ± 0.48	5.35 ± 0.59	3.79 ± 0.52	3.95 ± 0.65
	Organic matter (g kg <sup>-1</sup> )	230.6±13.5	234.8±18.6	213.7±38.0	242.2±20.5	266.6±30.2

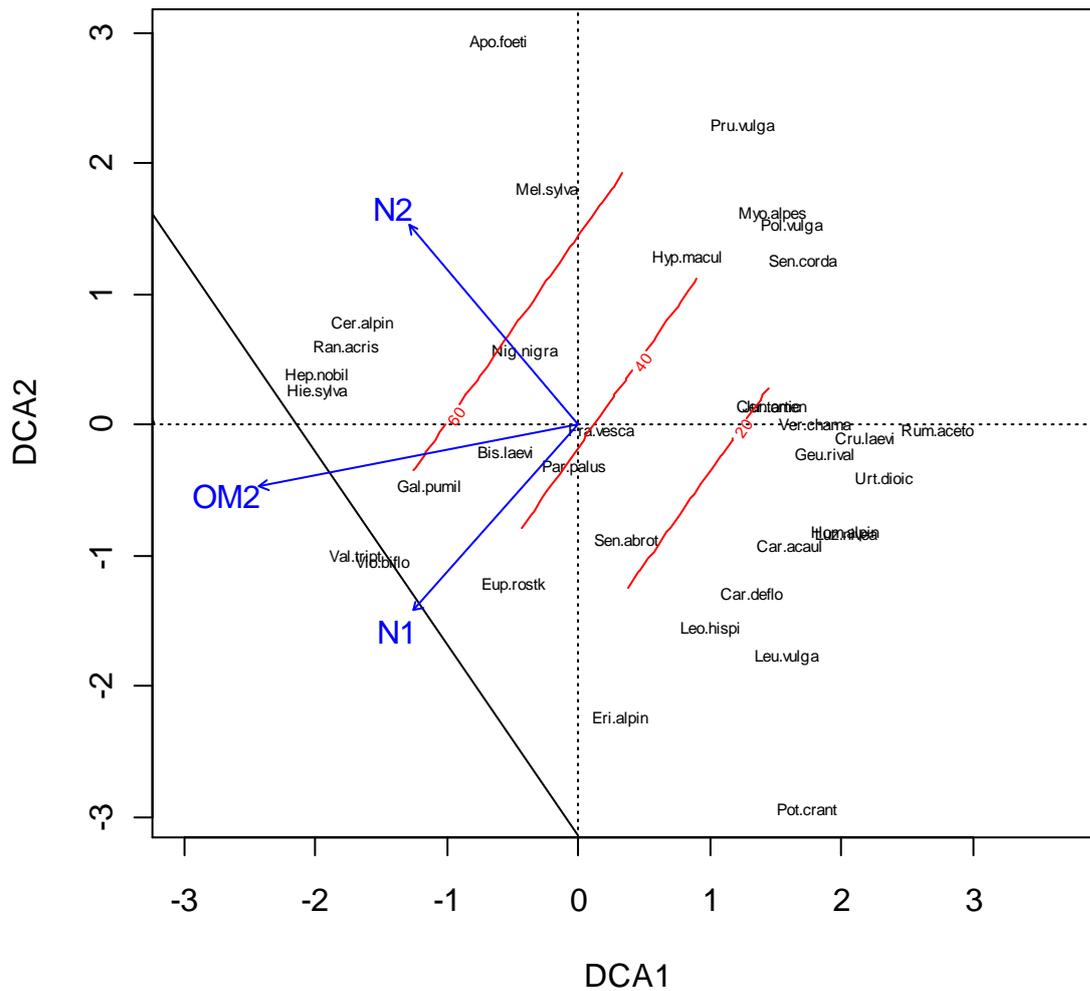


**Figure A.7.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vector is significant ( $P < 0.05$ ) fitted soil proprieties vector. With DCA and samples dataset we found three behaviors of dominant species:

Decreasing with wood cover: *Phleum alpinum* L., *Alchemilla xanthochlora* Rothm., *Briza media* L., *Trifolium pratense* L., *Poa alpina* L., *Acinos alpinus* (L.) Moench, *Luzula campestris* (L.) DC., *Festuca rubra* L..

Maximum percentage at intermediate wood cover: *Agrostis stolonifera* L., *Koeleria pyramidata* (Lam.) Domin, *Carex caryophyllea* La Tourr., *Horminum pyrenaicum* L.

Increasing with wood cover: *Sesleria varia* (Jacq.) Wettst., *Brachypodium pinnatum* (L.) Beauv., *Anthoxanthum odoratum* L. *Erica carnea* L..



**Figure A.8.** Ordination plot of rare species (specie cover < 1%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vectors are significant ( $P < 0.05$ ) fitted soil proprieties vectors. With DCA and samples dataset we found three behaviors of rare species:

Decreasing with wood cover: *Leucanthemum vulgare* Lam., *Leontodon hispidus* L., *Cruciata laevipes* Opiz, *Carduus defloratus* L., *Cerastium tomentosum* L.

Maximum presence at intermediate wood cover: *Polygala vulgaris* L., *Senecio cordatus* Koch, *Senecio abrotanifolius* L., *Euphrasia rostkoviana* Hayne, *Parnassia palustris* L..

Increasing with wood cover: *Fragaria vesca* L., *Galium pumilum* Murray, *Melampyrum sylvaticum* L., *Hieracium sylvaticum* (L.) L., *Cerastium alpinum* L..

**Table A.8.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	10	20	35	50	60	70	80	90	100
pH	3.59	4.08	3.92	3.48	4.51	4.30	4.54	4.51	4.95	4.22
	±	±	±	±	±	±	±	±	±	±
Nutrient	0.18	0.74	0.17	0.14	0.25	0.24	0.09	0.10	0.17	0.08
	3.53	2.92	2.80	2.58	2.50	2.49	2.50	2.44	2.51	2.37
Light	±	±	±	±	±	±	±	±	±	±
	0.05	0.24	0.19	0.16	0.10	0.05	0.05	0.02	0.05	0.07
Temperature	7.12	6.73	6.95	6.55	6.45	6.47	6.25	6.64	6.47	6.29
	±	±	±	±	±	±	±	±	±	±
Humidity	0.04	0.15	0.13	0.11	0.03	0.11	0.09	0.07	0.23	0.14
	1.76	1.60	1.91	1.99	1.98	1.68	1.94	1.74	2.17	2.04
Forage value	±	±	±	±	±	±	±	±	±	±
	0.21	0.05	0.15	0.10	0.10	0.30	0.23	0.19	0.18	0.27
	4.28	3.95	3.90	3.68	3.79	3.96	3.90	3.67	4.07	3.48
	±	±	±	±	±	±	±	±	±	±
	0.09	0.21	0.15	0.14	0.01	0.17	0.08	0.03	0.13	0.19
	2.93	3.10	2.83	2.55	2.63	2.54	2.59	2.51	2.40	2.03
	±	±	±	±	±	±	±	±	±	±
	0.09	0.25	0.12	0.12	0.07	0.08	0.17	0.36	0.25	0.32

## A.5. SITE E – Salanzada summer farms.



Altitude (m a.s.l.): 1000-1050

Mean annual temperature (°C): 6.75

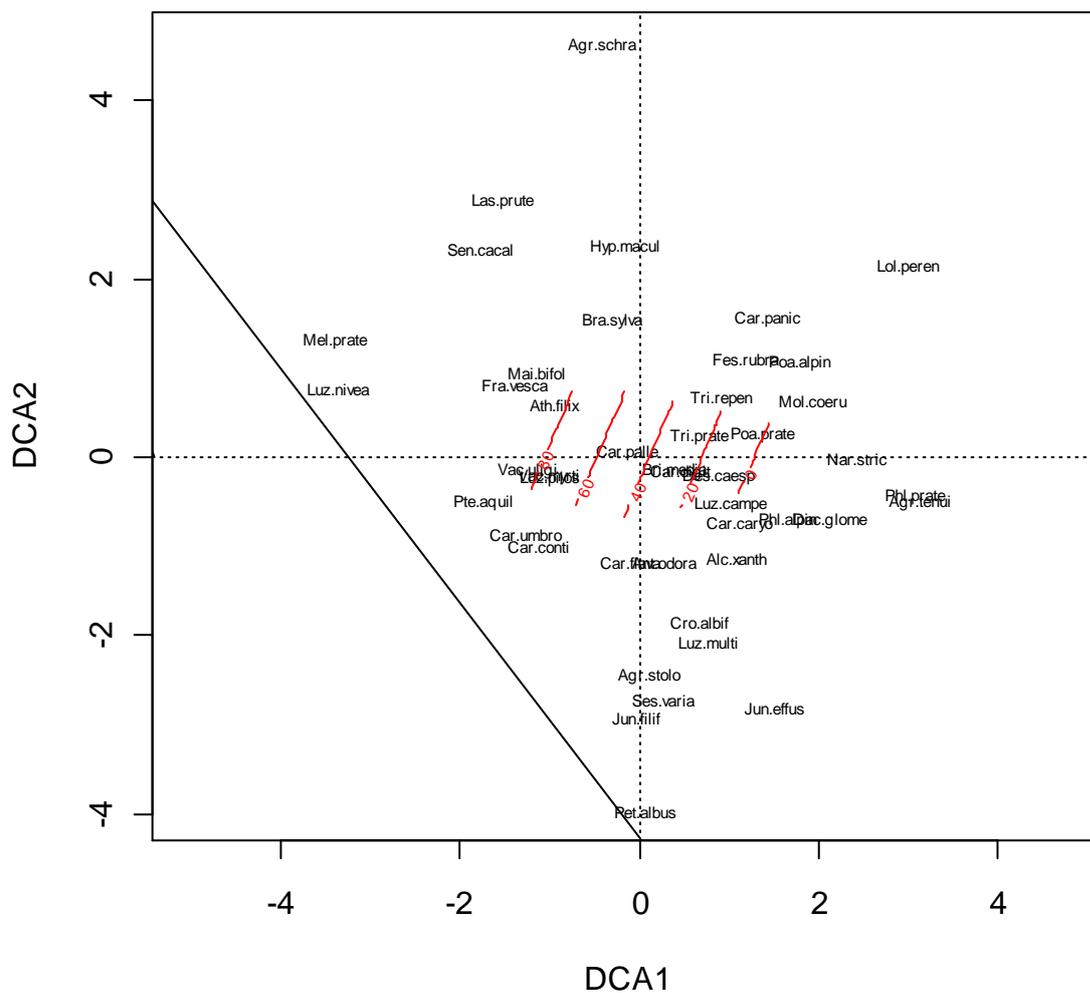
Precipitation (mm year<sup>-1</sup>): 800

Main wood and shrub species:

*Alnus viridis* (Chaix) DC. with *Berberis vulgaris* L., *Crataegus monogyna* Jacq., *Frangula alnus* Miller and *Betula pendula* Roth.

**Table A.9.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5-20 cm).

Depth	Soil properties	wood cover classes				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	4.88 ± 0.09	5.03 ± 0.06	4.82 ± 0.11	4.52 ± 0.09	4.76 ± 0.11
	pH (KCl)	4.40 ± 0.11	4.31 ± 0.12	4.36 ± 0.05	3.96 ± 0.07	4.42 ± 0.06
	N content (g kg <sup>-1</sup> )	6.38 ± 0.87	11.12±1.77	6.85 ± 0.36	7.68 ± 1.01	10.03±1.58
	Organic matter (g kg <sup>-1</sup> )	202.9±29.9	306.0±57.2	195.7±12.6	206.5±22.0	247.7±40.5
5 – 20 cm	pH (H <sub>2</sub> O)	4.79 ± 0.09	4.71 ± 0.11	4.67 ± 0.10	4.44 ± 0.14	4.50 ± 0.14
	pH (KCl)	4.11 ± 0.09	4.11 ± 0.08	4.13 ± 0.06	3.95 ± 0.07	4.02 ± 0.06
	N content (g kg <sup>-1</sup> )	3.40 ± 0.68	5.83 ± 0.93	4.00 ± 0.42	4.25 ± 0.56	5.40 ± 0.80
	Organic matter (g kg <sup>-1</sup> )	110.7±15.4	165.0±16.4	120.0±7.3	127.7±12.0	160.8±18.9



**Figure A.9.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination. With DCA and samples dataset we found three behavior of dominant species:

Decreasing with wood cover: *Poa pratensis* L., *Agrostis tenuis* Sibth., *Dactylis glomerata* L., *Carex caryophyllea* La Tourr., *Molinia coerulea* (L.) Moench, *Poa pratensis* L., *Poa alpina* L., *Trifolium pratense* L., *Trifolium repens* L..

Maximum percentage at intermediate wood cover: *Carex pallescens* L., *Briza media* L., *Carex digitata* L..

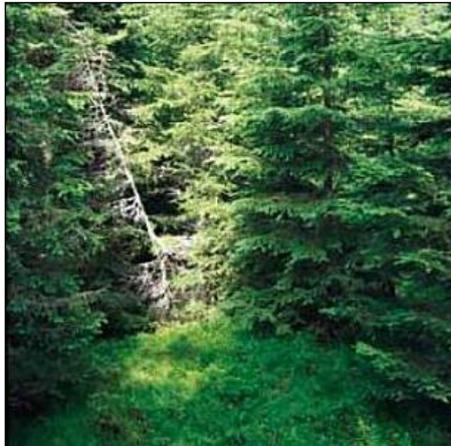
Increasing with wood cover: *Carex umbrosa* Host, *Pteridium aquilinum* (L.) Kuhn, *Athyrium filix-foemina* (L.) Roth, *Fragaria vesca* L., *Melampyrum pratense* L., *Luzula nivea* (L.) Lam. et DC..



**Table A.10.** Ellenberg's indicators and forage values (with standard error) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	5	25	35	50	60	70	80	90	100
pH	1.75	2.11	2.57	2.99	3.15	3.52	2.96	3.24	3.49	2.97
	±	±	±	±	±	±	±	±	±	±
Nutrient	0.17	0.22	0.19	0.31	0.08	0.34	0.33	0.61	0.09	0.41
	3.82	3.56	3.50	3.31	3.32	3.32	3.75	3.33	4.28	3.59
Light	±	±	±	±	±	±	±	±	±	±
	0.13	0.36	0.24	0.20	0.11	0.18	0.04	0.40	0.20	0.48
Temperature	6.92	6.50	6.07	5.81	5.48	5.25	4.82	5.06	4.89	4.89
	±	±	±	±	±	±	±	±	±	±
Humidity	0.04	0.29	0.13	0.22	0.20	0.33	0.43	0.05	0.14	0.48
	1.07	1.31	1.78	1.97	2.12	2.44	2.19	2.38	2.72	2.92
Forage value	±	±	±	±	±	±	±	±	±	±
	0.04	0.06	0.13	0.14	0.35	0.07	0.07	0.21	0.25	0.28
	4.39	4.19	4.27	4.24	4.19	4.29	4.09	4.40	4.88	4.28
	±	±	±	±	±	±	±	±	±	±
	0.07	0.09	0.15	0.08	0.43	0.06	0.44	0.30	0.04	0.74
	3.44	3.15	2.60	2.04	1.95	1.71	1.39	1.09	1.19	0.89
	±	±	±	±	±	±	±	±	±	±
	0.10	0.18	0.14	0.14	0.19	0.03	0.43	0.09	0.32	0.18

## A.6. SITE F – Sadole summer farms.

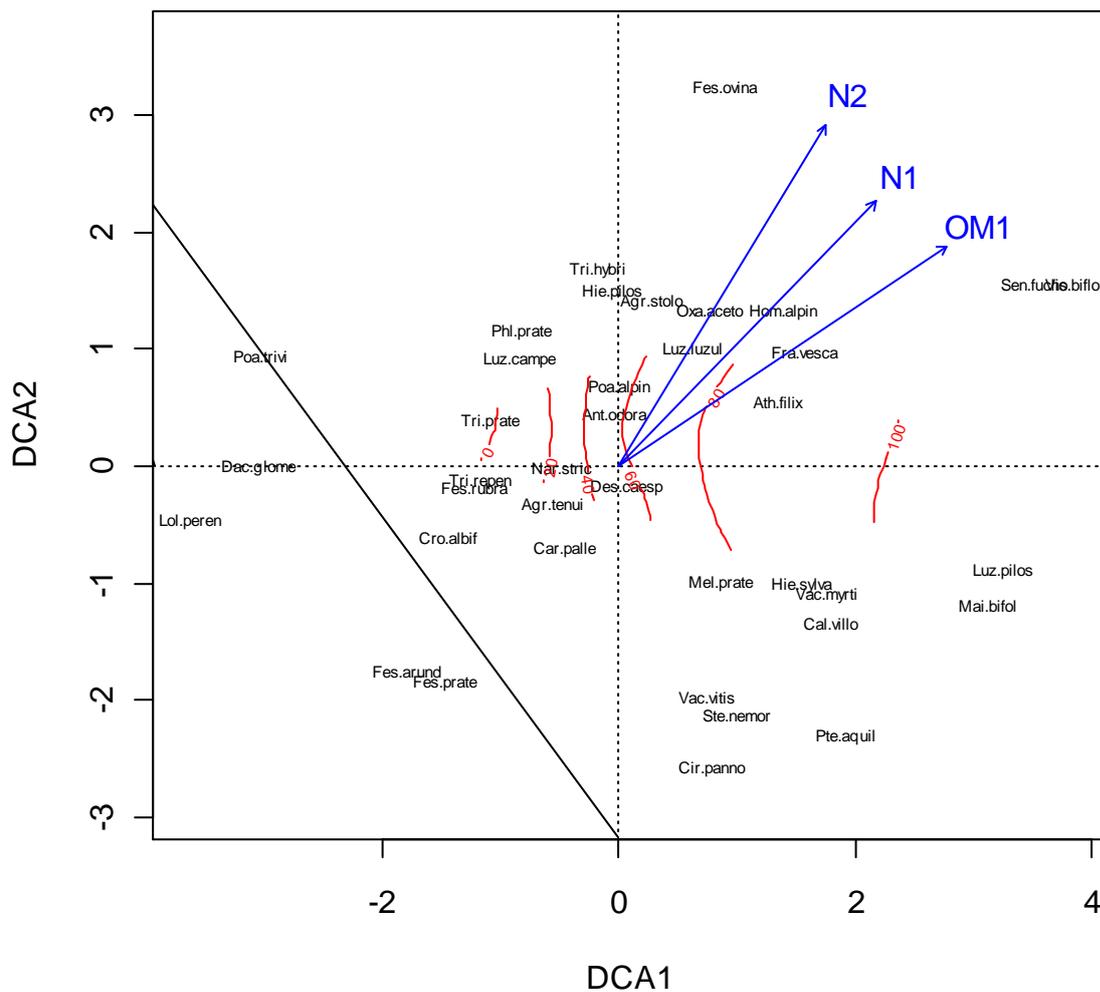


Altitude (m a.s.l.):	1500-1600
Mean annual temperature (°C):	4.0
Precipitation (mm year <sup>-1</sup> ):	1050

Main wood and shrub species: *Picea excelsa* (Lam.) Link

**Table A.11.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0-5 cm and 5-20 cm).

Depth	Soil properties	wood cover classes				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	4.57 ± 0.09	4.16 ± 0.16	4.18 ± 0.19	4.72 ± 0.07	4.57 ± 0.09
	pH (KCl)	3.87 ± 0.09	3.53 ± 0.14	3.55 ± 0.23	4.10 ± 0.14	4.17 ± 0.19
	N content (g kg <sup>-1</sup> )	6.75 ± 1.16	9.80 ± 1.87	9.88 ± 1.53	14.35±2.31	13.63±1.85
	Organic matter (g kg <sup>-1</sup> )	183.3±34.2	265.3±39.7	346.8±81.6	437.3±76.4	561.2±85.1
5 – 20 cm	pH (H <sub>2</sub> O)	4.30 ± 0.11	4.30 ± 0.09	4.32 ± 0.12	4.19 ± 0.11	4.31 ± 0.15
	pH (KCl)	3.91 ± 0.08	3.79 ± 0.09	3.77 ± 0.09	3.58 ± 0.12	3.66 ± 0.14
	N content (g kg <sup>-1</sup> )	3.85 ± 0.67	4.38 ± 1.59	4.00 ± 0.95	4.20 ± 1.04	8.60 ± 2.34
	Organic matter (g kg <sup>-1</sup> )	114.3±29.4	77.7±24.2	137.3±33.1	149.3±32.3	336.7±92.5



**Figure A.11.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vectors are significant ( $P < 0.05$ ) fitted soil proprieties vectors. With DCA and samples dataset we found three behavior of dominant species:

Decreasing with wood cover: *Dactylis glomerata* L., *Poa trivialis* L., *Festuca rubra* L., *Trifolium pratense* L., *Trifolium repens* L., *Luzula campestris* (L.) DC..

Maximum percentage at intermediate wood cover: *Nardus stricta* L., *Agrostis tenuis* Sibth., *Carex pallescens* L., *Anthoxanthum odoratum* L., *Poa alpina* L., *Hieracium pilosella* L..

Increasing with wood cover: *Melampyrum pratense* L., *Oxalis acetosella* L., *Homogyne alpina* (L.) Cass., *Fragaria vesca* L., *Hieracium sylvaticum* (L.) L., *Calamagrostis villosa* (Chaix) Gmelin, *Maianthemum bifolium* (L.) Schmidt, *Luzula pilosa* (L.) Willd..



**Table A.12.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	10	25	40	50	60	70	80	90	100
pH	2.48	2.64	2.98	2.59	2.67	2.36	1.78	1.98	1.73	2.38
	±	±	±	±	±	±	±	±	±	±
	0.27	0.23	0.11	0.20	0.05	0.08	0.01	0.41	0.36	0.62
Nutrient	3.70	3.47	3.08	2.87	2.69	2.88	3.24	2.81	3.16	2.92
	±	±	±	±	±	±	±	±	±	±
	0.27	0.19	0.08	0.08	0.16	0.19	0.50	0.86	1.03	1.11
Light	7.08	6.80	6.32	5.83	5.34	5.27	5.18	4.08	3.60	3.33
	±	±	±	±	±	±	±	±	±	±
	0.13	0.09	0.02	0.22	0.10	0.23	0.61	1.38	0.87	0.86
Temperature	0.46	0.31	0.56	0.47	0.59	0.56	0.89	0.61	0.78	1.33
	±	±	±	±	±	±	±	±	±	±
	0.09	0.06	0.09	0.07	0.08	0.07	0.04	0.03	0.13	0.14
Humidity	3.98	3.56	3.28	3.03	2.96	2.97	3.70	2.46	3.24	3.11
	±	±	±	±	±	±	±	±	±	±
	0.17	0.18	0.15	0.14	0.11	0.20	0.62	0.57	0.84	0.67
Forage value	4.12	3.83	3.10	2.78	2.28	2.28	1.93	1.76	0.89	0.34
	±	±	±	±	±	±	±	±	±	±
	0.52	0.27	0.13	0.10	0.13	0.17	0.41	1.16	0.39	0.08

## A.7. SITE G – Fosse summer farms.

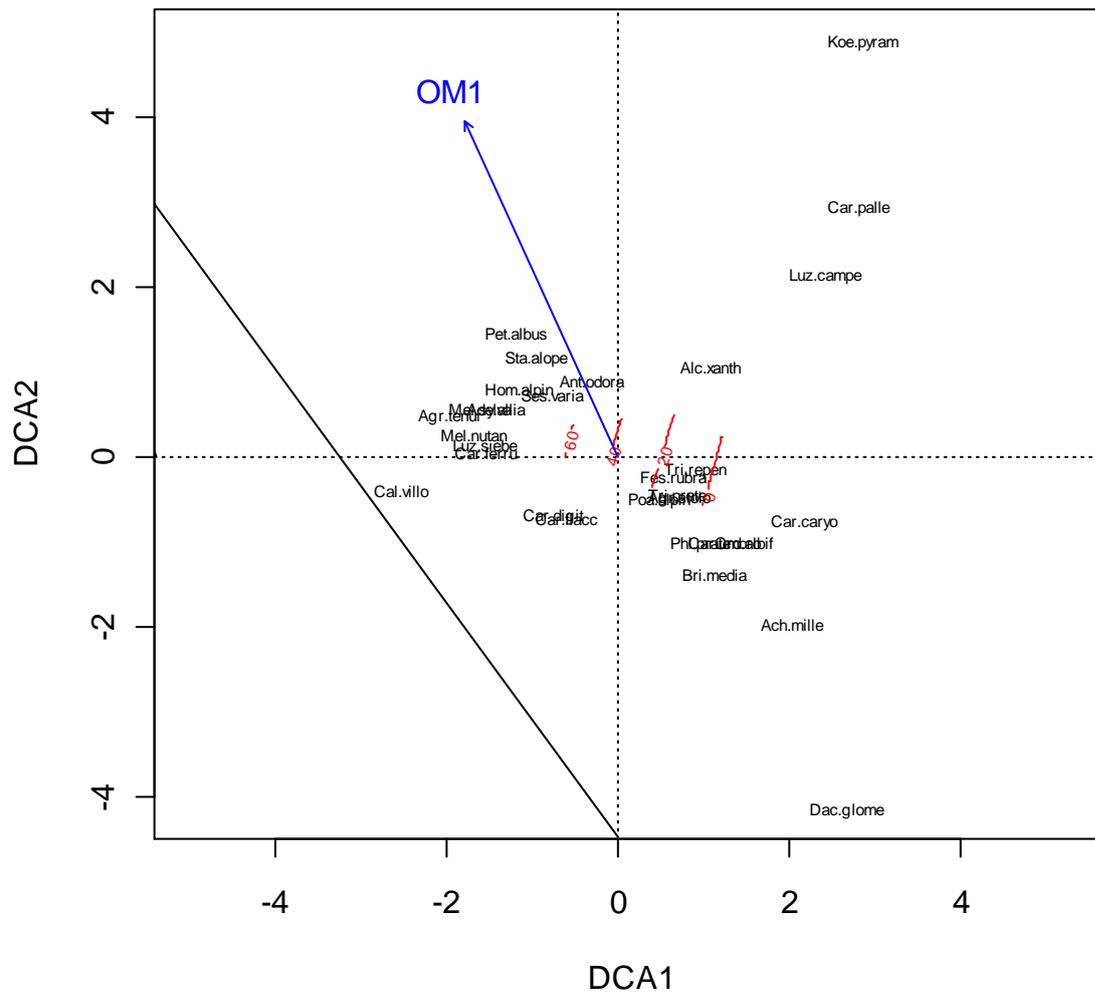


Altitude (m a.s.l.):	1700
Mean annual temperature (°C):	3.51
Precipitation (mm year <sup>-1</sup> ):	1050

Main wood and shrub species: *Larix decidua* Miller with *Rhododendron ferrugineum* L. and *Juniperus communis* L..

**Table A.13.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5–20 cm).

Depth	Soil properties	wood cover classes				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	5.85 ± 0.14	5.74 ± 0.12	5.83 ± 0.20	5.80 ± 0.12	5.66 ± 0.12
	pH (KCl)	5.81 ± 0.08	5.69 ± 0.20	5.91 ± 0.21	5.72 ± 0.08	5.51 ± 0.15
	N content (g kg <sup>-1</sup> )	8.93 ± 1.25	12.28±1.10	12.85±0.78	10.87±0.95	11.93±1.09
	Organic matter (g kg <sup>-1</sup> )	325.8±46.3	411.5±32.0	430.2±41.4	398.0±32.5	419.0±22.9
5 – 20 cm	pH (H <sub>2</sub> O)	6.08 ± 0.09	6.08 ± 0.19	6.28 ± 0.11	6.17 ± 0.09	6.17 ± 0.07
	pH (KCl)	6.03 ± 0.09	5.96 ± 0.23	6.26 ± 0.19	6.07 ± 0.15	6.04 ± 0.16
	N content (g kg <sup>-1</sup> )	5.65 ± 0.56	8.30 ± 0.82	7.72 ± 0.87	7.55 ± 1.13	8.28 ± 0.78
	Organic matter (g kg <sup>-1</sup> )	165.0±14.8	275.2±26.7	251.5±37.5	238.2±26.0	287.3±30.1

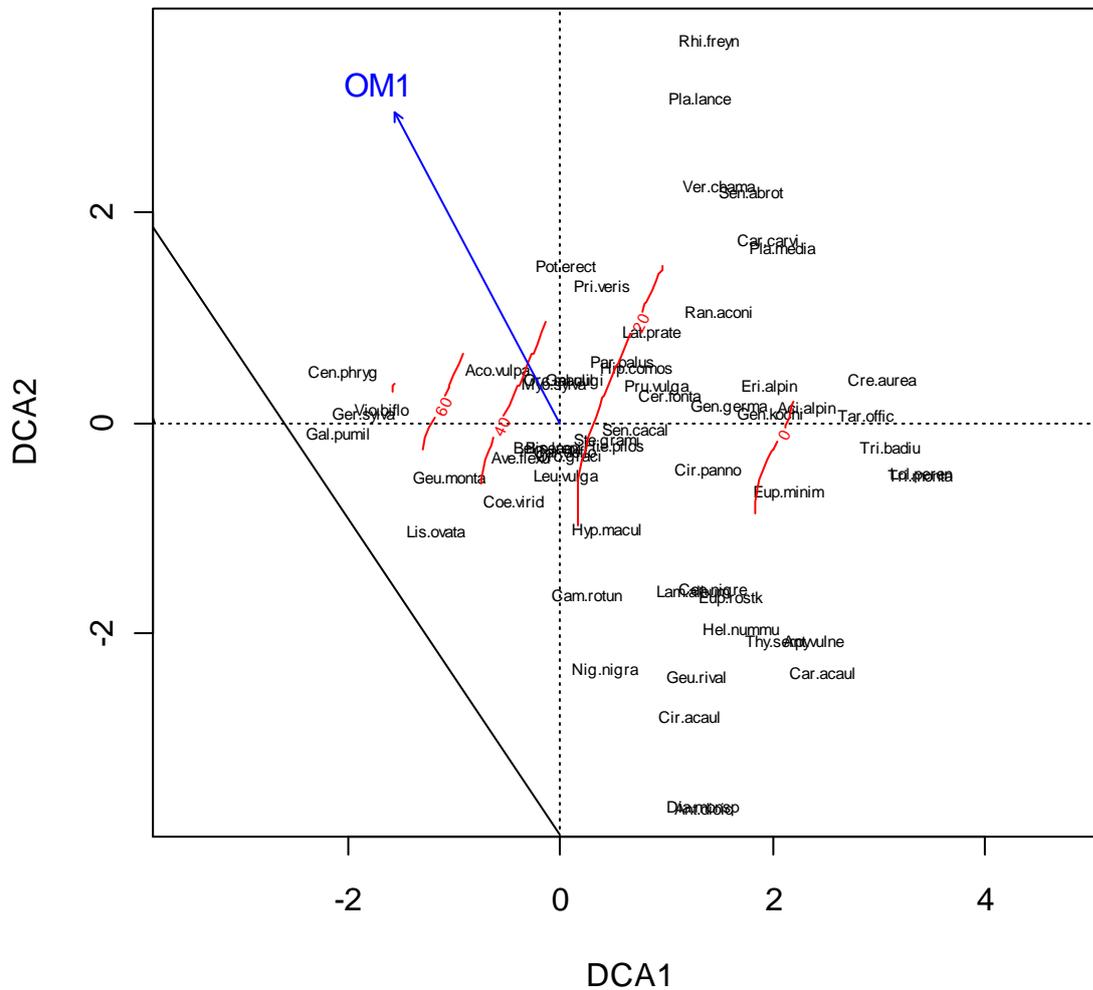


**Figure A.13.** Ordination plot of dominant species (specie cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vectors are significant ( $P < 0.05$ ) fitted soil proprieties vectors. At lower percentage of wood cover the differences between plots are higher than at higher wood cover. With DCA and samples dataset we found three behaviors of dominant species:

Decreasing with wood cover: *Carex caryophyllea* La Tourn., *Achillea millefolium* L. ssp. *sudetica* (Opiz) Weiss., *Luzula campestris* (L.) DC., *Phleum pratense* L., *Trifolium repens* L., *Alchemilla xanthochlora* Rothm., *Festuca rubra* L..

Maximum percentage at intermediate wood cover: *Trifolium pratense* L., *Poa alpina* L., *Carex flacca* Schrebe, *rAnthoxanthum odoratum* L.,

Increasing with wood cover: *Sesleria varia* (Jacq.) Wettst., *Carex ferruginea* Scop., *Luzula sieberi* Tausch, *Homogyne alpina* (L.) Cass., *Melampyrum sylvaticum* L., *Melica nutans* L., *Adenostyles alliariae* (Gouan) Kerner.



**Figure A.14.** Ordination plot of rare species (specie cover < 1%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination and the blue vectors are significant ( $P < 0.05$ ) fitted soil proprieties vectors. Also when rare species are take into account plots with lower wood cover have more variability on botanical composition. With DCA and samples dataset we found three behaviors of rare species:

Decreasing with wood cover: *Crepis aurea* (L.) Cass., *Taraxacum officinale* Weber (aggregato), *Euphrasia minima* Jacq. ex DC., *Acinos alpinus* (L.) Moench, *Thymus*

*serpyllum* L. s.s., *Helianthemum nummularium* (L.) Miller, *Carum carvi* L., *Plantago media* L., *Erigeron alpinus* L., *Gentianella germanica* (Willd.) Warburg, *Campanula rotundifolia* L., *Prunella vulgaris* L..

Maximum presence at intermediate wood cover: *Potentilla erecta* (L.) Rauschel, *Avenella flexuosa* (L.) Parl., *Biscutella laevigata* L., *Myosotis sylvatica* Hoffm., *Coeloglossum viride* (L.) Hartm., *Listera ovata* (L.) R.Br..

Increasing with wood cover: *Geranium sylvaticum* L., *Viola biflora* L., *Galium pumilum* Murray.

**Table A.14.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	5	10	20	30	40	50	60	70	80
pH	2.35	2.87	2.87	3.24	3.50	3.72	3.88	4.37	4.25	4.11
	± 0.055	± 0.07	± 0.13	± 0.22	± 0.08	± 0.14	± 0.02	± 0.05	± 0.36	± 0.18
Nutrient	3.49	3.03	3.02	3.02	2.96	3.10	3.15	2.92	3.01	3.19
	± 0.17	± 0.15	± 0.04	± 0.09	± 0.08	± 0.12	± 0.11	± 0.04	± 0.13	± 0.03
Light	7.04	6.49	5.95	5.80	5.86	5.71	5.51	5.53	5.42	5.31
	± 0.24	± 0.05	± 0.07	± 0.22	± 0.16	± 0.25	± 0.15	± 0.02	± 0.10	± 0.15
Temperature	0.98	1.16	0.82	0.83	0.91	1.11	1.06	1.42	1.50	1.51
	± 0.16	± 0.10	± 0.15	± 0.15	± 0.07	± 0.14	± 0.04	± 0.07	± 0.05	± 0.12
Humidity	4.04	3.76	3.37	3.56	3.58	3.76	3.69	4.08	4.08	4.24
	± 0.19	± 0.13	± 0.09	± 0.11	± 0.07	± 0.14	± 0.13	± 0.07	± 0.13	± 0.11
Forage value	3.67	3.18	3.60	3.04	3.04	2.53	2.27	1.77	1.64	1.25
	± 0.25	± 0.05	± 0.23	± 0.03	± 0.07	± 0.16	± 0.11	± 0.20	± 0.30	± 0.29

## A.8. SITE H – Trenca summer farms.



Altitude (m a.s.l.):	1700
Mean annual temperature (°C):	4.36
Precipitation (mm year <sup>-1</sup> ):	917

Main wood and shrub species: *Rhododendron ferrugineum* L. with *Juniperus nana* Willd. and *Larix decidua* Miller

**Table A.15.** Mean characteristic values and standard errors of soil samples collected at different wood cover classes and split into two depths (0–5 cm and 5–20 cm)

Depth	Soil properties	wood cover classes				
		C0	C25	C50	C75	C100
0 – 5 cm	pH (H <sub>2</sub> O)	3.71 ± 0.12	3.62 ± 0.13	3.42 ± 0.05	3.64 ± 0.15	3.54 ± 0.09
	pH (KCl)	2.55 ± 0.05	2.48 ± 0.06	2.36 ± 0.07	2.55 ± 0.16	2.33 ± 0.09
	N content (g kg <sup>-1</sup> )	11.53±1.00	11.38±1.30	11.95±0.43	9.97±1.80	12.72±1.31
	Organic matter (g kg <sup>-1</sup> )	318.6±41.7	326.9±38.8	359.6±9.4	341.7±33.6	455.8±61.8
5 – 20 cm	pH (H <sub>2</sub> O)	3.93 ± 0.09	3.86 ± 0.10	3.78 ± 0.12	4.00 ± 0.19	3.64 ± 0.10
	pH (KCl)	2.79 ± 0.03	2.81 ± 0.02	2.67 ± 0.04	2.84 ± 0.13	2.58 ± 0.14
	N content (g kg <sup>-1</sup> )	3.98 ± 0.30	3.77 ± 0.20	4.68 ± 0.38	3.65 ± 0.55	5.33 ± 0.60
	Organic matter (g kg <sup>-1</sup> )	133.4±10.8	140.9±6.0	160.4±9.3	138.8±11.6	189.0±13.7

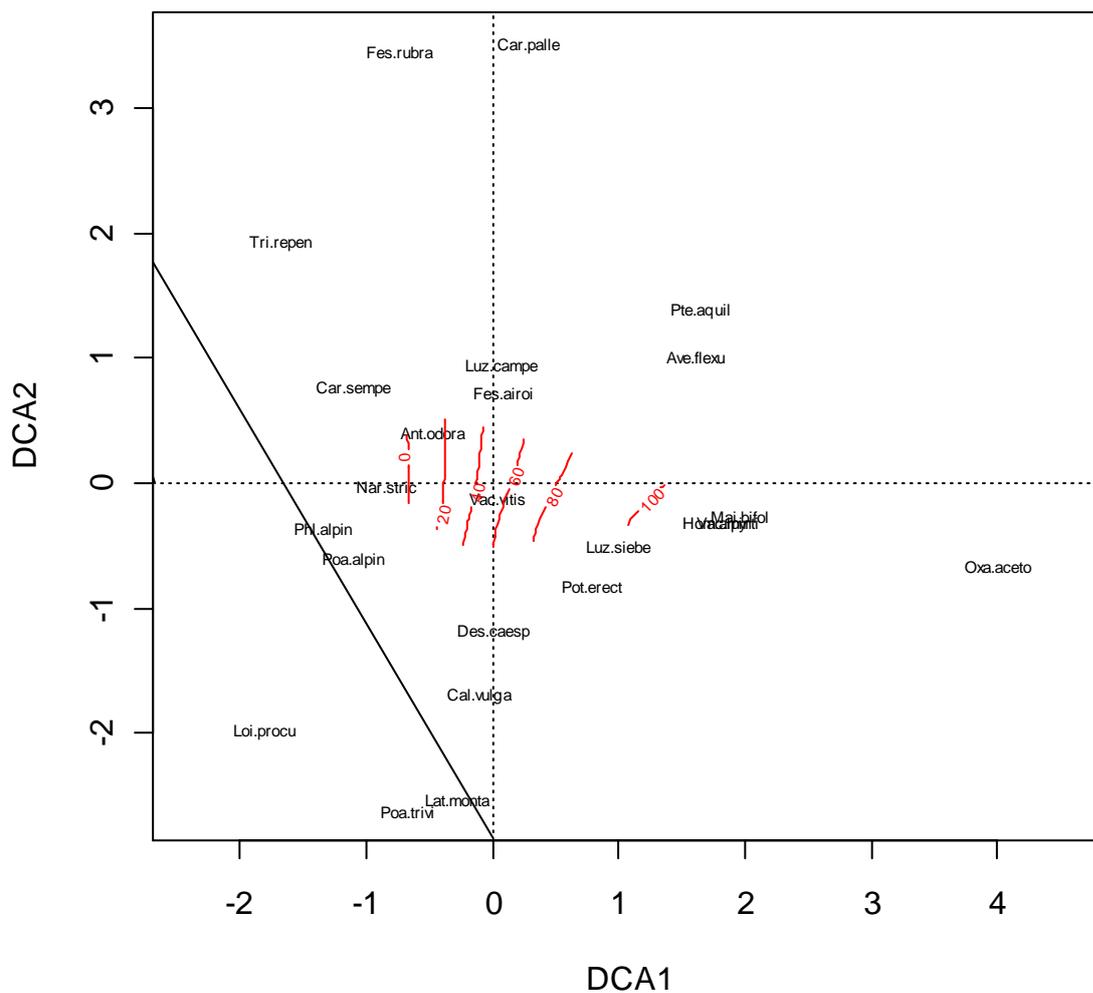


Figure A.15. Ordination plot of dominant species (species cover > 3%) along the first two axes of DCA. Red lines are the model surface of cover variable fitted to ordination. With DCA and samples dataset we found three behaviors of dominant species:

Decreasing with wood cover: *Phleum alpinum* L., *Poa alpina* L., *Nardus stricta* L., *Carex sempervirens* Vill..

Maximum percentage at intermediate wood cover: *Anthoxanthum odoratum* L., *Lathyrus montanus* Bernh., *Poa trivialis* L., *Festuca airoides* Lam. , *Luzula campestris* (L.) DC. , *Vaccinium vitis idaea* L.,

Increasing with wood cover: *Potentilla erecta* (L.) Rauschel, *Luzula sieberi* Tausch, *Avenella flexuosa* (L.) Parl., *Pteridium aquilinum* (L.) Kuhn,



**Table A.16.** Ellenberg's indicators and forage values (with standard errors) of plots survey at different wood cover.

Ellenberg's indicators values	wood cover (%)									
	0	10	20	30	40	50	60	70	80	100
pH	1.93	2.39	2.20	2.04	2.09	2.05	2.17	1.97	1.97	1.32
	±	±	±	±	±	±	±	±	±	±
Nutrient	0.04	0.24	0.08	0.05	0.08	0.02	0.20	0.11	0.17	0.28
	2.36	2.04	1.97	1.95	1.83	1.82	1.91	1.86	1.83	1.41
Light	±	±	±	±	±	±	±	±	±	±
	0.17	0.09	0.06	0.06	0.08	0.03	0.001	0.14	0.07	0.29
Temperature	7.09	6.88	7.07	6.65	6.17	6.09	5.59	6.07	5.75	3.14
	±	±	±	±	±	±	±	±	±	±
Humidity	0.07	0.10	0.08	0.29	0.16	0.16	0.35	0.20	0.24	0.84
	0.68	0.76	0.60	0.77	0.87	0.95	1.04	0.74	1.05	0.98
Forage value	±	±	±	±	±	±	±	±	±	±
	0.09	0.13	0.09	0.07	0.07	0.06	0.04	0.09	0.21	0.26
Forage value	2.00	1.79	1.57	1.72	2.00	2.13	2.41	2.09	2.35	2.24
	±	±	±	±	±	±	±	±	±	±
Forage value	0.12	0.18	0.17	0.16	0.05	0.16	0.06	0.33	0.17	0.08
	1.78	1.65	1.71	1.64	1.15	1.34	1.51	1.29	1.33	0.51
Forage value	±	±	±	±	±	±	±	±	±	±
	0.16	0.15	0.06	0.19	0.13	0.10	0.19	0.34	0.14	0.15



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