



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



Cristina Pornaro^{a,*}, Manuel K. Schneider^b, Stefano Macolino^a

^a Department of Agronomy Food Natural Resources Animals and Environment, Università degli Studi di Padova, viale dell'Università 16, Legnaro, Italy

^b Agroscope, Forage Production and Grassland, Reckenholzstrasse 191, 8046 Zurich, Switzerland

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ABSTRACT

Cessation of agricultural management and subsequent natural forest succession has been the primary land use change in the Southern Alps over the past 50 years. It is generally assumed that early stages of succession host more plant species than grazed pastures, but that this richness is partly lost as the density of woody species increases. Based on vegetation surveys on eight sites in the Italian Alps, we found the effects of forest succession on plant species richness to depend strongly on environmental conditions. The relationship between plant species richness and wood cover at the sites ranged from non-detectable over hump-shaped, to monotonically decreasing. Linear mixed-effects models indicate that high mean annual temperature is associated with a strong decrease in plant species richness and in the number of red-list species along the pasture-to-forest gradient. Sampling plant species composition at a range of scales allowed us to rule out artefacts caused by modified species–area relationships as a consequence of changes in wood cover. Multi-scale sampling also indicated that the primary loss of plant species richness by forest succession is in plant species with low abundance. Our data further allow assessment of the risk of species loss in mountain grasslands in the Southern Alps, which is highest on sites with higher mean annual temperature. These areas should receive concentrated attention and support for biodiversity conservation.

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1. Introduction

In the past half a century, the Southern Alps have undergone a tremendous and arguably unprecedented change in land use. Since 1960, about 800,000 ha of grassland have been abandoned throughout the Italian Alpine Arch, which corresponds to 45% of the surface originally covered by pastures and meadows (Bovolenta, 2004; Chemini and Gianelle, 1999). As a consequence of land abandonment processes, mountain regions have been experiencing radical landscape changes, as once managed areas are reverting to forests through the process of natural succession (Conti and Fagarazzi, 2004). According to the National Statistical Bureau (ISTAT), during the second half of the 20th century, forest areas in the Italian Alps increased by 14.9%, with an increase of 7.0% in the last decade of the century alone (Piussi and Pettenella, 2000).

Studies conducted in other regions demonstrated that the reduction and abandonment of cutting or grazing favours establishment of persistent, competitive species, most of which are woody shrubs or trees. In pastures, woody species establish first in close association with the plants or vegetation patches that facilitate their establishment, for example forest edges or forest islands

and individual trees within the grassland (Van Uytvanck et al., 2008). The process of forest succession is highly non-linear over time. Finegan (1984) observed that 4–11 years after the cessation of grazing activities, only few woody plants were growing in a scattered pattern. However, after 10–20 years, stable woody vegetation had established across the entire originally grazed surface (Finegan, 1984). The pace of establishment also depends on which dominant woody species are present: each woody species has a specific response to environmental factors (for example De Gasperi and Motzkin, 2007; Tasser and Tappeiner, 2002; Van Gils et al., 2008). The nutrient status of the soil is another determinant of the rate of shrub encroachment in abandoned pastures. On nutrient rich and productive sites (such as pastures), it takes 30–45 years before woody species become dominant in former open vegetation (Smit and Olf, 1998). Del Favero et al. (1998) found that *Fagus sylvatica* established at different rates on various substrates because of the competition by other shrubby and woody species. In contrast, Van Gils et al. (2008) observed that the establishment of *F. sylvatica* on abandoned farmland depended on the intensity of grazing by sheep, the distance from seed sources, the presence of protective forest or shrubby shade and the wind exposure of saplings, but not on the soil substrate. Hence, on sites where some grassland management remains, grazing increases the complexity of successional processes. Van Uytvanck et al. (2008) found that trampling

* Corresponding author. Tel.: +39 049 8272859; fax: +39 049 8272839.

E-mail address: cristina.pornaro@unipd.it (C. Pornaro).

by herbivores favor the germination of tree seeds but damages saplings not protected by established shrub or other unpalatable vegetation. Also forest management may have an important role on the succession of natural forests influencing the composition of trees, saplings and herbaceous species, and the competition between all of them (Del Favero et al., 1998).

Natural reforestation changes vegetation composition and species richness in pastures. Most studies report a decrease in the number of species with increasing forest cover (Anthelme et al., 2001; Fischer and Wipf, 2002; Kesting, 2009; Manning et al., 2006). However, there is no consensus on whether the decrease is steady over the entire investigated wood cover gradient or whether the decrease reaches a maximum at intermediate stages of forest succession. This lack of agreement arises from the fact that most studies analysed forest succession at only one site or at a number of sites covering a very limited range of environmental gradients.

In order to identify environmental drivers of the effect of forest succession on plant species richness, we investigated pasture-to-forest gradients on eight contrasting sites in the Italian Alps. All eight investigated sites are only periodically inhabited and used by livestock between May or June and September, depending on altitude and annual weather conditions. Seven sites are grazed by dairy cows, one is grazed by sheep. On all sites, grazing persisted until present, but stocking rates and labour input are not sufficient to prevent the natural succession of forests. The reasons for this are, for example, that cows are increasingly fed on concentrates instead of grass, and that farmers invest less time into the removal of saplings.

In our study we address three basic questions. The first is: how is plant species richness affected by wood cover? We hypothesize that a low percentage of wood cover outcompetes a comparatively larger number of shade-sensitive grassland species and hence species richness decreases with wood cover.

The second question is: does the effect of wood cover on plant species richness depend on environmental site conditions? We hypothesize that environmental conditions have direct effects on the grassland vegetation present before abandonment and also affects the dynamics that occur thereafter (e.g. the woody species becoming dominant). In order to test this second hypothesis, we use generalized linear mixed-effects models which allow the analysis of effects of environmental site conditions in a hierarchical manner.

The last question is: are the observed patterns of species richness an artifact of changing species–area relationship with increasing wood cover? We therefore investigate to what extent forest succession alters scale-dependent heterogeneity in species composition. Such alteration would seriously affect the interpretation of species numbers collected on equal sample sizes along the successional gradient.

2. Materials and methods

2.1. Survey sites

This study was carried out on eight sites located in the Eastern Alps of Italy, between 2006 and 2010 (Fig. 1). The sites represented typical pastures, which were formerly open grassland but are now in the process of forest succession. Pastures with an important portion of old-grown trees (i.e. traditionally wooded pastures) were not investigated. Sites were selected to assure that each of the primary establishing woody species in the region were present. Each site was dominated by a particular woody species (Table 1). In order to identify the different stages of woody species establishment, we used aerial photographs and forest settlement plans developed

for 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 cadastral unit to be totally established woodland. Inside the area delineated as pasture land, we identified different stages of forest succession based on analysis of aerial photographs. The resulting map was used in the field to identify sampling locations with different percentage of wood cover.

All study sites are still partly communally grazed by livestock during the summer; in all sites the actual stocking rates are lower than the potential ones. Average grazing intensities are low but locally highly variable especially along the wood-cover gradient. The vegetation is heterogeneously structured, including 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 3.5 °C and 6.8 °C (Table 1). The rainfall shows a pronounced intra-annual pattern with May and November being the periods when most of the rainfall occurs. Four sites are situated on calcareous substrate and four sites are situated on siliceous substrate. Additional site characteristics are presented in Table 1.

2.2. Species sampling

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

2.2.1. Small-area sampling (1 m²) along transects

At each site, six transects were established perpendicular to the edge of the forest, in order to include different stages of woody species establishment. Each transect started in an area of pure grassland without woody species and finished in completely established woodland. The lengths of the transects varied between sites depending on the dominant wood species. Along the transect line, five quadrats of 1 m² were placed in order to represent one of five wood cover classes: 0%, 25%, 50%, 75%, and 100%. In each quadrat, the percentage of wood cover was visually estimated and all species present were recorded without their relative abundance, three times per year over 2 years.

2.2.2. Large-area sampling (100 m²) in wood-cover strata

Thirty additional quadrats of 10 m × 10 m were established at each site in strata with particular levels of wood cover. Care was taken that the selected locations of the large area quadrats had homogenous levels of wood cover that were well distributed between 0% and 100%. Along transects, it was frequently impossible to find areas of suitable size with a homogenous wood cover. Strata of shrub and tree cover were delineated using orthophotos in which sampling locations were predetermined at random. Where the orthophoto provided insufficient information to assess the real state of reforestation, the exact locations was refined in the field. For each plot, a complete floristic survey was carried out: all herbaceous species were recorded, and their relative abundance values were visually estimated. Additionally, cover values were estimated using a 0–100% scale for each of the two vegetation layers (wood and shrub). Using these estimates, the distribution of species numbers was analysed along the entire gradient of wood cover. For every plot, the abundance of all vascular plants was recorded three times per year over 2 years. Species richness was calculated as the cumulated number of all six surveys and the abundance of each species was calculated as the mean of the estimates of all six surveys.

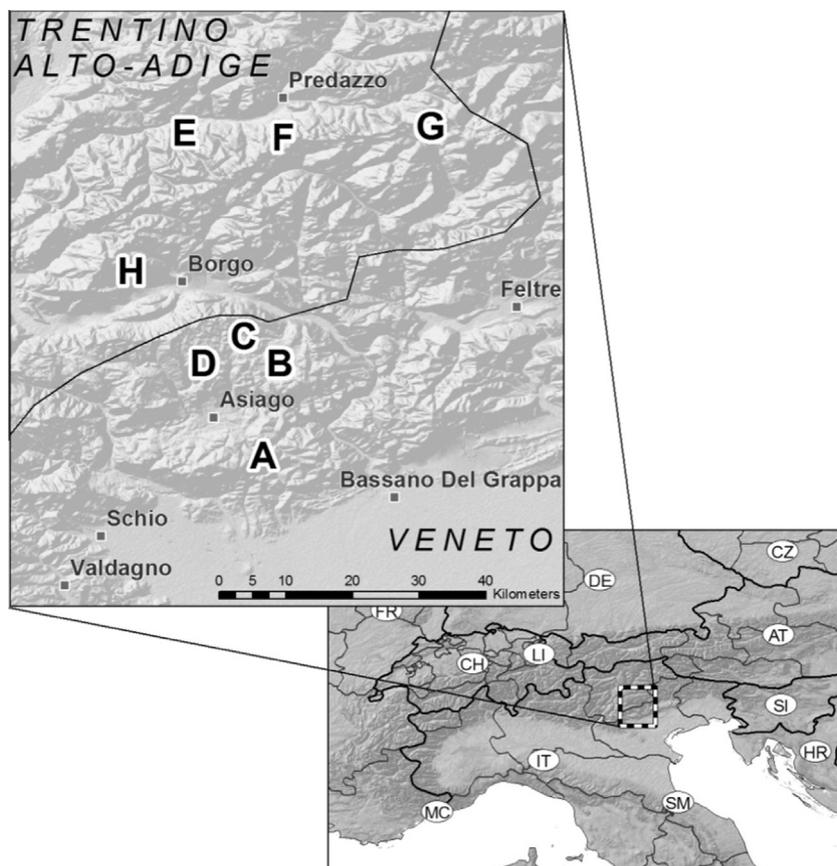


Fig. 1. Location of the eight study sites in the Eastern Alps of Italy.

Table 1
Main characteristic of the eight study sites.

ID site	A	B	C	D	E	F	G	H
Dominant woody species	<i>Fagus sylvatica</i>	<i>Picea abies</i>	<i>Larix decidua</i>	<i>Pinus mugo</i>	<i>Alnus viridis</i>	<i>Picea abies</i>	<i>Larix decidua</i>	<i>Rhododendron ferrugineum</i>
Substrate	Carbonate	Carbonate	Carbonate	Carbonate	Silicate	Silicate	Silicate	Silicate
X coordinates*	5,078,100	5,092,270	5,096,180	5,091,965	5,127,085	5,126,725	5,128,975	5,105,810
Y coordinates*	699,850	701,070	697,195	693,095	690,120	700,305	715,490	685,140
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 ⁻¹)	2.6	6.9	14.3	5.4	4.9	5.3	7.3	7.8
Organic matter (g kg ⁻¹)	128.6	212.2	309.5	295.0	156.7	148.7	245.5	226.0
Total number of species (approx. γ diversity)	190	147	128	81	141	128	116	106
Red list species**	5	4	1	2	4	0	2	0

* Projection system is IGM95/UTM zone 32 N.

** According to Moser et al. (2002).

2.2.3. Nested quadrats sampling

At each site, three out of six transects used for small-area sampling were selected, and a series of nested quadrats of 1, 4, 10, 25, 50 and 100 m² were established, being careful to have homogeneous wood cover within each series of quadrats. In each nested quadrat, the presence of all vascular plants was recorded. Specific attention was given to maintain a constant sampling effort across all quadrats.

2.3. Additional plot and site properties

In order to validate wood cover estimates, photosynthetically active radiation (PAR) measurements were carried out using a

Li-Cor PAR meter (Li-Cor Inc., 1996). In each small-area plot, eight readings were taken at different positions just above the herbaceous canopy. All the measures were collected from August 9 to 14 between 11.30 a.m. and 1.30 p.m. under clear sky conditions. 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 each small area plot, soil was sampled at two depths: from 0 cm to 5 cm, and from 5 cm to 20 cm. Soil pH (1:5 soil: water solution), N content (Kjeldahl total nitrogen [g kg⁻¹]), and soil organic matter (Springer–Klee method [g kg⁻¹]) were measured using the Italian standard soil analysis techniques (G.U., 1999). Only soil

Table 2

Estimated coefficients of explanatory variables in generalized linear mixed-effects models to plant species richness on 1 m² and 100 m² and significances based on likelihood-ratio tests. Estimated coefficients are at log scale because of the use of a Poisson GLMM.

Sampling area	1 m ²		100 m ²	
	Estimate	Pχ ²	Estimate	Pχ ²
Intercept	2.86		3.64	
Cover	-1.95	<0.001	-1.71	<0.001
Cover ²	-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: mean annual temperature	-0.11	n.s.	0.09	n.s.
Cover: pH	0.28	<0.01	-	n.s.
Cover: organic matter	-0.06	<0.01	0.05	<0.001
Cover: nitrogen content	1.45	<0.001	-0.53	<0.01
Cover: rain	-	n.s.	0.52	<0.001
Cover ² : mean annual temperature	-	n.s.	0.10	n.s.
Cover ² : organic matter	0.12	<0.01	0.11	<0.01

samples collected at 0% wood cover were used in this study. They were averaged and used to estimate general soil characteristics of each site.

For each large-area plot, slope and wetness index were calculated using SAGA GIS 2.0.3 based on a 20 m digital terrain model (Ministero dell'ambiente, Rome). Slope was calculated using the algorithm by Zevenbergen and Thorne (1987). The wetness index calculation was based on the D_{∞} 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), and the interpolated mean monthly temperatures were summed.

Mean annual precipitation was derived from the weather station (ARPAV and Meteotrentino data) nearest to each site.

2.4. Statistical analysis

Species richness (S) was calculated as the number of species in each sample.

The influence of species rarity on the response of species diversity to forest succession was investigated using the data from the 100 m² samples. This was done by calculating effective species numbers D 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 number of 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 down-weighting of the species with low abundance. Effective species numbers of order $q = 1$ and $q = 2$ are equivalent to the $e^{S_{\text{Shannon}}}$ and $1/\text{Simpson}$, 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 in order to avoid biased results from higher abundance of dominant arboreal or shrubby species systematically at higher levels of forest succession. In addition, red-list species were counted in the 100 m² samples and correlated against wood cover. Because no regional red list for plants exists for the investigated area, red-list status was based on the Swiss red list of plant species (Moser et al., 2002).

Generalized linear mixed models (GLMMs) were built to explain observed variation in species diversity (richness and effective species numbers) depending on wood cover and environmental conditions. The evaluated environmental descriptors were mean annual temperature, pH, soil organic matter content, soil nitrogen content, rainfall, altitude, slope, and wetness index. Spearman's correlation coefficients between these eight environmental descriptors were calculated and displayed in Table 3. 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). Species numbers were assumed to follow a Poisson distribution. Overdispersion of data was accounted for by the inclusion of an observation-level variance term. Separate GLMMs were built for the 1 m² and 100 m² sampling datasets, with site as the random effect. Wood cover was centred in order to have a more stable model. A full GLMM, including all environmental variables, was simplified based on Akaike's Information Criterion (AIC). Significances were determined by likelihood-ratio tests (LRT) of reduced versus full models.

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

$$\text{the exponential model } S = a \cdot A^b \quad (2)$$

$$\text{the Monod or Clench model } S = a \cdot A/(b + A) \quad (3)$$

$$\text{and the cumulative Weibull model } S = a \cdot (1 - e^{-bA^c}) \quad (4)$$

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

In all three 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 Monod and the cumulative Weibull model. Parameter b describes the increase of species richness with area. Parameter c is an additional factor of modulation of the richness increase in the cumulative Weibull model.

Non-linear mixed-effects models of all the three forms were fitted to data. Wood cover and its square were modelled as fixed effects, transect and site were included as nested random effects (Pinheiro and Bates, 2000). Significances were determined by likelihood-ratio tests of reduced versus full models. All calculations

Table 3

Spearman's correlation coefficients between environmental descriptors evaluated in the full GLMM. Coefficients are reported for significant correlations only.

Environmental descriptors	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Mean annual temperature								
(2) pH	-0.48							
(3) Soil organic matter content	-0.88	0.69						
(4) Soil nitrogen content	-0.77	0.33	0.81					
(5) Rainfall	-0.31	0.23	0.24	0.22				
(6) Altitude	-0.77	0.33	0.69	0.81	/			
(7) Slope	/	0.22	/	-0.40	-0.30	/		
(8) Wetness index	/	/	/	/	0.26	0.39	/	-0.77

were performed in R 2.13.1 (R Core Team, 2011) using libraries `vegan`, `nlme` and `lme4`.

3. Results

3.1. Impact of environmental factors on the effect of reforestation on species richness

A total of 177 vascular plants were found in the 1 m² quadrats: 155 herbaceous, six shrubby and 16 arboreal species (Nanophanerophytae life-form for shrubby species and Phanerophytae life-form for arboreal species; following Pignatti, 1982). The species richness per 1 m² ranged from 2 to 23 (Fig. 2). The total number of plant species in the 100 m² quadrats in the eight investigated sites was 348: 324 herbaceous, seven shrubby and 16 arboreal species.

Sites strongly differed in mean alpha diversity at both sampling scales (i.e. the mean number of species present at a single sampling quadrat of either 1 m² or 100 m²). Mean alpha diversity was highest in the relatively warm site A, dominated by *F. sylvatica* L. with 59 plant species per 100 m². Lowest mean alpha diversity, with only 27 plant species at 100 m², was found in site F, dominated by *Picea abies*. Approximate gamma diversity (i.e. the total number of plant species found at site level) ranged from 81 plant species at the coldest site dominated by *Pinus mugo* Turra to 190 plant species at one of the warmest sites dominated by *F. sylvatica* (Table 1). Species pertaining to red lists were more frequently found on sites with higher mean annual temperature (Table 1). At the warmer sites A, B and E 4 or 5 red-listed species were found in compared to a maximum of 2 species in the other sites. In addition, the relative frequency of red-listed species was negatively correlated to wood cover (Spearman's $R = -0.7$, $p = 0.02$).

The relationship between wood cover and the number of species was linear to hump-shaped and, in general, negative (Fig. 2). The maximum number of species was found at low to intermediate levels of wood cover. The hump was well captured by including the

square term of wood cover into the model, which was significant at both sampling scales (LRT: $P_{\chi^2} = 0.0018$ for the 1 m² samples and $P_{\chi^2} = 0.0004$ for the 100 m² samples). There were marked differences at the site level and between sampling scales (Fig. 2). In sites B, E, F and H a hump-shaped relationship was found at both sampling scales. Sites A, D and G, showed a hump-shaped relationship between species richness and wood cover at the 100 m² sampling scale only. Site C exhibited a weakly negative linear relationship between species richness and wood cover at both scales.

Including covariates into the GLMM demonstrated that a number of environmental variables have significant influence on species richness alone or on the effects of wood cover on species richness (Table 2). The most important environmental factors were mean annual temperature (1 m² sampling LRT: $P_{\chi^2} = 0.001$; 100 m² sampling LRT: $P_{\chi^2} = 0.01$), soil pH (1 m² sampling LRT: $P_{\chi^2} = 0.001$; 100 m² sampling LRT: $P_{\chi^2} = \text{n.s.}$), organic matter (1 m² sampling LRT: $P_{\chi^2} = 0.01$; 100 m² sampling LRT: $P_{\chi^2} = 0.001$), and N content of the soil (1 m² sampling LRT: $P_{\chi^2} = 0.001$; 100 m² sampling LRT: $P_{\chi^2} = 0.01$). These four covariates had significant effects on species richness alone, as well as on the effect of wood cover on species richness. Precipitation, altitude, slope and wetness index had no significant effects on species richness or on the effect of wood cover on species richness at either scale.

Because several of these environmental factors are highly correlated (Table 3), subsequent model simplification showed that the most parsimonious GLMM (i.e. having the lowest AIC) at both scales were models with cover, its square, and mean annual temperature as fixed effects (Table 4). This means that mean annual temperature captures differences between sites well and can be used as a surrogate of site properties to predict species richness in dependence of wood cover. Because the model contains only three variables, the prediction can be visualized as a 3D surface.

At the 1 m² scale, mean annual temperature and percent wood cover had a significant effect on species richness (respectively $P_{\chi^2} < 0.05$ and $P_{\chi^2} < 0.001$). The interaction between wood cover

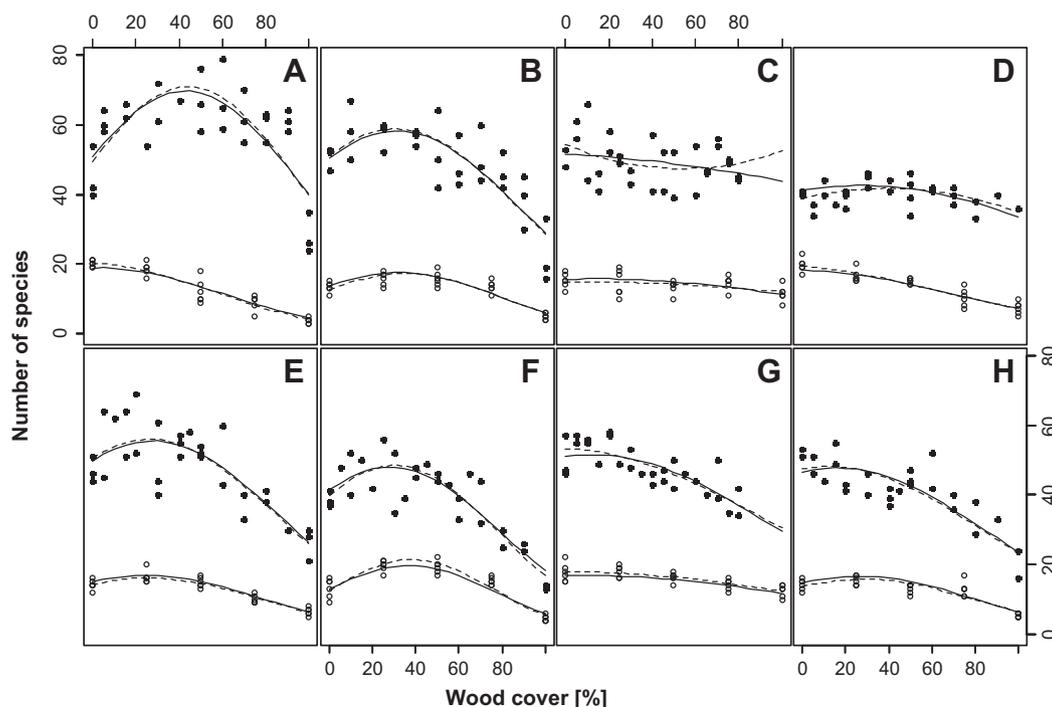


Fig. 2. Effect of wood cover on number of species for the eight sites. Dashed lines are generalized linear model regressions and solid lines are generalized linear mixed model regressions. Empty symbols represent 1 m² samples dataset, while full symbols represent 100 m² samples dataset. For explanation of the codes A/H, see Table 1.

Table 4
Estimated coefficients of explanatory variables in generalized linear mixed-effects models to plant species richness on 1 m² and 100 m² with wood cover and mean annual temperature as fixed effects. Significances are based on likelihood-ratio tests. Estimated coefficients are at log scale because of the use of a Poisson GLMM.

Sampling area	1 m ²			100 m ²		
	Df	Estimate	P_{χ^2}	Df	Estimate	P_{χ^2}
Intercept	2	2.71		2	3.94	
Cover	1	-0.85	<0.01	1	-0.48	<0.001
Cover ²	3	-1.45	<0.01	3	-1.27	<0.001
Mean annual temperature	1	0.06	<0.05	1	0.10	<0.05
Cover × mean annual temperature	1	-0.19	<0.001	1	0.01	n.s.
Cover ² × mean annual temperature		-	-	1	-0.26	<0.05

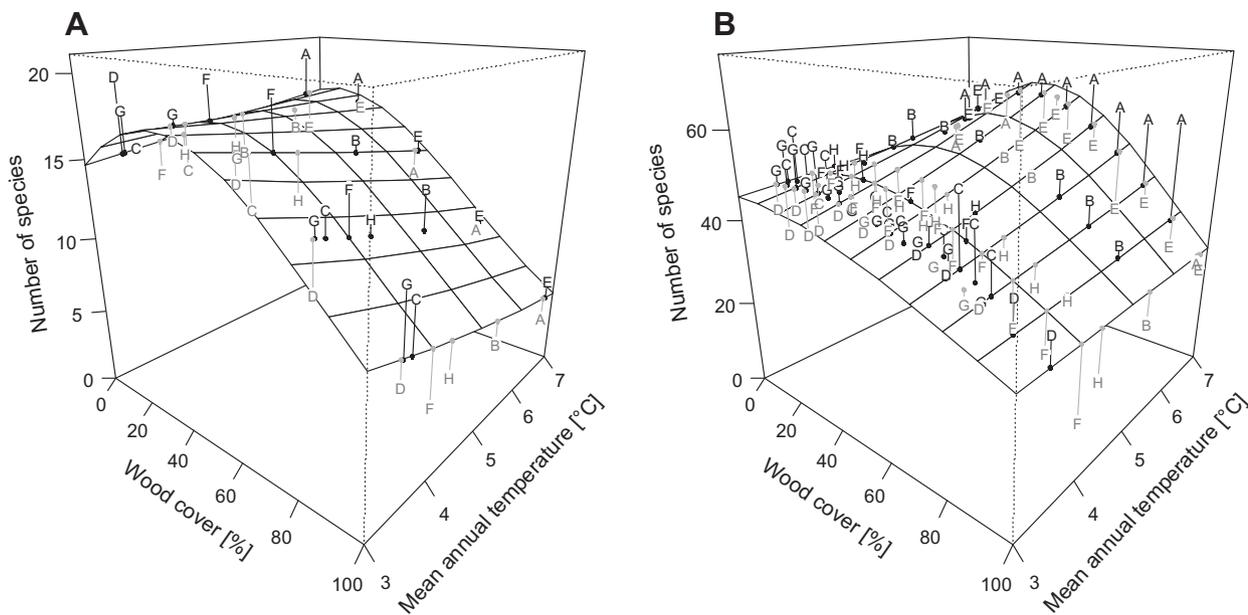


Fig. 3. Effects of wood cover and mean annual temperature on the number of species on 1 m² (a) and 100 m² (b). Surface is predicted from a generalized linear mixed-effects model fitted to the data. In (a), letters are mean values of the five repeated transects at each wood cover level for each site. In (b), letters are values of each plot at the respective wood cover for each site.

and mean annual temperature was also significant ($P_{\chi^2} < 0.001$). Fig. 3a illustrates this interaction. The estimated effect of wood cover on species richness was more linear at higher mean annual temperature, while at lower temperature the effect of wood cover on species richness had a maximum value at intermediate percentages of wood cover.

In contrast, a significant interaction between the squared wood cover and mean annual temperature ($P_{\chi^2} < 0.05$) was found in the 100 m² data. The estimated response of species richness to wood cover was therefore hump-shaped at high mean annual temperature, while at low mean annual temperature, the decrease was linear (Fig. 3b).

3.2. Effect of species abundance on the relationship between species richness, wood cover and mean annual temperature

Differences in model surfaces between the two sampling methods may be explained by species which have low abundance and are therefore more likely to be detected at the 100 m² scale than at the 1 m² scale. In order to investigate this further, effective diversities of orders $q = 1$ and $q = 2$ were derived from the species data at the 100 m² scale. GLMMs were fitted to original species numbers and effective diversities of orders $q = 1$ and $q = 2$. Wood

cover and its squared value also had a significant effect on effective diversity of order $q = 1$ and $q = 2$ (both $P_{\chi^2} < 0.001$) but mean annual temperature and the interaction between cover and mean annual temperature were no longer found to be significant (Table 5). Fig. 4 illustrates the gradual disappearance of the peak of species richness at high mean annual temperature and intermediate wood cover, as species with low abundance were sequentially down-weighted by calculating effective species numbers.

3.3. Effect of wood cover on the species–area relationship

A range of possible models and fixed parameters were evaluated to describe the species–area relationship in the investigated pastures (Table 6). A non-linear mixed cumulative Weibull model with wood cover and its square as fixed effects of parameter a was found to be the most parsimonious model. Correlations between parameters were relatively small, so a diagonal variance–covariance matrix (Pinheiro and Bates, 2000:157) performed best. We also tested various autocorrelation structures, but none substantially improved the fit.

Fig. 5 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 from site E was selected for

Table 5

Estimated coefficients of explanatory variables in generalized linear mixed-effects models to effective diversity of orders $q = 1$ and $q = 2$ on 100 m² with wood cover and mean annual temperature as fixed effects. Significances are based on likelihood-ratio tests. Estimated coefficients are at log scale because of the use of a Poisson GLMM.

	Effective diversity ($q = 1$)			Effective diversity ($q = 2$)		
	Df	Estimate	P_{χ^2}	Df	Estimate	P_{χ^2}
Intercept	2	3.01		2	2.64	
Cover	1	-0.28	<0.001	1	-0.27	<0.001
Cover ²	3	-1.26	<0.001	3	-1.18	<0.001
Mean annual temperature	1	0.10	n.s.	1	0.13	n.s.

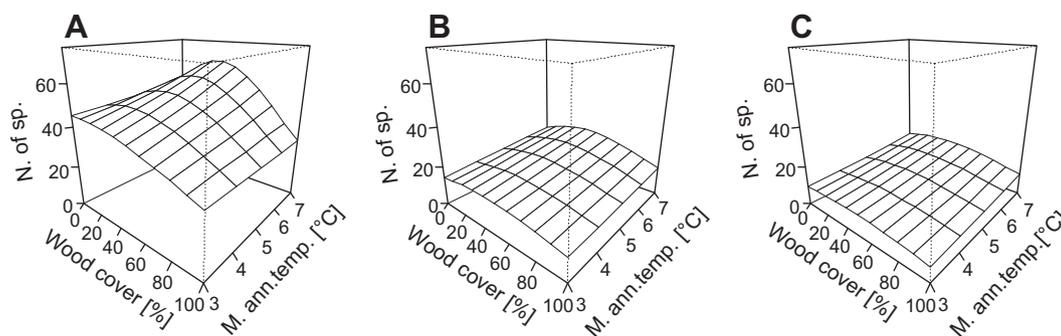


Fig. 4. Effects of wood cover and mean annual site temperature on the number of species on 100 m² (a) and the effective number of species of orders $q = 1$ (b) and $q = 2$ (c) on 100 m². Surface resulted from generalized linear mixed-effects models fitted to data.

Table 6

Parameter estimates for mixed non-linear cumulative Weibull model effects of wood cover and its square on parameter a . Significances are based on likelihood-ratio tests.

Parameter		Coefficient	Standard error of estimate	P	Standard deviation (site level)	Standard deviation (plot level)
a	Intercept	44.694	3.153	0.0001	8.334	5.072
	Cover	0.102	0.052	0.05		
	Cover ²	-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

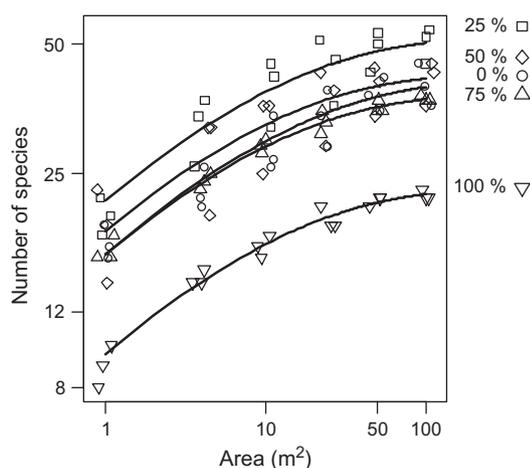


Fig. 5. Species–area relationship of site E on log–log scale. Symbols show richness estimates by nested-square sampling, lines show predictions from a non-linear mixed cumulative Weibull model on the entire dataset from all eight sites (Table 1).

display because this site exhibited the 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 sigmoid 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 the data significantly better than the power and Monod model ($P_{\chi^2} < 0.0001$).

Fig. 6 illustrates the effect of wood cover on the random effects in the model described in Table 6. The graph illustrates visually that wood cover has a strong effect on parameter a (i.e. the asymptote of the SAR curve). Parameters b (the slope) and c (the sigmoidal tempering) were marginally and non-significantly altered at the highest levels of wood cover. Hence, wood cover primarily altered the asymptote of the SAR curve (i.e. the number of species observed at 100 m², but had only marginal effects on the shape of the curve.

4. Discussion

4.1. Plant species loss by forest succession

Our investigations show that species richness generally decreases with increasing cover of woody species. This is consistent with most studies that investigated consequences of land use changes in the Alpine region (see for example Dullinger et al., 2003a, 2003b; MacDonald et al., 2000; Öckinger et al., 2006). Despite this general acknowledgement, very few studies explicitly investigated the relationship between wood cover and species richness. 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 (1950 m a.s.l.). He found no effect of the cover of green alder on species numbers up to a cover of 10% but a constant decrease in species numbers at higher values of green alder

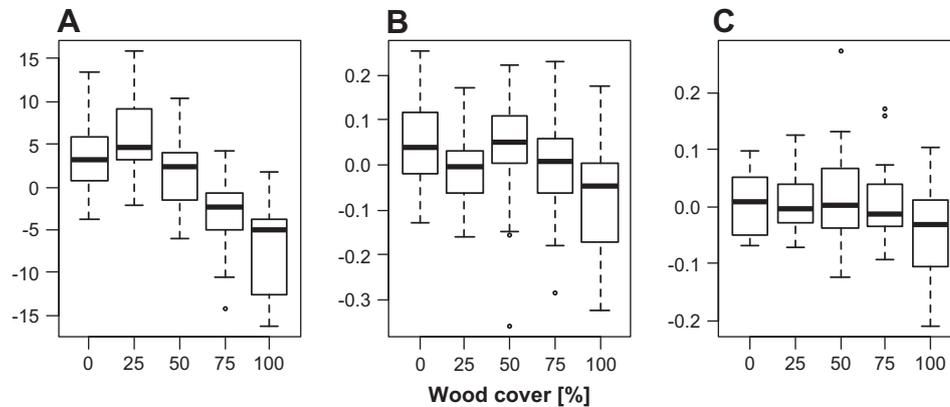


Fig. 6. Boxplots of random effects against wood cover at eight sites in the Italian Alps. Random effects are estimated for parameters *a*, *b* and *c* of a cumulative Weibull model using non-linear mixed modelling.

cover. On a site in Central Germany, Kesting (2009) found that shrub cover increased species richness at lower percentages, but decreased it at intermediate to higher percentages. Our study at eight contrasting sites in the Italian Alps suggests that mean annual temperature modulates the relationship between number of species and wood cover. Since plot size was 15 m × 15 m in Anthelme et al. (2001) and 10 m × 10 m in Kesting (2009), it is reasonable to compare these studies to our 100 m² data (Fig. 3b). Kesting (2009) reported a mean annual temperature of 8.7 °C for their site. Because Anthelme et al. (2001) only provide coordinates of a nearby village, we evaluated a range of possible locations at 1950 m a.s.l. around the published position. Based on the data by Hiebl et al. (2009), we then derived a mean annual temperature of around 2.5 °C. Using these estimates we evaluated the consistency of our model with the two published studies. We found 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 (2009) agree very well with Fig. 3b, which shows a less unimodal relationship at low mean annual temperature.

It is well known that mean annual temperature is highly correlated to other variables (Table 3). Mean annual temperature depends on altitude, exposition and latitude and affects soil formation and ecosystem productivity. For example, low mean annual temperature limits decomposition of soil organic matter and causes its accumulation (Brady and Weil, 1999). Ecosystem functioning has consequences for plant species richness. As shown by Moser et al. (2005) and Ziliotto et al. (2004) altitude (or mean annual temperature) has a negative effect on species richness, in general, irrespective of habitat type. Our data confirm that mean annual temperature had a substantial reducing effect on the total number of species (approximate γ diversity). Furthermore, the initial succession of reforestation is faster at warmer sites, while establishment of woody species on abandoned grasslands is slower for higher altitude (Tasser et al., 2007).

The strong correlations between the explanatory variables resulted in multicollinearity, which we addressed by model reduction based on AIC. Model reduction resulted in a simple model with mean annual temperature as the only site variable, suggesting that other environmental descriptors do not explain substantially more of the variability in the data because they are collinear to mean annual temperature. This means that mean annual temperature can stand in as a proxy for other environmental variables such as soil organic matter content and soil fertility which also have significant effects on species richness, but are collinear (Table 2).

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 woody species. Using a multilevel model in our study permitted us to draw more general conclusions about the observed patterns than possible from a study on a single site. In fact, ecological studies often involve variation among units of investigation, in our case among sites. Variation among these units of investigation can be conveniently quantified as random effects in a mixed-effects model (Bolker et al., 2009). A mixed-effects model is an 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 the site level and at the plot level, and therefore, data from different sites can be analysed together. The information available at the site level can then be used to investigate how site properties affect patterns observed at the plot level (e.g. how site temperature affects species loss).

The second advantage of employing a mixed-effects model is that the result is a much more parsimonious model representation. Considering individual sites as part of a 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 sites with weak data. In general, differences between GLM and GLMM on regressions were not very important (Fig. 2). However, where evidence was relatively weak, such as at site C, the relationship obtained by GLMM was much more realistic than that of GLM, which had a polynomial pattern with the concave side up.

4.3. Detection of plant species loss depends on observation scale

Ecological studies often sample data only at one scale of observation that 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 m² or 100 m²) could be misleading. At the 1 m² observation scale, highest species richness was found at intermediate wood cover on sites with lower mean annual temperature, whereas at the 100 m² scale, highest species numbers was found at intermediate wood cover on sites with high mean annual temperature. This discrepancy can be explained by the presence of species with low abundance at warmer sites (and be simulated by

down-weighting species abundance using effective species numbers; Fig. 4). This pattern would not have been detected if observation was conducted 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 the minimum area required to capture species richness along the pasture-to forest gradient is about 25 m² (Fig. 5). If sampling areas are smaller than 25 m², it is possible to considerably underestimate species richness. Furthermore, we found that the percentage of wood cover (i.e. the transition from grasslands into forests) changed the asymptotic level of the SAR, but not its slope. This is reassuring for a whole body of literature (e.g. Anthelme et al., 2001; Fischer and Wipf, 2002; Kesting, 2009) who investigated effects of forest succession with equal-area sampling without considering that the observed effects could just be an artefact of changing SAR.

Reporting scale-effects on the relationship between species richness and wood cover on the one hand and finding no significant effect of wood cover on the SAR on the other may appear contradictory. The discrepancy arises from the differences in analysis. In the first, we analyse effects of site characteristics on the relationship between species richness and wood cover, in the latter, we test effects of wood cover on the SAR and detect only marginal effects of wood cover on the shape of the SAR.

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

5. Conclusions

A multi-site study, as the one presented here, is necessary to understand complex phenomena such as forest succession in mountain pastures. Our investigations show that sites with higher mean annual temperature are the most vulnerable to loss of species by forest succession. These sites also had low soil organic matter content and low soil fertility. The observed high loss at warmer sites is most likely a result of the number of species with low abundance. In agreement, species pertaining to red lists were more frequently found on sites with higher mean annual temperature and at lower degrees of wood cover. Sites with higher mean annual temperature, presumably because of the larger magnitude of changes in abiotic and biotic conditions, should therefore receive primary attention of land managers and conservation bodies with the aim of halting biodiversity loss.

On the other hand, highest total species numbers and high numbers of red-list species were found at low to intermediate percentages of wood cover. Hence, the target of land management should not be pastures completely free of shrubby or woody species. Rather, management strategies should be promoted, by which a low percentage of shrubs or trees on pastures can be maintained in the long term. Because of the higher heterogeneity created by grazing than by mechanical operations (Adler et al., 2001; Wrage et al., 2011), a combination of animal grazing with mechanical interventions to regulate shrub cover may maintain the highest species numbers.

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