Multivariate analysis of the response of overgrown semi-natural calcareous grasslands to restorative shrub cutting

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Summary

Monitoring and evaluation of restoration management often suffers from poor sampling design and a lack of statistical rigour, seldom considering the spatial and temporal variability of habitat. We tested the effectiveness of shrub cutting on the restoration of calcareous grasslands, using a 4-year Before–After/Reference-Control-Impact (BARCI) project design with replicated patches of habitat. Departure from the control sites and convergence with the reference sites were analysed using the Principal Response Curves (PRC) method.

The structure of communities was compared 1 year before and 3 years after management on control scrubland, restored ex-arable land, restored and reference grassland. Results show that shrub cutting did not suffice to restore the community composition of the semi-natural calcareous grasslands. The restored ex-arable areas maintained a community structure extremely similar to the control scrubland.

The restored grassland, on the other hand, deviated from the control and slightly converged to the reference grassland, though not significantly. The dominant scrub species that was cut, \textit{Prunus spinosa}, showed higher cover values compared to the reference grassland in any of the treatments, even the first year after cutting. Species typical of xeric and/or calcareous grassland were more abundant in the reference than in the restored grassland, while arable and ruderal species were more frequent in the restored sites.

This study has demonstrated that the BARCI approach is a powerful tool for the evaluation of restoration management, as it was possible to evaluate not only...
departure from the unmanaged control, but also convergence with the reference community. The PRC method provided a comprehensive overview of the response of the 153 species involved in the study. Our results also indicate that the second PRC should be considered, when significant, to not exclude important information from the assessment.

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Introduction

Semi-natural grassland communities are of great interest for their landscape value, abundance of rare and threatened species and high species richness (Willems, 2001). However, semi-natural grasslands have strongly decreased due to changes in land use throughout Europe (Poschlod, Bakker, & Kahmen, 2005; Stoate et al., 2001). Abandonment or insufficient management of pastures and meadows are the main factors that trigger the secondary succession of semi-natural grasslands into increasingly dense scrublands, characterised by lower species richness and fewer rare species (Barbaro, Dutoit, & Cozic, 2001), contributing to an overall homogenisation of the landscape (Debussche, Lepart, & Dervieux, 1999), and leading to a loss of habitat and species diversity (Gallego Fernández, García Mora, & García Novo, 2004).

From 1992 to 2005, 45 projects on semi-natural dry grasslands (Festuco-Brometalia) have been financed by the European Union (LIFE Projects Database, 2005). Moreover, there is plenty of information about grassland restoration projects (Muller, Dutoit, Alard, & Grévilliot, 1998; Ormerod, 2003; Ruiz-Jaen, & Aide, 2005); however, we often cannot correctly measure the ecological outcomes of those projects. Many authors have recently emphasised the need for a powerful sampling design and statistical analysis to evaluate restoration experiments, considering spatial-temporal variability of habitat (Block, Franklin, Ward, Ganey, & White, 2001; Chapman, 1999; Chapman & Underwood, 2000; Michener, 1997; Palmer et al., 2005;
The most powerful approach, Before–After / Reference-Control-Impact design (BARCI, Lake, 2001), includes references and control sites in order to unambiguously assess the success of a restoration programme. Using this design, data from the restored sites and reference sites (areas which have the desired end conditions) should be compared to control locations (degraded areas that are not being restored) (Chapman & Underwood, 2000).

Several studies have incorporated restored, reference and control sites to evaluate the restoration of semi-natural grasslands (Pywell et al., 2002; Pykälä, 2005) but no study, to our knowledge, applied a complete Before–After sampling design (BARCI).

Principal Response Curves (PRC) method is a multivariate repeated measurement technique that is able to show differences between time series in a single diagram. This method is widely applied in aquatic ecotoxicology and environmental sciences (see Frampton, Van den Brink, & Gould, 2000; Heegaard & Vandvik, 2004) and is considered as an analysis that has a potential for wider application in experimental community ecology (Leps & Smilauer, 2003; Vandvik, 2004).

In this study we evaluated the success of shrub cutting on the restoration of semi-natural calcareous grasslands as an example of a highly spatial heterogeneous and species rich communities, using a 4-year BARCI design. Departure from the control sites and convergence with the reference sites in terms of plant community composition were analysed by the PRC method. The null hypothesis that was tested using Monte Carlo permutation tests was that the temporal trends in the species composition is independent of the treatment (i.e. Reference-Control-Impact).

**Materials and methods**

**Study area**

The research was conducted near the Monte Labbro summit (42°49′13″ N, 11°31′33″ E) a prevalently calcareous massif on the Uccellina-Monte Amiata ridge, Tuscany (Lazzarotto, 1993) (Fig. 1). The area is included in a proposed Site of Community Interest (pSCI, "Monte Labbro and Upper Albegna Valley"). Mean annual rainfall in the area is 1002–1469 mm and the average annual temperature is 12.1–12.5 °C. Monte Labbro summit has been grazed for centuries, mostly by sheep, and human traces in the area date back to the Bronze Age (Pisto, 1989). After the 1960s the grazing pressure decreased and arable land abandoned.

The study area, ranging in altitude from 1089 to 1193 m, is characterised by a mosaic of community types: semi-natural calcareous grasslands with low or no presence of shrubs; overgrown grasslands (*Prunus spinosa* scrublands); dense scrublands on small abandoned ex-arable areas located on karstic *doline* where stones have been removed in the past, with *Prunus spinosa*, *Rubus ulmifolius* and *Cytisus scoparius* and small woodlands with *Acer campestre* and *A. monspessulanum*. The area is mainly grazed by sheep (stocking rate estimated as 2/3 sheep ha⁻¹ yr⁻¹; Comunità Montana, unpublished data).

The restoration project, financed by a LIFE-Nature Project, started in the area in 1999 and has been managed by a single authority (Comunità Montana (Fig. 1). Study area. Different treatments location are localised by different symbols. Woodlands, not included in the analysis, are represented in grey.)
del Monte Amiata Zona "I1", Area Grossetana) since then. The project included the cutting of shrubs on overgrown grasslands and dense scrublands on abandoned ex-arable areas.

**Sampling design and data collection**

To test the effects of shrub cutting on the community structure by means of species composition of overgrown grassland we conducted a 4-year BARCI experiment with replicated patches of habitat to take into account natural spatial variability (Chapman, 1999; Dutilleul, 1993; Johnson, 2002; Ruiz-Jaen & Aide, 2005).

In late June 2000, we allocated 40 1 × 1-m square plots by randomly generating spatial coordinates in the study area to gather the field data before the intervention. According to the LIFE project design, in the period comprising late summer of 2000 to early spring 2001, the labourers manually cut all shrubs (except all individuals of Juniperus communis and some of Prunus spinosa, Rosa canina and Crataegus monogyna, for faunistic conservation purposes) in three out of five patches of overgrown grassland and in the two patches of scrubland on ex-arable areas, for a total area of about 8 ha. Shrub clearing was not repeated.

Plots were divided into four treatments, namely: control scrubland, restored ex-arable, restored grassland and reference grassland (Fig. 1 and Table 1). The number of plots are proportional to the dimension of patches (Elzinga, Salzer, Willoughby, & Gibbs, 2001). We decided to differentiate two management treatments (restored ex-arable and restored grassland) because we expected a different response following the shrubs cutting, due to differences in topographic features and land use history. Two patches of overgrown grasslands dominated by Prunus spinosa were taken as a "control" to assess the changes regarding community structure of the restored ex-arable and restored grassland, as they represented "sites similar to the site being restored prior to restoration" Chapman (1999). As reference, we adopted the interspersed patches of semi-natural calcareous grasslands recognised in the study area, to avoid the bias of changing spatial context and environmental distance from the site to be restored (White & Walker, 1997).

Floristic measurements were made annually in the 40 plots in late June—early July from 2000, before the treatment, to 2003. All vascular plants growing in each plot were recorded and their cover estimated using a point-quadrant method (Moore & Chapman, 1986) with a density of 100 pins/m². Species present in a plot but not touched by any pins were recorded with an arbitrary cover of 0.1%. The nomenclature of species follows Pignatti (1982) and Bechi (1998) for Cerastium arvense ssp. arvense var. etruscum.

**Statistical analysis**

Departure of the managed plots from control site and convergence to reference site conditions were analysed by the PRC technique (van den Brink & ter Braak, 1997, 1998, 1999). This technique, derived from the redundancy analysis (RDA) constrained ordination method, plots the principal components of the effects of treatment, expressed as deviations from the control treatment, against time (van den Brink & ter Braak, 1998).

PRC consists of the canonical coefficients of a partial redundancy analysis, in which the input data set are the sample-by-species matrix of log-abundance values, the sample-by-year matrix of covariables, and sample-by-treatment in year matrix of explanatory variables. The canonical coefficients of partial RDA (C(dt)) quantify the compositional differences between the control and other treatments (d) at each sampling date (t): temporal trends can be visualised by plotting $C_{dt}$ against time. The species weight $b_k$ indicates the affinity that each individual taxon has with the overall community response as displayed in the PRC diagram: species with high positive values follow the overall community response as indicated by the

**Table 1.** The total number of 40 plots is divided into four treatments: each treatment has been replicated at least in two patches

<table>
<thead>
<tr>
<th>Treatments</th>
<th># Patch</th>
<th># Plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control scrubland: dense scrubland where shrubs were not cut</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Restored ex-arable: scrubland on ex-arable sites where shrubs were cut</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Restored grassland: shrubby grassland where shrubs were cut</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Reference grassland: target grassland with scattered shrubs where shrubs were not cut</td>
<td>5</td>
<td>16</td>
</tr>
</tbody>
</table>

Plot amount always allows to perform statistical analysis with a $p<0.05$ significance.
PRC, while for negative values the community response is the opposite.

After extraction of the first PRC, which shows the dominant response present in the data set, a second, third, fourth, etc. PRC can be extracted (van den Brink & ter Braak, 1998). The second PRC shows the main deviations from the first PRC and may be important if not only a single dominant response but also several sub-dominant responses are present (van den Brink, van den Brink, & ter Braak, 2003). Adding the second PRC, if significant, to the interpretation of data set can enhance visualisation of the responses (van den Brink & ter Braak, 1998). The change in log-abundance as calculated per PRC must be summed across components to yield the joint fit of the change. The fitted change can be plotted against time to yield fitted response curves for individual species or species groups. For instance, if a species has an equal weight with both PRC, they can be summed to yield the fitted change for that species. If species weights are different, the PRC should be weighted by these species weights to obtain the fitted response.

Before multivariate analysis, the abundance of communities was ln(20×1)-transformed, to down-weight the influence of dominant species (van den Brink, Hattink, Bransen, van Donk, & Brock, 2000).

The significance of the PRC diagrams was tested by Monte Carlo permutation tests (999 permutations), permuting whole time series in the partial RDA from which the PRC were obtained. Monte Carlo permutation tests were also performed for all sampling data (199 permutations), by testing each treatment against all other treatments. Statistical analysis was performed using the CANOCO 4 software package (ter Braak & Šmilauer, 1998).

**Results**

**General results**

One hundred and fifty-three vascular species were found in the 4 years of observations. Mean species number in control sites was low while restored grassland, with a maximum of 37 species m⁻² recorded in one plot in 2002, showed higher values (Table 2). In 2000, the pre-treatment year, reference grassland was dominated by *Bromus erectus* while the other treatments were dominated by *Prunus spinosa*, though with different percentages of cover (control scrubland 44–100%; restored ex-arable 28–100%; restored grassland 3–100%).

**Community changes**

Differences between the treatments in time accounted for 21.9% of the between-plot variation in species composition, which was highly significant (Table 3). The two restored treatments showed the highest variation in composition and cover (Fig. 2 and 3). Fig. 2 shows the first Principal Response Curve (PRC 1), indicating a similar community composition for the control scrubland and the restored ex-arable land for the pre-treatment year. In the same year of observation, the control scrubland was very different from the restored grassland and especially from the reference grassland. The differences between restored grassland, control scrubland and reference remained significant throughout the observation period (p<0.01).

The restored ex-arable plots had strong similarity with the control plots: in 2002 and 2003, however, they were significantly different from the control (p<0.01), while remaining distinct from the restored and reference grassland. In 2000, the restored grassland was clearly different from the reference grassland, towards which it evolved after shrub cutting. The differences between restored grassland, control scrubland and reference remained significant throughout the observation period (p<0.01).

In the second PRC (Fig. 3) the curves of the restored ex-arable and reference grassland stayed fairly close to the line of the control scrubland during the whole period, while the restored grassland showed a clear separation from the control scrubland from 2000 to 2003.

<table>
<thead>
<tr>
<th>Species richness and year of observation (mean±SE; species m⁻²) for each treatment</th>
<th>Species m⁻² (mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2000</td>
</tr>
<tr>
<td>Control scrubland</td>
<td>9 ± 1.3</td>
</tr>
<tr>
<td>Restored ex-arable</td>
<td>14 ± 1.5</td>
</tr>
<tr>
<td>Restored grassland</td>
<td>25 ± 0.4</td>
</tr>
<tr>
<td>Reference grassland</td>
<td>25 ± 0.3</td>
</tr>
</tbody>
</table>
Species response

*Bupleurum baldense*, *Festuca brevipila*, *Petrorhagia prolifera* and *Poa annua*, showing high species weight (\(b_k\)) only for the PRC 1 (Fig. 2) and low for PRC 2, strongly changed in cover values in restored grasslands approaching the cover observed in the reference grassland, especially in 2002 and 2003. Additionally, although *Galium coruddifolium* and *Thymus longicaulis* showed an increase with respect to 2000, their cover remained lower than in the reference grassland. *Rubus ulmifolius* and *Galium album* with negative \(b_k\) values, were more abundant in the restored ex-arable areas than in the control and other treatments.

In the second PRC (Fig. 3), *Phleum pratense*, *Aira elegans*, *Anthoxanthum odoratum* and *Tordylium maximum* (high \(b_k\) values, low values for PRC 1) showed resilience to shrub cutting in the restored grassland.

*Fig. 4* shows species weights for PRC 1 and PRC 2. Species with the same weights in the two PRC, except for sign, showed a pattern similar to that of the PRC at the corners of *Fig. 4*. The response of a species indicated by the first two PRC diagrams can be calculated by adding up the PRC using the species weights as the weighing factor. The diagrams in the corner indicate the response if the weight in each diagram is the same, i.e. that lie on the lines that have a 45° angle with the first and second axes. For example, *Prunus spinosa* had similar abundance values in the control and restored plots in 2000, showing a decrease subsequent to shrub cutting (negative \(b_k\) for the PRC 1 and positive \(b_k\) for PRC 2, upper left quadrant, \(PS\)). In the following years, *P. spinosa* cover in restored ex-arable sites returned to values similar to those of control scrubland, while in restored grassland it remained distinct from both control and reference sites during the whole period.

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**Table 3.** Percentages of the total variance that can be attributed to time and treatment regime for the data set; the table indicates the fraction of variance captured by the first and second principal components response (PRC)

<table>
<thead>
<tr>
<th>% Variance accounted for by</th>
<th>% Variance explained by treatment regime captured by</th>
<th>(p(999) permutations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Treatment</td>
<td>First PRC</td>
</tr>
<tr>
<td>2.1</td>
<td>21.9</td>
<td>58.4</td>
</tr>
</tbody>
</table>

The treatment component includes the interaction between treatment and time. The remaining fraction of variance is residual.

**Figure 2.** First Principal Response Curves diagram, showing the effects over time of shrub cutting on restored sites. Species weight on the right (\(b_k\)) can be read as the affinity of every species with the shown diagram.
Many species, such as *Cynosurus echinatum*, *Crepis neglecta*, *Eryngium campestre* and *Orlaya grandiflora* had lower cover in the control plots compared to all treatments (see upper right quadrant of Fig. 4). In particular, *Bromus erectus* and *Trifolium incarnatum* ssp. *molinerii*, corresponded to the species with higher cover in the restored grassland than the reference grassland. In contrast *Anthyllis vulneraria* ssp. *praepropera*, *Sedum album*, *Centaurea deusta*, *Phleum ambiguum* and *Thymus longicaulis* (see lower right quadrant of Fig. 4) had higher cover in the reference than in the restored grassland, representing the guiding species for the desired community (reference grassland).

Discussion

Community changes

Although more years of study are necessary to definitively declare the outcome of the restoration actions, from the present result we can assert that in the short-term shrub cutting and a low grazing pressure did not suffice to restore the community composition of semi-natural calcareous grasslands. As in similar experiments, the greatest changes were recorded in the second year after management practices (Dzwonko & Loster, 1998). The restored ex-arable areas maintained a similar species composition to the control scrubland, although in the last 2 years of observation they differed significantly. Conversely, although they still differed significantly, the restored grassland diverged from the control and slightly converged to the reference grassland. Even the first year after cutting, the dominant scrub species, *Prunus spinosa*, did not reach cover values similar to the reference grassland in any of the managed plots. This may be related to the mode of reproduction of *Prunus spinosa* which has vigorous vegetative suckering (Snow & Snow, 1986) with resprouting after cutting.

In the restored grassland, the annuals *Bupleurum baldense*, *Petrorhagia prolifera* and *Poa annua*, together with *Festuca brevipila* showed a positive response to the management, with cover values closer to the reference grassland. Significantly, the most abundant species in reference grassland are typical of xeric and/or calcareous grassland, such as *Inula montana*, *Sanguisorba minor*, *Teucrium chamaedrys* and *Thymus longicaulis* and the species of high conservation value *Phleum ambiguum* and *Centaurea deusta* (Italian peninsula endemism). Arable and/or wasteland species *Tordylium maximum*, *Orlaya grandiflora* and *Plantago lanceolata* were more frequent in the restored than in the reference grassland.

Gaps created by shrub cutting in the two managed communities were colonised mostly by *Trifolium incarnatum* ssp. *molinerii* (with an increase of 17.5% and 7.9% in restored grassland...
and restored ex-arable respectively from 2000, the year before management, to 2002 and Dactylis hispanica which are also present in the seed bank of open grassland and prairies afforested with conifers (Maccherini & De Dominicis, 2003). In ex-arable plots, the gaps were also colonised by Brachypodium rupestre and in restored grassland by Bromus erectus as well. These species, are almost absent in the germinable soil seed bank of these calcareous grasslands (Maccherini & De Dominicis, 2003). Hemi-cryptophytes with regenerative strategy principally by seeds (Grime, 1979) prevail while annual species such as Linum bienne, Campanula rapunculus and Veronica agrestis, massively present in the soil seed bank of open and afforested grassland, were scarcely present or absent from restored patches.

**Methodological considerations**

This study has demonstrated that the BARCI approach is a powerful tool to evaluate restoration...
efforts. Without control sites, it can be demonstrated that a managed site changed in the predicted manner, but the transformation cannot be unambiguously attributed to the restorative treatment; further, without reference sites, the extent to which the restored sites follow a trajectory toward some specified target state cannot be assessed (Chapman, 1999). Resemblance of control and restored sites in terms of community composition can only be verified after statistical comparison since control sites should be “…as similar as possible to the restored site” (Block et al., 2001). Even if we tested that the control scrubland was statistically different from the restored grassland before the intervention occurred, the control scrubland still remained a real ”control” to which the restored sites can be compared (Chapman, 1999) in time. In fact, the adoption of the BARCI approach allowed to effectively assess how and when the restored communities, evaluated by means of species composition, followed in the years a trajectory from the control scrubland toward the desired reference grassland condition.

The PRC method provided an overview of the response of the 153 species involved in the treatment. Normally only the results of the first PRC are presented: if only the first PRC had been considered in this paper, the emphasis would have been put on the pre-treatment differences between the control and restored grassland plots. When pooled with the second PRC these differences still hold, but the combination of the two provides a clear treatment axis from the upper left quadrant to the lower right quadrant (Fig. 4). Although this is one of the first ecological studies to include the second PRC, its results clearly indicate that, when significant, the second PRC should be considered, otherwise important information is left out of the assessment.

Implications for the conservation of calcareous grasslands

Different opinions have been expressed regarding the restoration of grassland overgrown by trees or shrubs: Dzwonko & Loster (1998), for example, suggest that cutting alone is not sufficient to maintain grassland communities. However, encouraging results have been obtained in the restoration of overgrown grassland by clearing shrubs, mowing or grazing (Barbaro et al., 2001; Bobbink & Willems, 1993; Willems & Bik, 1998; Zobel, Suurkask, Rosen, Partel, 1996). On the other hand, the restoration of formerly arable land seems to be a slow and difficult process if target species are not sown, and if soil fertility and the establishment rate of competitive, early successional species is not artificially reduced (Bissels, Holzel, Donath, Otte, 2004; Hutchings & Booth 1996; Kleijn 2003; Walker et al. 2004).

Our study showed that after shrub clearing neither the overgrown grassland nor the scrubland on former arable land reached a community composition similar to the reference grasslands. The scarcity of seeds of target species in the soil seed bank (Bissels et al. 2004; Maccherini & De Dominicis 2003) together with the documented high soil fertility typical of overgrown areas (Berendse 1998; Marrs 1993) might be responsible for the negative result obtained. Despite the presence of grazing livestock, the grazing pressure appeared too low to determine a consistent progressive shifting to the reference grassland after shrubs cutting. Rigorously testing the efficacy of the adopted intervention is hence crucial to correctly address the scheduling and management for future intervention.

For these reasons, other factors not considered in the present management project, such as increased grazing pressure, the introduction of target species, the reduction of nutrient status and prescribed burning used in the past to control shrub encroaching, should be considered for the restoration of these grasslands.

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References


Assessing grassland restoration success


