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Changes in vegetation composition of hay meadows between 1993 and 2009 in the Picos de Europa and implications for nature conservation

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Abstract
The Picos de Europa are a range of predominantly Carboniferous Limestone and Sandstone mountains mainly in the Cantabrian Region of northern Spain. The highest peaks are precipitous and reach 2600m. There are complex gradients between Lusitanian, Alpine and Mediterranean environmental zones as well as variable soil types. In combination with the long history of traditional agricultural management, a wide range of diverse habitats and species are present. The herb-rich hay meadows have long been recognised as having a high nature conservation value but, as elsewhere in European mountains, such grasslands are threatened by changing agricultural practices. Accordingly, in 1993, 92 quadrats were recorded using a restricted list of indicator species from a stratified random sample. The sample was repeated in 2009 by the authors. Only approximately 3% of meadows had changed land use but farmyard manure was no longer used, probably because of the lack of labour. A range of measures produced by statistical analysis of the vegetation data showed many significant changes consistent with the increased use of slurry as well as re-seeding of some fields. The grass swards had not only become denser with fewer species present but there was also a loss of sensitive indicators especially of calcareous conditions and open vegetation. By contrast, competitors had increased and the vegetation had become simpler with a balance of vegetation types shifting to more fertile conditions. These changes have mainly been in the more fertile meadows used for silage. The core of about 35% of herb-rich meadows mainly cut for hay has remained relatively stable but the results show that they are at risk if the current trend continues. If management practices that form the core of traditional agriculture are not maintained, one of the most important resources of herb-rich meadows in Europe will be lost.

Introduction
The Picos de Europa mountains in northern Spain, are a range covering approximately 600 km^2. The highest peaks reach 2600m and consist of precipitous Carboniferous Limestone cliffs, screes and grasslands. At lower altitudes the mountains and valleys are mainly Carboniferous Sandstones and Shales which have more rounded slopes but some cliffs are also present There are also some smaller areas of Conglomerates giving local variations in soil conditions. The western side of the massif is hyper-oceanic because of the Atlantic influence and there is a major environmental gradient towards the east with a progressively stronger Mediterranean influence, especially in the valleys and on south facing slopes This gradient is reflected in the transition in the European Environmental Zones (Metzger et al., 2005) from Lusitanian in the west, to Alpine South in the highest mountains and Mediterranean mountains on the lower slopes especially in the east. The range forms the highest part of the Cantabrian chain which extends almost 400 km south west from the Pyrenees. It is geographically relatively isolated from other mountains in the Iberian Peninsula, as reflected by the number of endemics, some of which are in common with the Pyrenees. Otherwise the region contains a complex mixture of phytogeographical elements containing Alpine, Atlantic, Lusitanian and Mediterranean species as well as many plants with wide ranges throughout Europe. A correspondingly wide range of habitats is also present.

Earlier studies (Goldsmith & Garcia, 1983; Farino, 1987, 1988; Rivas-Martinez et al*.,* 1984) describe the particular botanical significance of the hay meadows and the diversity of the region. This exceptional diversity results from the combination of pedogenic and climate factors interacting with centuries of management by man. Traditionally, the practice of altitudinal transhumance enabled the species rich meadow areas to produce hay in the spring and summer which provided fodder for overwintering livestock (Ruiz & Ruiz, 1986; Bunce, Bell & Farino, 1998).

Over the last 50 years the structure of the cultural landscapes has changed because of differences in management systems and social factors. The pace of these changes has accelerated since Spain joined the European Union in 1992 but especially in the last ten years. Farming systems have moved away from self-sufficiency towards profit based agriculture particularly as a result of subsidy incentives through the Common Agricultural Policy (CAP) (Hindmarch & Pienkowski, 2000). For example, there has been a shift from traditional cattle breeds (*Tudanca*) to high output breeds (Charolais and Limousin for beef; Friesian and Swiss Grey for milk) with concomitant ecological implications. These breeds are ill suited to the swards of steep mountain pastures and therefore rarely taken to higher altitudes in the valley (Waterhouse, 1996). A decline in the farming population has also resulted in a reduction in land management through cutting or burning. The result has been scrub encroachment on the steeper slopes because of lack of grazing. The need for a higher productivity in meadows and pasture through silage production has led to the increasing use of slurry.

Silage production also allows earlier and more frequent cutting and does not enable plants to set seed (Frame, 2000; Farino, 2009).

The reduction in grassland area raises concerns over the loss of biodiversity and endemic species linked to the flora and associated fauna - hence the present paper (Baura et al., 2006; Hopkins, Bunce & Smart, 2000). The remaining meadows on steeper slopes tend not to be fertilized and have lower land management pressure due to a variety of social factors especially the aging population.

Cultural landscapes, such as those in the Picos de Europa, in which component ecosystems have developed over many centuries in response to management practices, are now recognized as of major conservation importance (Birks et al., 2004; Bergland, 1991; Selman, 1994) However throughout Europe they are under threat mainly because of changes in traditional management systems. Changes in the floristic diversity and composition of hay meadows are a reflection of multiple drivers in the landscape and are the subject of the present study which reports the changes in mountain meadows and pastures over almost 20 years.

Methods
Location
The initial botanical survey following the Flora Europaea (Tutin et al.,1993) took place in April 1993 with a replicate survey completed in May 2009 (a seasonal difference of three weeks) covering the Deva valley, Cantabria, Picos de Europa. The sample comprised 23 stratified random square kilometres with four random points in each square, in groups of four following meadow delineation on the Mapa Topografico de Espana Camaleňo (81-1) and Potes (81-2) and a detailed survey in 1992 by Charlotte Mason College, Ambleside. The meadow altitudes ranged from 290m to 1085m, a range of 795 m. In 2009, map detail was further enhanced using 'Sigpac' (http://sigpac.mapa.es/fega/visor) as used by the Cantabrian Regional Government, enabling a combination of aerial photography and maps to locate the sites.

The intervening years were covered by data from small scale research projects by the students of the College, now the University of Cumbria, mainly covering meadow composition in selected meadows near to Baró, San Pelayo, Brez and Lon in the Camaleňo District.

Sampling
Each point on the map was the central point for a $4m^2$ quadrat laid out according to diagonals (Bunce & Shaw, 1973) with a 5m minimum distance from any track or field boundary. Notes of location and field sketches were made in 1993 to facilitate replication. 86 indicator species with minimal taxonomic problems were selected following research by Farino (1992) and through discussions with the

authors with an estimation of % grasses, % moss and % bare ground. Species abundance was categorised on the Braun-Blanquet scale of 1 – 5:
1: 1 individual present; 2: <5% cover; 3: 5-25% cover; 4: 26-50% cover; 5: 51-100% cover. For calculations of comparative abundance, a point percentage was used (1:1%; 2: 2.5%; 3:15%; 4:37.5%; 5:75%)

In 1993, a pilot study of eight quadrats where all species were identified was used to assess the reliability of indicator species as a measure of meadow composition. The results confirmed that indicators were reliable and could be used to determine the status of meadows.

In both surveys, the co-ordinates of the point were recorded (in 2009, the grid reference was given using GPS), the altitude (also using GPS in 2009 but from the map in 1993), the mean height of the vegetation was estimated, with grazing and application of farmyard manure also noted. In 1993, further environmental variables were added including: slope angle and aspect, water content, pH and depth of soil. The distance from villages and tracks was also recorded. Surveyors in 1993 working in pairs comprised trained botanists from Wageningen Agricultural University, the authors and professional botanists who quality assured the samples (Buit & Moonen, 1993; Prince, 1993).   By 2009, two of the professional botanists were still present for quality assurance together with the first author. Thus, there were three replicate surveyors.

The surveys comprised identical indicator species although in 2009, each of the grasses was identified whereas in some of the 1993 quadrats, only selected grasses were recorded and entered into the data analysis, with all grasses in both surveys being given an abundance category.

In 2009, all 92 quadrats were surveyed and categorised as 'match', 'near match' or 'no match'. 'No match' included meadows with a change of land use (e.g. new building) or where there was insufficient or spurious data in the previous survey to locate the meadow. 'Near match' was in the same meadow but a different location and 'match' was as close as possible to the original quadrat.

Quantitative data
Records from the survey in 1993 showed that 12 of the meadows (13%) were manured whereas only one (1%) was recorded in 2009.This major decline confirms local observations that farmyard manure is no longer used probably because of lack of labour and is now dumped in heaps on the edges of villages. Such manure releases nitrogen slowly as compared with the use of slurry which moves quickly into plants.

Analysis
A number of descriptive statistics were facilitated using Excel (Microsoft) with multivariate analyses through TWINSPAN (Two Way INdicator SPecies ANalysis) and DeCORANA (Detrended Correspondence Analysis) (Hill, 1973;

1979a, 1979b). This was used via the Community Analysis Package (Pisces) in 2009 and via mainframe computers in 1993 for species –sites analysis with CANOCO (Braak, 1986).

The data were also processed using MAVIS (Modular Analysis of Vegetation Information System, available from the internet) for Ellenberg Scores and C-S-R comparisons (Grime, 1979). The mean Ellenberg values per plot indicate implicit shifts along the gradients of incident light, moisture, pH and substrate fertility (Ellenberg et al., 1991) using unweighted indicator species. These values have been used in a number of vegetation change studies across Europe (McCollin et al., 1999; Smart, 2000) with validated correlations between Ellenberg values and environmental parameters (Hill and Carey, 1997; Ersten, Alkemade & Wassen,1998). Changes in ecological conditions over time can therefore be hypothesised from mean score changes between the two sample dates.

Results

Match
In 2009, 93% of meadows were a 'match' (47%) or a 'near match' (46%) relative to 1993 data i.e. these were reliable, replicate samples.   In three of the meadows i.e. 3%, there was a change of use, for example, to a garden or to a new building, indicating that there had been some complete loss of meadows. These samples were therefore excluded.

Abundance
The abundance of many species had changed. Using attribute percentages for the categorical data,   a paired t-test was performed to determine if there had been a significant reduction in abundance between 1993 and 2009. The mean difference in species (mean difference = 6.25; SD = 7.64, N = 88) t=7.68, one tail, (p= 0.0005), provides evidence ($t>t_{crit}$) that there is a significant reduction in abundance and that the vegetation had become less complex.

Using matched species records, a positive or negative value was calculated overall and 95% of quadrats showed decreasing values in categories of abundance, confirming a major change in the structure of the vegetation. The % overall cover of grass in the quadrats was stable but the Braun-Blanquet scale is only sufficiently sensitive to detect major changes.

The change in the mean cover abundance of seven species between 1993 and 2009 indicative of diverse meadows is given in Table 1.These species are not able to compete against more vigorous species and all but one are indicators of more diverse groups in the TWINSPAN analysis.

Table 1: Changes in seven species between 1993 and 2009

The complete species list together with occurrences in both years is shown in Appendix One but the list of species with over five records is given in Table 2. There is no evidence of inaccurate identification of species as the total number of species were recorded at both dates was similar.

Only seven species showed increases in species abundance from 1993 to 2009 and one of these, *Trifolium repens* L., is indicative of more fertile conditions. Otherwise the changes reflect decreases in species abundance probably differentially through the population thus *Bellis perennis* L. and *R. bulbosus* were probably lost due to the increased density of the swards of more fertile grasslands. Although *D. carota* declined to a great extent, subsequent observations have suggested that this could be due to the difference in the dates of recording between the two years. All the other declines are of more sensitive species indicative of herb-rich grasslands.

Table 2: Occurrences of species in 92 quadrats surveyed in both 1993 and 2009
N2009 and N1993: number of quadrats (out of 92) where a species was present in 2009 and 1993 respectively for records > 5. Species with 10 or more records in 1993 and a decline of more than 50% in occurrence are in bold. There are no records from 2009 with an increase of 50% occurrence.

The table therefore emphasises that the frequency of species has declined between the sample dates.

Number of species
In the viable quadrats in 2009, species number varied in the range 2 – 17 with the mean being 10.39; in the matching quadrats in 1993, species number ranged between 3 and 22 (mean 12. 36).   This suggests that the mean number of indicator species has declined by 1.97 over 16 years, a decrease of 16%.
A paired t-test was performed to determine if there had been a significant reduction in species between 1993 and 2009. The mean difference in species (mean difference =-2.03; SD = 4.36, N = 89) t=-4.40, one tail, (p= 0.0005), provides evidence (t>t_{crit}) that there is a significant reduction in number of species.
Therefore the overall species richness has declined in parallel with the other measures discussed above.

Altitude and mean height of vegetation
In the 1993 data, a Pearson product moment correlation coefficient (r) of -0.75 (p=0.01) suggests a negative correlation between mean height of vegetation and altitude, i.e. at the time of sampling, the higher the altitude, the lower in height of the vegetation, suggesting that altitude is influential at a broad scale. However, by 2009, r = +0.006 (p<0.1) suggesting that altitude had become less influential, possibly because of increasingly uniform management. Seasonal variation may not be apparent in these results.

Ellenberg and C-S-R scores
Overall, the quadrat data showed an increase in competitor (C) species, a decrease in stress tolerator species (S) and an increase in ruderals (R). However, the increase in ruderals is not considered a reliable indicator because of the limited number of species in the indicator list. The Ellenberg scores showed an increase in wetness and in fertility indicators between 1993 and 2009, and a decrease in pH and light indicators. The decline in calcareous indicators is probably because sensitive species have been outcompeted by more vigorous plants. These results confirm the direction of change indicated by the previous tables. It should be noted that MAVIS excludes non-British species but fewer than 10 % of species are involved.

Multivariate analyses
In 1993, TWINSPAN categorised the meadows into four groups based on species richness and species composition: The interpretation below of the TWINSPAN output for 1993 and 2009 meadows is partly on the indicators but mainly by the balance of frequencies between the groups. Some diverse indicator species did not appear in 2009 as discriminants (e.g. *R. minor, L. bienne)* and *D.carota* does not appear at all in 2009 as a major indicator species. Note that meadows are cut for hay or silage but pastures are only grazed.

The percentage figures are as of 2009 as this represents the current resource.

A   Very fertile meadows (9%) dominated mainly by grasses; virtually all cut for silage.
B   Fertile meadows (22%) mainly dominated by grasses but with some species more resistant to high nitrogen levels - usually cut for silage.

C **Quite fertile meadows (10%)** low sward, cut for silage or hay.
D **Neutral meadows   (8%)** containing some diverse indicator species, usually cut for hay.

Note that the following are some distance on the ordination diagram from A, B, C and D

E **Infertile meadows (36%)** containing the highest number of species in managed meadows and usually cut for hay.
F **Calcareous pastures (15%)** characterised by calcareous grassland species and some heathland plants, not cut for hay

Table 3 below summarises the directional change in meadow composition.

Table 3: Distribution of quadrats by TWINSPAN classes, 1993 and 2009

Figure 1 illustrates the mean positions of all quadrats (1993 and 2009) on two axes using TWINSPAN and emphasises the major shifts that occurred in the vegetation, notably an almost complete shift from B to A over the two periods. The directional change in 2009 is illustrated by arrows. If axis 1 represents the degree of calcareous influence and axis 2 represents fertility, then there is a directional increase in fertility towards more intensively used, fertile meadows dominated by *Medicago* species and grasses and a change towards less calcareous tolerating species. The major changes have taken place in groups A – D, which are at the most fertile end of the spectrum. Groups E and F are relatively stable although the calcareous pastures have declined by 22%.

This change is due to the increased use of slurry causing shading out the sensitive species of open conditions and therefore reducing the calcareous indicators present. This confirms the conclusions reached in the sections above.

Figure 1: TWINSPAN plots on two axes, classes A – F

The mean pH values (by MAVIS) of the TWINSPAN classifications are 6.3 and 6.2 for meadow classes F and E respectively, falling to 5.8 for the other four categories.

DeCORANA of sites
Table 4 shows a summary of the DECORANA plot for the sample sites in 1993 and 2009. The samples show a tendency towards fewer calcareous plots in 2009 thus confirming the results discussed above.

Table 4: Summary DECORANA Ordination Plot

A paired t-test was performed to determine if there had been a significant change in samples between 1993 and 2009 on each of axes 1, 2 and 3.   The mean differences in sample scores for N = 89 are shown in table 5.

Table 5: t-test for paired samples on DeCorAna axes, 1993 and 2009

Thus, on axes 1 and 3, one tail, (p = 0.0005), provides evidence (t>t$_{crit}$) that there are significant changes in the samples between 1993 and 2009. The level of confidence is reduced on axis 2 (p=0.05). There are, therefore, major shifts in the balance of the vegetation between the two sampling dates.

The **CANOCO** and environmental data analyses (1993) showed little significant difference between the groups but trends could be seen:

- The herb-rich infertile meadows occur mostly on sites with deep, dry or humid soils at varying slope angles (mean angle = 10 degrees), altitudes 450 – 1060 m with no dominant aspect;
- Neutral meadows are found on relatively steep slopes (mean angle 13 degrees but up to 45 degrees) mainly on humid, medium depth soils but with 16% on sites with dry soils, 330 – 940 m altitude;
- Very fertile and fertile meadows are mainly on deep soils on dry and humid ground but on flatter land with slope angles below 5 degrees;
- The height of the vegetation is lower in infertile meadows and calcareous pastures than in the improved and partially improved meadows.
- The infertile meadows had the lowest number of fields with manure.
- Infertile meadows and calcareous pastures are situated at higher altitudes.
- Slope aspect did not appear to be influential.
- Very fertile and fertile meadows are closer to tracks.

Validity of methodology
Table 6 shows a summary of categories in the first eight quadrats of the 1993 survey (all species were identified).

Table 6: Picos de Europa meadows – extra species, first eight quadrats (Q) (1993 data)

Only in quadrat 2 do the number of extra species exceed the number of species on the indicator list. The most common extra species and associated number of quadrats are *Carex sp.* (3), *Carex* cf. *caryophyllea* cf. Latourr. (3), *Festuca rubra* L. (3), *Geranium columbinum* L. (4), *Poa pratensis* L. (3), *Taraxacum/Leontodon* agg. (5), *Trifolium dubium* Sibth. (5), *Veronica arvensis* L. (4). This validates the methodology employed.

Discussion
In 1993, 35% of the meadows were still classed as herb-rich, diverse infertile meadows. However, by 2009, these diverse meadows comprise fewer discriminating high diversity meadow species with decreases in abundance of many species including *R. minor* and *R. bulbosus*. However, as the area of unencroached meadow land recorded by aerial photographs has also decreased (Dykes, 2009) the core area of high quality meadows has probably remained stable; the major losses of species have been in the more fertile

grasslands. These losses have implications for insect species and emphasises that measures are required to maintain a viable component of high value meadows.

The observed declines may be a function of the increased use of slurry, shading out the sensitive species but perhaps also because of a decrease in small ruminants (Busqué, Bedia & Cabaňas, 2008). The shift to species poor meadows is probably indicative of the increasing intensity of management over the years with diversification of income streams and reliance on subsidies, although this trend is hard to quantify. The patterns of grazing in the higher altitude meadows may also have changed to a more extensive nature again leading to the loss of species that require open swards, as would be apparent from observational data and supported by current research (Busqué, Bedia & Cabaňas, 2008).

Small scale research projects in the intervening years confirm this pattern for the valley in 68 matched meadows around the villages of Lon, Brez and Baró with the biggest changes observed between 2000 and 2002. However, there were local differences in grazing intensity with reported excessive sheep grazing in the Lon meadows, which showed the most apparent continuing decline in high diversity meadow indicators. The number of high diversity, infertile meadows has been shown to increase with greater distances from villages. In addition almost half were on steeper ground presumable because of difficulty of access by machines. The distance to the nearest track affects the level of management within very fertile and fertile meadows but this was not influential for infertile meadows.

There has been a significant change in species abundance and in the mean number of species over 16 years together with a noticeable decrease in the use of manure in the meadows. The decrease in floristic diversity is not mirrored in grass cover and indeed, the negative correlation seen between altitude and the mean height of vegetation in 1993 was not apparent in 2009 suggesting that altitude has less effect on the vegetation in the valley than formerly. The detailed effects of management are difficult to quantify without detailed interviews of farmers which are not feasible in such a study. Anyway such studies are notoriously difficult to carry out objectively but the present results agree with the trends observed elsewhere in Europe in both lowland and mountain areas.

Stands of stress tolerators may have been weakened by the greater abundance of competitor (and ruderal species). Mean Ellenberg scores showing a change to wetter, more fertile and more acidic but shadier conditions, imply an increased substrate fertility by the local use of nitrate fertilisers (replacing manure) with more encroachment in the meadows by scrub and woody species.

Thus, grass swards have become denser over the sixteen years of this period with fewer species and a loss of sensitive indicators especially of calcareous conditions and open vegetation.   This is consistent with the increased use of inorganic fertiliser and slurry. Competitors have increased and the balance of the vegetation is now towards more fertile conditions.

The methodology would appear to be robust, with the use of indicator species allowing a rapid assessment of quadrat botanical composition. The extra species identified in the first eight

quadrats would not have enhanced the meadow management outcomes permitted by the indicator species approach.

The changes in vegetation in the Deva valley over this period illustrate the ongoing changes from subsistence through market oriented to subsidised farming with European subsidies now accounting for more than 40% of farm income in the valley (Busqué, Bedia & Cabaňas, 2008). A highly extensive system dominated by mainly allochthonous mixed breeds of beef cattle have replaced a diversity of livestock (Rook et al., 2004) The mid valley areas have undergone cultural and landscape degradation with severe shrub encroachment in many higher, steeper meadows inaccessible by mechanised vehicles (Farino, 2009) Some fire abuse or misuse has led to soil degradation through podsolisation.   Communal grazing land is available for small ruminants: goats, in particular, grazed marginal steep land in the past but now they are housed in large modern barns for milk production for cheese and thus there has been an increase in land covers of tree and shrub across the whole of the valley on the steepest slopes (Dykes, 2009). However, many grassland areas in the lower valley have persisted in extent as close proximity to settlements facilitates access and regulates management intensity (Reger, Otte & Waldhart, 2007).

Thus, the maintenance of semi-natural grasslands depends on traditional farming techniques (Baura et al., 2006) and the conservation of traditional landscapes is linked to biodiversity and these landscapes exhibit sustainable management practices (Calvo-Iglesias, Fra-Paleo & Diaz-Varela, 2009). The future stability of the cultural landscape depends on economically and environmentally viable alternatives to prevent the abandonment of the rural environment and reduced biodiversity of hay meadows. Knop et al., (2006) conclude that the Swiss agri-environment scheme targeted at hay meadow conservation preserves biodiversity. However, the development of Natura 2000 sites based on European Directives do not guarantee the maintenance and biodiversity for all areas as many habitats and species are outside protected areas. (Mücher, Hennekens & Bunce, 2009). New CAP guidelines and a new Spanish Sustainable Rural Development Law have not been implemented in the valley and research has highlighted the Deva valley as a social-ecologic system in need of a participatory management system (Rescia, et al., 2008). There needs to be policy implementation with the participation of farmers who are the recipients of subsidies and the 'real' managers of the landscape.

Conclusion
The change in farming practices and consequent decrease in heterogeneity of the landscape in the Deva valley is undisputed and is undoubtedly linked to the decline of biodiversity observed in the present study. There has been a significant negative change in the abundance and number calcareous and sensitive indicators with corresponding increases in competitor species and a major change in the structure of the vegetation. Currently despite the National Park policy, there are no policies such as agri-environmental schemes which are needed to maintain the management practices that have been used in traditional agriculture and which would maintain the diminishing resources of herb rich meadows which are so

important for nature conservation If the core practices from traditional farming are not maintained, then this vital resource for nature conservation will be lost.

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Appendix 1
<u>Occurrences of species in 92 quadrats surveyed in both 1993 and 2009.</u>
N2009 and N1993: number of quadrats (out of 92) where a species was present in 2009 and 1993 respectively. 0 = species absent. Species with 10 or more records in 1993 and a decline of more than 50% in occurrence are in bold. There are no records from 2009 with an increase of 50% occurrence.

	N2009	N1993	change
Acinos alpinus Moench	0	0	0
Ajuga reptans	7	15	-8
Alchemilla glabra (agg.) Neygenf.	0	0	0
Anagallis arvensis L.	1	0	1
Anthoxanthum odoratum	43	48	-5
Anthyllis vulneraria	7	14	-7
Aquilegia vulgaris L.	0	0	0
Asphodelus albus	8	3	5
Astrantia major L.	0	1	-1
Bellis perennis	42	64	-22
Caltha palustris L.	1	0	1
Capsella bursa-pastoris Medik.	1	4	-3
Cardamine raphanifolia Pourr.	0	2	-2
Centaurea sp.	22	34	-12
Cerastium fontanum	15	21	-6
Colchium autumnale L.	1	3	-2
Dactylis glomerata	26	43	-17
Daucus carota	16	39	-23
Echium vulgare	8	5	3
Erica vagans	4	6	-2
Fagus sylvatica L.	0	0	0
Erythronium dens-canis L.	0	0	0
Gentiana verna L.	0	3	-3

Geranium dissectum	20	13	7
Geranium sanguineum	6	3	3
Helianthemum sp.	11	17	-6
Helleborus virdis L.	2	2	0
Heracleum sphondylium L.	2	2	0
Hieracium pilosella	16	31	-15
Hippocrepis comosa	6	5	1
Holcus lanatus	36	33	3
Juncus inflexus L.	0	0	0
Juncus effusus L.	0	1	-1
Leucanthemum vulgare Lam.	1	0	1
Linum bienne	15	20	-5
Lithodora diffusa	5	3	2
Lotus corniculatus	55	60	5
Luzula campestris	11	27	-15
Malva moschata L.	2	4	-2
Medicago sp.	27	36	-9
Medicago sativa	11	8	3
Narcissus asturiensis (Jord.) Pugsley	0	0	0
Narcissus triandrus L.	0	1	-1
Ononis repens L.	0	4	-4
***Origanum* sp.**	4	11	-7
Parentucellia latifolia (L.) Caruel	1	2	-1
Pinguicula grandiflora L.	0	0	0
Plantago media	12	18	-6
Plantago lanceolata	80	82	-2
Potentilla montana Schur ex	4	0	4

Nyman			
Potentilla erecta	1	11	-10
Potentilla reptans	5	11	-6
Primula veris	7	8	-1
Primula vulgaris Hill	1	2	-1
Prunella vulgaris	4	10	-6
Quercus pyrenaica Willd.	4	0	4
Quercus ilex L.	0	2	-2
Ranunculus gramineus L.	0	2	-2
Ranunculus bulbosus	39	61	-22
Ranunculus ficaria	5	8	-3
Rhinanthus minor	24	24	0
Romulea bulbocodium (L.) Sebast.& Mauri	0	3	-3
Rosa sp.	2	3	-1
Rubus fruticosus	4	5	-1
Salvia verbenaca	6	4	2
Sanguisorba minor	44	55	-11
Saxifraga granulata	7	10	-3
Sedum sp.	8	11	-3
Sherardia arvensis	22	26	-4
Thalictrum minus L.	0	0	0
Thymelea sp.	1	0	1
Thymus sp.	1	5	-4
Trifolium pratense	62	74	-13
Trifolium ochroleucon	10	0	10
Trifolium repens	62	44	18
Trollius europaeus L.	0	0	0

Urtica dioica L.	1	1	0
Valerianella sp.	4	0	4
Grasses not specified in both years			
Brachypodium pinnatum P.Beauv.			
Briza media L.			
Bromus mollis L.			
Bromus sterilis L.			
Lolium perenne L.			
Nardus stricta L.			
Poa bulbosa var. *vivipara* Koch			

Figure 1: TWINSPAN plots on two axes, classes A – F

Heather E Prince: Figure 1.

Mean positions of classes on axes 1 and 2
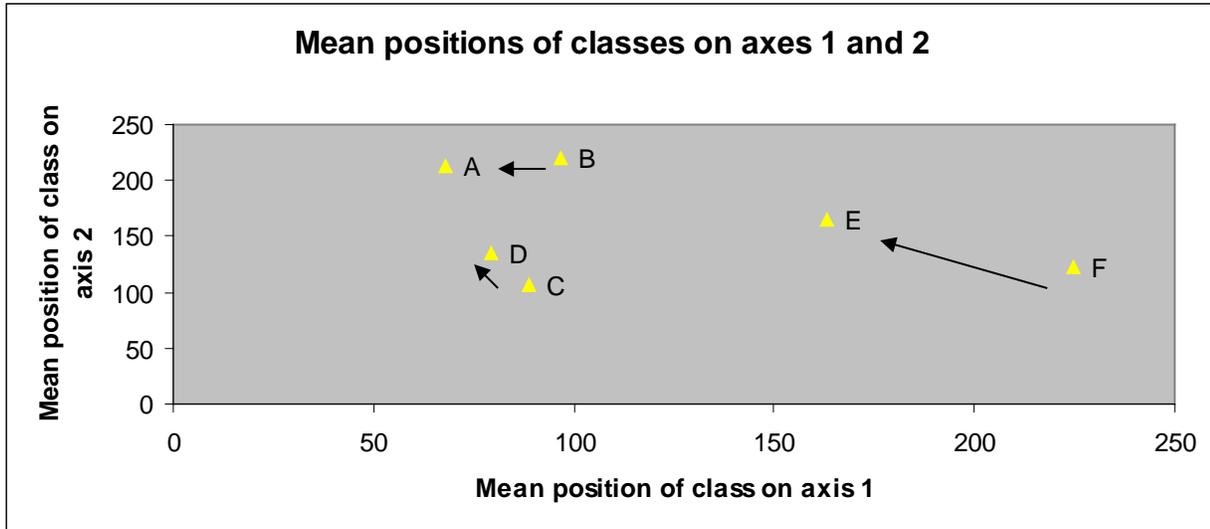
Table 1: Changes in seven species between 1993 and 2009

Species	Years	Mean abundance (%)	% Change
Rhinanthus minor L.	1993	1.17	+0.81
	2009	1.98	
Linum bienne Mill.	1993	0.45	-0.27
	2009	0.18	
Lotus corniculatus L.	1993	4.47	-1.99
	2009	2.48	
Daucus carota L.	1993	1.49	-1.48
	2009	0.01	
Ranunculus bulbosus L.	1993	4.34	-3.03
	2009	1.31	
Sanguisorba minor Scop.	1993	4.63	-3.05
	2009	1.58	
Sherardia arvensis L.	1993	1.14	-1.11
	2009	0.3	

Table 2: Occurrences of species in 92 quadrats surveyed in both 1993 and 2009
N2009 and N1993: number of quadrats (out of 92) where a species was present in 2009 and 1993 respectively for records > 5. Species with 10 or more records in 1993 and a decline of more than 50% in occurrence are in bold. There are no records from 2009 with an increase of 50% occurrence.

	N2009	N1993	change
Ajuga reptans L.	7	15	-8
Anthoxanthum odoratum L.	43	48	-5
Anthyllis vulneraria L.	7	14	-7
Asphodelus albus Boiss	8	3	5
Bellis perennis	42	64	-22
Centaurea sp.	22	34	-12
Cerastium fontanum Baumg.	15	21	-6
Dactylis glomerata L.	26	43	-17
Daucus carota	16	39	-23
Echium vulgare L.	8	5	3
Erica vagans L.	4	6	-2
Geranium dissectum L.	20	13	7
Geranium sanguineum L.	6	3	3
Helianthemum sp.	11	17	-6
Hieracium pilosella L.	16	31	-15
Hippocrepis comosa L.	6	5	1
Holcus lanatus L.	36	33	3
Linum bienne	15	20	-5
Lithodora diffusa (Lag.)I.M. Johst.	5	3	2
Lotus corniculatus	55	60	5
Luzula campestris (L.)DC.	11	27	-15
Medicago sp.	27	36	-9

Medicago sativa L.	11	8	3
***Origanum* sp.**	4	11	-7
Plantago media L.	12	18	-6
Plantago lanceolata L.	80	82	-2
Potentilla erecta (L.) Raeusch	1	11	-10
Potentilla reptans L.	5	11	-6
Primula veris L.	7	8	-1
Prunella vulgaris L.	4	10	-6
Ranunculus bulbosus	39	61	-22
Ranunculus ficaria L.	5	8	-3
Rhinanthus minor	24	24	0
Rubus fruticosus L.	4	5	-1
Salvia verbenaca L.	6	4	2
Sanguisorba minor	44	55	-11
Saxifraga granulata L.	7	10	-3
Sedum sp.	8	11	-3
Sherardia arvensis	22	26	-4
Thymus sp.	1	5	-4
Trifolium pratense L.	62	74	-13
Trifolium ochroleucon Huds.	10	0	10
Trifolium repens	62	44	18

Table 3: Distribution of quadrats by TWINSPAN classes, 1993 and 2009

	2009	1993	2009	1993	Species
A	16	15	1	94%	6%	*Medicago* sp -grass
B	14	1	13	7%	93%	*Ranunculus* spp.- *T. pratense - C. fontanum*
C	18	6	12	33%	66%	*H. pilosella - Sedum sp.*
D	39	22	17	56%	44%	*G. dissectum - R. bulbosus - A. odoratum*
E	66	34	32	52%	48%	*R. bulbosus - L. corniculatus - T. pratense*
F	28	11	17	39%	61%	*E. vagans - S. minor - R.minor*

Table 4: Summary DECORANA Ordination Plot

	Neutral	Calcareous/neutral	Calcareous
Moist	*1*/2	*2*/1	*2*/2
Dry	*12*/6	*126*/120	*8*/16
Very dry	*8*/6	*12*/20	*10*/8

First number *italic* (1993 plots)/second number (2009 plots)

Table 5: t-test for paired samples on DeCorAna axes, 1993 and 2009

	Mean difference	SD	t	p
Axis 1	27.00	55.42	4.60	0.0005
Axis 2	-19.45	61.16	-3.00	0.05
Axis 3	41.09	60.75	6.38	0.0005

Table 6: Picos de Europa meadows – extra species, first eight quadrats (Q) (1993 data)

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
No. of species on indicator list	17	6	16	11	13	12	11	10
No. of extra species	7	11	11	5	11	6	6	6