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Improvement of plant diversity and methods for its evaluation in Mediterranean basin grasslands

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Abstract. It is widely known that the Mediterranean basin sustains a remarkable biodiversity. Its rich vascular flora, comprising up to 11,700 endemic known plants which is more than four times the number found in all the rest of Europe, highlights the basin as one of the globe's biodiversity hot spots, with tremendous importance for ecosystems integrity and life *per se*. Grasslands is the biome with the major contribution to this nature's high value. Current signs of grassland biodiversity deterioration or loss inevitably alarm scientific and institutional mechanisms to withstand further decrease, in order to avoid irreversible changes of the total biodiversity and quality of life. The present paper emphasises on plant diversity, and describes the levels of diversity in Mediterranean grasslands, stresses the need to shift from point to total diversity, investigates the causes and effects behind biodiversity deterioration and loss, reviews the methods for biodiversity improvement, proposes a typical methodological set for evaluating biodiversity, and includes this set into a general scheme for a spatial and temporal monitoring programme. It is concluded that although significant theoretical and empirical knowledge has been accumulated up to now concerning either the halting of biodiversity loss or improving biodiversity of the Mediterranean basin grasslands, still this knowledge is pending to be put into full action from institutional structures and mechanisms.

Keywords. Point diversity – Total diversity – Monitoring – Mediterranean grasslands.

Amélioration de la diversité végétale et méthodes pour son évaluation dans les prairies du bassin méditerranéen

Résumé. Il est certain que le bassin méditerranéen possède une biodiversité remarquable. Sa flore vasculaire riche de plus de 11 700 plantes endémiques connues, c'est-à-dire quatre fois plus que dans le reste de l'Europe, caractérise le bassin comme un des points chauds de biodiversité de la planète, avec une importance extrême pour l'intégrité des écosystèmes et de la vie elle-même. Les prairies constituent le biome qui contribue le plus à cette valeur élevée de la nature. Des signes récents de détérioration ou de perte de la biodiversité des prairies ne peuvent qu'alarmer les mécanismes scientifiques et institutionnels sur la façon d'affronter une diminution supplémentaire, dans le but d'éviter des changements irréversibles sur l'ensemble de la biodiversité et la qualité de vie. La présente étude souligne la diversité végétale, décrit les degrés de diversité des prairies méditerranéennes, met l'accent sur le besoin de passer de la diversité biologique ponctuelle à la diversité biologique totale, recherche les causes et les conséquences de la détérioration et de la perte de biodiversité, passe en revue les méthodes pour l'amélioration de la biodiversité, propose une méthodologie typique pour évaluer la diversité biologique et l'englobe dans un ensemble plus général pour un programme de suivi du changement de la biodiversité dans le temps et l'espace. On peut en conclure que malgré l'importante connaissance théorique et pratique qui a été accumulée jusqu'à maintenant en ce qui concerne, soit la maîtrise de la perte de biodiversité, soit l'amélioration de la biodiversité des prairies du bassin méditerranéen, il reste encore à mettre en œuvre cette connaissance par les structures et mécanismes institutionnels.

Mots-clés. Diversité ponctuelle – Diversité totale – Suivi – Prairies méditerranéennes.

I – Introduction

Biological diversity (or biodiversity) holds a central thesis in Biology presiding ecological thought, research and action. Biodiversity offers an array of benefits to society, such as:

(i) valuable gene pools supporting productivity, enhancement of stress tolerance of domesticated species that provide new medicines, energy and industrial feedstock; (ii) ecological services (climate amelioration, water purification, soil stabilization, flood control); and (iii) contribution to outdoor recreation that supports human well-being (Huston, 1993). Using some functionalistic point of view, biodiversity is an important ecosystem's property often determining its overall function (Loreau *et al.*, 2002).

As humans are integral parts of Mediterranean grassland ecosystems, inevitably, biological diversity is continuously exposed to negative anthropogenic impacts, thus confining its magnitude and social importance (Thompson, 2005). This paper shortly describes the levels of diversity in Mediterranean grasslands, stresses the need to shift from point to total diversity, investigates the causes and effects behind biodiversity deterioration and loss, reviews the methods for biodiversity improvement, proposes a typical methodological set for evaluating biodiversity, and includes this set into a general scheme for a spatial and temporal monitoring programme.

II – Moving from *point* to *total* grassland diversity

Measures of diversity provide grassland managers and conservationists with numerical abstracts of energy exchange and niche diversification, both adjusting and determining the three levels of biodiversity: (i) genetic diversity; (ii) species diversity; and (iii) ecosystems diversity.

The integration of genetic variation into changing community diversity has been an objective of experimentation aiming at pointing out the important role of Mediterranean grasslands in conserving valuable gene resources (Heywood *et al.*, 2007). On the other hand, scientific emphasis on species diversity has been placed not because the other levels of diversity are not so important, but rather because of our ability to easily identify species in the field that overemphasizes the use of species diversity. Species diversity measures are quite attractive in quantitative ecological studies in Mediterranean grasslands, as it is affected by several factors as altitude (Vrahnakis *et al.*, 2002; Herrera and Bazaga, 2008), latitude (Médail and Diadema, 2009), nutrients' availability (Vourlitis and Pasquini, 2009), disturbances like ploughing (Montalvo *et al.*, 1993), grazing (Alados *et al.*, 2004) and fire (Vrahnakis *et al.*, 2004; Capitano and Carcaillet, 2008) or combinations of them (Merou and Papanastasis, 1997), with determinants often being downscaled to the available energy (Storch *et al.*, 2005). Ecosystem diversity includes not only structured species distributions but also combinations of species functions and interactions usually expressed in large spatiotemporal scales. Often, ecosystem diversity is translated into higher structures than species levels, like community or landscape (Carranza *et al.*, 2007). Mediterranean ecosystem diversity is not easy to quantify since the majority of the communities or ecosystems often have blurred transitions between them, and are governed by a set of multidimensional environmental agents.

In accordance with its multidimensionality, and in order to improve Mediterranean grassland biodiversity, we have to integrate all three levels of diversity and find ways to measure it and express it by numbers [although Purvis and Hector (2000) argued about this need]. According to Magurran (2004), diversity measurement is based on three assumptions, all relevant to equal representativeness: i.e. equity in the relative importance of species (*all species are equal*), equity in the relative importance of individuals (*all individuals are equal*), equity in the use of units expressing species abundances (*all units are uniform*). In addition, the improvement of biodiversity must be in accordance with the principles of *ecosystem management*, that comes to replenish and expand the sustainability principle by anticipating that resource managers treat grasslands, for example, as ecosystems and the main goal uniformly adopted is to sustain the capacity of the ecosystem rather than merely maintain and sustain structures, products and services *per se*. Consequently, it is urgent to shift from conservation efforts solely focussed on key-stone (or flagship) species (*point diversity*) to the conservation of the whole apparent

diversity (*total diversity*). This does not mean that we have to abandon conservation efforts for the protection of plant or animal species of special importance (e.g. rare, threatened or endangered species). We should rather include in our management planning the dimension of total diversity and set relevant priorities.

III – Grassland diversity: Causes of decline/loss and methods for improvement

Livestock grazing is a key factor in sustaining high total biodiversity in the Mediterranean (Noor Alhamad, 2006). Accumulated experimental evidences have shown that the "intermediate disturbance" hypothesis (Sousa, 1984), that anticipates the highest levels of biodiversity under intermediate levels of disturbance, corresponds to the majority of Mediterranean natural ecosystems experiencing grazing (Alados *et al.*, 2004; Fadda *et al.*, 2008). Altering the upper or lower limits of *intermediate grazing* has proven to result in biodiversity decline. However, while overgrazing was the main source of ecosystem functioning decline in the past, the lowering of grazing intensity (undergrazing) in terms of space and time or both, or the cease of grazing has gradually become the general rule in many Mediterranean countries (Peco *et al.*, 2005; Fadda *et al.*, 2008 and references therein).

Land use and cover undergo dramatic alterations not only in the Mediterranean (Symeonakis *et al.*, 2007), but in central and northern Europe as well (Rodwell *et al.*, 2007), usually at the expense of grasslands (Hodgson *et al.*, 2005; Chuvardas and Vrahnakis, 2009). For example, in the area of Lagadas, northern Greece, model simulation (CLUE-S) showed that all major land use/cover types would be increased from 1993 to 2013, especially at the expense of species-rich grasslands that tend to be extinct (Chuvardas and Vrahnakis, 2009). One of the major driving forces for these alterations may be found in EC's system of subsidies that favour intensification and expansion of arable lands (Hodgson *et al.*, 2005; Chuvardas and Vrahnakis, 2009).

The total grassland area in the EU has decreased by 13% from 1990 to 2003 (FAO, 2006). The BIODDEPTH EU research project has shown that biodiversity loss of European grasslands would cause serious decline of their productivity, reduction of energy available to the other parts of the food chain, thus threatening the overall ecosystem health (Hector *et al.*, 1999). The areas of Mediterranean mountain pastures (mostly pseudalpine and alpine natural and semi-natural grasslands) have undergone rapid declines mostly due to human population decrease that led to land abandonment, cessation of traditional practices, livestock pressure reduction, scrub and shrub encroachment and increase of catastrophic wildfires (Lasanta *et al.*, 2009). Human population decrease along with rapid modernization resulted in dramatic decline of traditional activities that used to be key factors for controlling, among others, species-rich communities in the past.

Before planning or making decisions on the method for grassland biodiversity improvement, it is essential to consider biodiversity as part of the total grassland resources improvement including human activities. By applying a holistic approach, a set of management objectives may emerge; part of them will have a species-specific conservation interest. Additionally, the interrelations between total grassland biodiversity improvement and species-specific conservation must be fully understood.

Although grassland biodiversity objectives may have spatial and temporal references and may vary from time to time and site to site, a general rule maybe the maintenance of the biological and ecological setting of the *main* plant communities as well as the maintenance of adequate bare ground, and scattered shrubs, phryganic elements and trees that will benefit different forms of wildlife and increase total biodiversity. The particular openness and patchiness that is desirable for biodiversity objectives may be adjusted by the species conservation objectives; for example, vegetation gaps followed by shrub/phryganic areas will alternate by 1 m² for insects up to 1 ha for mammals or birds (Croquet and Agou, 2006).

Grazing and mowing are the traditional practices for keeping high openness and patchiness in Mediterranean grassland landscapes. Which is the best option (grazing or mowing) is not a straightforward answer since despite the general recommendation of grazing as more efficient traditional method for improvement grassland biodiversity (Butaye *et al.*, 2005), evidences exist favouring mowing compared to grazing (Fischer and Wipf, 2002). What is the best selection is matter of consideration of the history and the nature of a plant community (Grime *et al.*, 2000), and in cases where a long history of livestock grazing is experienced, like the majority of natural or semi-natural Mediterranean grasslands, the precautionary approach of avoiding changes in long-established management should be adopted (Crofts and Jefferson, 1999).

Major consumers of grassland vegetation are sheep, cattle and horses. It is estimated that the 80 million ha of European grassland area sustain 150 million cattle and 150 million sheep, which is nearly equal to 15% of the global animal production (FAO, 2004). Grazing animals may directly affect vegetation either by establishing a species-specific trampling regime, or by their selective feeding behaviour. Consequently, it is essential in Mediterranean species-rich grassland communities to use mixed flocks of cattle/sheep or sheep/goats. Goats normally consume (annual) twigs, leaves, and fruits from shrub species, thus controlling shrub expansion; most sheep flocks in the Mediterranean have traditionally few goats – an "ecologically" approved method for biodiversity improvement (Papanastasis, 1999).

The period when grazing is exercised is of considerable importance since plants exhibit different phenology during the year, depending on their life cycles (annual, perennial plants), specific photosynthetic pathways (C3, C4 plants), and responses to environmental drivers of phenology (early-season, late-season plants). Consequently, it is a quite complicate task to make decision upon the proper grazing period for maximizing total grassland biodiversity – site specific studies must be conducted and a strict monitoring framework must be implemented as to gain adequate knowledge and experience that according to Holecheck *et al.* (2000) are irreplaceable.

The calculation of the proper stocking rate is a fundamental topic in grassland management. It is suggested that moderate grazing of grasslands is a key factor in maintaining their productivity, biodiversity and environmental value (Tsiouvaras *et al.*, 1998) and a minimum stubble height of 6 cm at the end of the grazing period is indicative of moderate grazing (Papanastasis, 1985).

The adjustment of grazing duration is directly related to the determination of the stocking rate that contributes to total grassland biodiversity increase, since an inverse linear relation is supposed to hold between these two grazing parameters (NCC, 1986). More specifically, as far as xerothermic summers of Mediterranean areas are concerned, this implies that stocking rates during summer must be significantly lower than in winter. Generally, intense grazing for short time durations is suggested when the control of weed and strongly competitive species is of prior importance (Crofts and Jefferson, 1999).

The adoption of a grazing system is mainly based on the matching of forage supply and nutritional demands; for Mediterranean grasslands, it is often determined by the number of animals, morphology of the ground, available area, tradition, and financial resources. Set or continuous stocking is often met in Mediterranean zones (mostly in uplands or subalpine zones) where intensified grazing is practiced. Set stocking grazing significantly contributes to the increase of total biodiversity since it is responsible for the generation of high patchiness in the landscape, it controls the invasion of highly competitive species, and it supports the development of perennial and annual plants without causing any special disturbance to microfauna (Colas and Hébert, 2000). The spatial distribution of stocking is adjusted by the existence and location of infrastructures (roads, shelters), salting and watering points; thus offering the opportunity for a uniform spatial use of the vegetation, while the temporal adjustment is dictated by the seasonal progressiveness of vegetation. The response of these two systems in shifting total biodiversity is a matter of the dominant life cycles and forms of the vegetation and conservation goals as well. In terms of optimum overall grassland performance,

rotational grazing is suggested to be used in the perennial grasslands of Mediterranean Australia, while for annuals the extensified set stocking is suggested (Sanford *et al.*, 2003). Set stocking is also proposed for perennial grasslands dominated by stoloniferous, rhizomatous perennial grasses or plants with bulbous rooting systems and for annuals dominated by species with ability to self-bury seeds or having prostrate life forms (chamaephytes). Rotational stocking is preferable when species or habitats of specific conservation management goals are to be achieved. This way, a stricter monitoring framework has to be established. Transhumance, i.e. the cyclical, annual movement of livestock to exploit seasonal growth of distinctive rangelands (Vallentine, 2001), is a special type of extensified grazing having significant benefits for total biodiversity, though nowadays it is no longer traditionally exercised as in most Mediterranean areas animals are transferred by trucks (Ispikoudis *et al.*, 2004).

Mowing, as a method to manage grasslands for biodiversity improvement, is not widespread in Mediterranean areas, at least in respect to the rest of Europe and especially compared to sites where it is traditionally practiced (Britaňák *et al.*, 2009). Since non selection of forage material is taking place, mowing results in less gaps of vegetation and patchiness in respect to grazing and finally leads to more sudden and uniform canopies. For effective biodiversity improvement, several questions must be addressed concerning timing, frequency, distribution, and method of cutting (Pearson *et al.*, 2006). As a general rule, late (single) cutting is suggested for semi-natural grasslands of high biodiversity concern (in respect to intensively used meadows). In addition, the protection of animal species, (insects and birds) that require highly structured vegetation for feeding and refuge, and the disposal of available time for late-flowering plants to bloom are highly ensured. On the other hand, early cutting is suggested when alien aggressive plant species are to invade (Pearson *et al.*, 2006), while sustained early cutting may result to richness reduction (Smith, 1994).

The majority of Mediterranean grasslands are not natural plant communities, but they have derived from natural forests after extensive historical clearings, seeding, flooding, fertilisation, burning, grazing or combination of them (Papanastasis, 1999). If intensive grassland management is abandoned then natural succession will favour shrubs like *Quercus coccifera*, *Pyrus amygdaliformis*, *Juniperus oxycedrus*, *Rosa* spp., *Ulmus* spp., *Ulex* spp., *Crataegus monogyna*, *Prunus spinosa* and *Buxus sempervirens* that expand and dominate on typical Mediterranean grassland landscapes. According to Fuhlendorf (1997), shrubs exhibit specific characteristics that support significant competitive advantages over herbaceous species: (i) increased levels of seed production; (ii) persistent seeds or seed banks; (iii) effective seed dispersal; (iv) exponential growth rate; (v) tolerance to nutrient or water stress; (vi) chemical or physical deterrents to browsing; (vii) vegetative reproduction; and (viii) extended longevity. Thanks to their invasion strategies (by seeds or most commonly, vegetatively by sprouts or rooting of sinking branches) and surviving strategies (unpalatability, poisonousness, spininess, hairiness, strong regrowth potential after being browsed or cut) they gradually dominate in the landscape (Spatz and Papachristou, 1999). It is well-documented that as shrub assemblages become thicker, total biodiversity declines (Merou and Vrahnakis, 1999; Alados *et al.*, 2004; Vrahnakis *et al.*, 2005; Vrahnakis 2008). Biodiversity is favoured by adjusting shrub cover to 10-15%, a value typically found in Mediterranean grasslands (Papanastasis, 1999), 10-40% (Vrahnakis, 2008) or 50% (Papachristou, 1997) for kermes oak shrublands, or below 20% for scrubs (Pearson *et al.*, 2006). In addition, shrubs provide additional forage to animals, especially under prolonged xerothermic conditions of Mediterranean summers (Papachristou and Papanastasis, 1994). For example, the contribution of *Quercus coccifera* may reach up to 40% of the total forage production (Platis, 1994). Furthermore, they provide habitat and refuge to wild fauna and shelter to herbaceous species vulnerable to grazing, improve microclimate, protect against soil loss and erosion, and enhance the aesthetic value of the landscape (Papanastasis, 1999). Goat browsing, often on a rotational basis, offers opportunities to halt shrub expansion and improve biodiversity. Nevertheless, when shrub vegetation is dominated by species with unfavourable characteristics (spines, anti-nutritional agents like tannins, etc.), browsing alone is not enough to halt shrub encroachment. In this case, and if the improvement

of biodiversity is the main goal, mechanical clearing is a proper technique, while control by burning or herbicides must be avoided for biodiversity purposes. Generally, to increase plant diversity on grasslands, old stands of shrubs must be removed since they are sources of excessive nutrients accumulation that favour the development of fixed competitive hierarchies of low diversity value. Mechanical clearing must be followed by rotational grazing, further clearing, thinning, slashing, ploughing, mulching or hoeing to maintain openness and to achieve a desirable vegetation form and structure that balances both forage accessibility and consumption by grazing animals and high biodiversity value. Rotational grazing by goats (or sheep and goats) may be applied on a 15-day basis with a stocking rate able to remove 60% of the current season growth (Tsiouvaras, 1984). To benefit wild fauna of poor dispersal ability mechanical treatment must be applied by cutting one section at a time in a frequency of 1/15 of the grassland each year or 3/15 every third year (RSPB, 2004).

Total grassland biodiversity is often threatened by the domination of species considered as weeds, like bracken (*Pteridium* spp.), thistles (Asteraceae like *Cardus* spp., *Carlina* spp., *Cyrcium* spp., *Arctium* spp., *Cnicus* spp., *Echinops* spp., etc.), etc. These species are strong competitors, extract toxic substances, while modify surrounded space by the heavy shade they provide; the latter constraints the development of photophilous species like orchids. Once they establish they are very difficult to control; effective confinement of their persistence and expansion is made only during early stages of development. Pearson *et al.* (2006) suggested the following control techniques for bracken: cutting 2-3 times a year before the full expansion of leaves, cutting young shoot during springtime, grazing by horses and cattle during autumn when levels of toxicity are low and grasses are short, and for thistles: cutting the plant at a height of 5-10 cm by taking care of seed dispersion.

While the reversion of grasslands to agricultural land is a common phenomenon in Mediterranean areas (Chuvardas and Vrahnakis, 2009), the opposite is rather uncommon. In the few cases that arable land is to reverse to grassland, then adjacent natural vegetation is allowed to invade or grassland plants are established by seeding followed by appropriate follow-up management; the latter is necessary since without human intervention the reversion is made by low rates mostly due to excess soil nutrients (Bakker and Berendse, 1999). Biodiversity improvement is further helped by sowing seed mixtures of indigenous plant species. Experienced gained from a reversion programme in the White Carpathians suggested: (i) an optimum seed rate of 17-20 kg ha⁻¹; (ii) with less than 17 kg ha⁻¹ the sward is sparse; and (iii) with more than 20 kg ha⁻¹ the project becomes cost inefficient (Jongepierová and Mitchley, 2009). The White Carpathian paradigm offers some principles of good practices readily applicable to the Mediterranean conditions, i.e. (i) the use of regional seed sources; (ii) the use of agricultural or other subsidies to fund grassland re-creation; (iii) the assessment of local conditions (soil, seed bank, characteristics of plant species in the immediate surroundings) before applying the re-creation method; (iv) the cost-effectiveness of the re-creation project; (v) the monitoring of the re-created sites after sowing; and (vi) the monitoring of the re-creation of functioning ecosystems (e.g. improvement of total biodiversity).

IV – Grassland diversity: Evaluation and monitoring

Monitoring of biodiversity includes a number of different objectives such as species richness, habitat quality, ecosystem integrity, etc. Therefore, it selects information on various spatial and temporal data, for a species, a community or a habitat, which are further evaluated and assessed for significance. The purpose of an effective monitoring scheme/programme is to collect and analyse data in a way that the gradual change can be detected and therefore assist decision making (Hill *et al.*, 2005). When monitoring of total grassland biodiversity is to be designed, a clear evaluation scheme must be followed. Given the enormous literature on floristic diversity measures, problems of a standard and widely accepted quantification are often met. Nevertheless, it is believed that the five-step procedure based on Southwood (1978),

Routledge (1979), Krebs (1999) and Magurran (2004) suits better to quantify Mediterranean grassland floristic diversity:

(i) Construction of Whittaker's plots of log abundance on species rank that illustrate the dominance-diversity curve which in turn indicates which models of species-abundance relations might be applied to specific data.

(ii) Explore the best fit of a series of statistical distributions [species abundance models *sensu* Magurran (2004)] to obtain an insight of the community organization. Distributions like geometric series, log series, log normal, or broken-stick may fit to abundance data.

(iii) Estimation of species richness to obtain an overall view of the potential species number that a grassland community may sustain. To obtain a measure of species richness, a series of non-parametric and parametric estimations must be undertaken. This is judged necessary as there is no clear consensus about the best method of species richness estimation (Henderson, 2003). The selection of methods may be based on their suitability for incidence (presence-absence) data. A final figure of species richness may be extracted by averaging the methods' suggested richness values. Non parametric species richness estimations may include Chao Pr. Ab., Chao & Lee 2, 1st order jackknife, 2nd order jackknife, and bootstrap, while the approach of Michaelis-Menten is a suitable parametric estimation. Detailed descriptions on the above and mathematical formulas of the estimators are found in Magurran (2004). Jackknife and bootstrap techniques are based on repeated estimations of species richness on sub-samples of data. The jackknife technique removes the sub-samples one at a time, while in the bootstrap sub-samples are selected at random with replacement.

(iv) Use of non-parametric or/and parametric indices to quantify diversity, like the well-known *Shannon-Wiener information* and the *reciprocal of Simpson's* non-parametric indices of diversity, or the parametric *Q-statistic* which is based on the distribution of species abundances (Kempton and Taylor, 1978). Simpson's index of diversity emphasizes the weight of the common, most abundant species in a sample, while Shannon-Wiener's index the weight of the rare species and is more sensitive to species richness. Alternatively, the non-parametric Berger-Parker's index of dominance, which is a measure of the numerical importance of the most abundant species, may be used.

(v) Use a measure of equitability, like the non-parametric of Shannon-Wiener's (J_H), or other.

For estimation of the confidence limits of estimates, the use of the percentile bootstrap technique is suggested, with the 95% of confidence intervals being the 2.5 and 97.5 percentiles of the bootstrap distribution of the estimate (Southwood and Henderson, 2000).

For purposes of species richness comparison the sample rarefaction method (Sanders, 1968, corrected by Hulbert (1971) and Simberloff (1972); Heck *et al.*, 1975) may be used. The method estimates how the species number in a selected sample changes with the number of individuals. It uses the number of individuals in the selected sample as a measure of effort. Two versions of the rarefaction curve may be applied: a finite (sampling performed without replacement) and an infinite (sampling performed with replacement). The method estimates the expected number of species in a random sample of n individuals [$E(S_n)$] as (Krebs, 1999):

$$E(S_n) = \sum_{i=1}^S \left[1 - \frac{\binom{N-N_i}{n}}{\binom{N}{n}} \right]$$

where S : total number of species found in the community, N_i : number of individuals of species i , $N = \sum N_i$, n = value of sample size chosen for standardization ($n \leq N$), $\binom{N}{n}$: $N! / n! (N - n)!$, i.e. number of combinations of n individuals that can be chosen from a set of N individuals.

Often, a statistical pair-wise comparison of the indices calculated separately for each community is needed. Generally, non-parametric indices of diversity face important difficulties of statistical nature when they are used for comparisons. The randomization test proposed by Solow (1993) solves such difficulties by re-sampling 10,000 times from a distribution of species abundances produced by a summation of two samples. At first the difference (delta, δ) between observed indices of diversity from two samples is calculated. Then two t -tests are to be performed: (i) a two-sided t -test, where the null hypothesis that diversities are equal is tested, by estimating the number of simulated $|\delta|$ (after 10,000 randomizations) that are greater than the observed $|\delta|$, and so the estimated probability that diversities are equal is calculated; values lower than 0.05 (i.e. a proportion of 500 over 10,000 randomizations) indicates that diversities are statistically different; and (ii) a one-sided t -test where the null hypothesis that the diversity of sample 1 is statistically higher than diversity of sample 2 is to be tested by estimating the number of simulated δ (after 10,000 randomizations) that are greater than the observed δ , and so the estimated probability that diversity of sample 1 is greater than sample 2 is calculated; values lower than 0.05 indicate that diversity of sample 1 is greater than sample 2, while values over 0.95 (i.e. a proportion of 9550 over 10,000 randomizations) indicate that sample 2 is more diverse than sample 1.

Finally, to compare the communities in terms of their overall diversity and to define their comparability the Rényi's family equation (Rényi, 1961, 1970; Hill, 1973) may be used. By generating a family of diversity indices, it is possible to define comparable communities. This becomes useful when non-parametric indices of diversity are used for diversity ordering (Tóthmérész, 1995). In a plant community composed of N species, p_i is the proportional abundance (often measured as number of individuals) of the i -th species ($i = 1, 2, \dots, N$) such that $0 \leq p_i \leq 1$ and $\sum_{i=1}^N p_i = 1$. Hill (1973), considering that traditional diversity indices measure different aspects of the partition of abundance between species, proposed a generalized formulation of diversity by defining a continuum of possible diversity measures. For a relative abundance vector $P = (p_1, p_2, \dots, p_N)$ Rényi (1970) defined a generalized entropy of order α as

$$H_\alpha = \frac{1}{1-\alpha} \ln \sum_{i=1}^N p_i^\alpha, \alpha \geq 0$$

While traditional diversity indices supply point descriptions of community structure, according to the Rényi's parametric diversity family there is a continuum of possible diversity measures that differ in their sensitivity to changes of the relative abundances of dominant and rare species as a function of parameter α (Ricotta, 2003). Accordingly, rather than as a single-point summary statistics, diversity is seen as a scaling process from community species richness to its dominance concentration that takes place not in the real but in the topological data space (Podani, 1992). Indeed, for $\alpha = 0$, $H_0 = \ln N$, where N is the total number of community's species; for $\alpha = 1$, $H_1 = H$ (where H is the Shannon-Wiener's index of diversity); for $\alpha = 2$, $H_2 = \ln 1/D$ (where D is the Simpson's index of diversity), and for $\alpha = \infty$ $H_\infty \approx \ln 1/d$ (where d is the dominance index of Berger-Parker). The biological justification for using the Rényi's family equation in studying plant diversity is found in Ricotta (2003).

V – Conclusions

Biodiversity, as a conservation issue, is often more concerned on species rarity than on maximizing the number of species in a given area. Nevertheless, and in conjunction with the principle of *ecosystem management*, it is important to sustain all the components of biodiversity and shift from point to total diversity. This does not mean that conservation efforts targeting plant or animal species of special importance have to be abandoned, but it stresses the need to include the dimension of total diversity in management planning and set such priorities.

The main causes of total biodiversity decline of natural or semi/natural grasslands of the Mediterranean basin have common socioeconomic basis: (i) unregulated (irrational) grazing; (ii) land use/type changes; and (iii) abandonment of traditional human interventions, that finally all lead to habitat shrinking and loss (shrub and tree encroachment). Developmental planning and policy making must take seriously under consideration the predictions on species-rich grassland elimination (and the decrease of the area of open shrublands) in the future Mediterranean landscape, given that the ecosystems they support are important for sustaining the ecological integrity and the social reference of the landscape.

A monitoring scheme for the evaluation of grassland biodiversity may include the: (i) construction of a Whittaker plot; (ii) exploration of a series of statistical distributions; (iii) estimation of species richness; (iv) use of parametric or/and non-parametric quantification of biodiversity; and (v) quantification of equitability. For purposes of species richness comparison and for pair-wise comparison of the indices, the sample rarefaction and the Solow's randomization methods may be used respectively. Finally, by constructing the diversity ordering diagram we obtain insights about communities' comparability in spatial and temporal references.

A clear spatial and temporal adjustment of grazing towards grassland ecosystem natural potential is necessary to improve biodiversity. The regulation of grazing activity to promote total biodiversity is a quite complex exercise, especially for natural and semi-natural Mediterranean grasslands, since several grazing-related parameters must be adjusted: (i) type of grazing animals; (ii) grazing periods; (iii) stocking rate; (iv) duration of grazing; and (v) grazing system. Other methods of grassland biodiversity include mowing, mechanical control of shrub expansion, control of aggressive weeds, and conversion of agricultural land to grassland.

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