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Original article

Short-term effects of nitrogen enrichment, litter removal and cutting on a Mediterranean grassland

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ABSTRACT

Semi-natural grassland communities are of great interest in conservation because of their high species richness. These communities are being threatened by both land abandonment and nitrogen eutrophication, and their continued existence will depend upon correct management. However, there is a distinct lack of studies of the ecological mechanisms that regulate species diversity and productivity in Mediterranean grasslands. We have conducted a 3-year field experiment in a species-poor grassland in central Italy to investigate the effects of nitrogen fertilization coupled with removal of plant litter and artificial cutting on species diversity and community productivity. Vegetation cutting reduced living biomass but increased species diversity. In fact, cutting had positive effects on the cover of almost all of the annual and biennial species, while it had a negative effect on the dominant perennial grasses *Brachypodium rupestre* and *Dactylis glomerata*. Litter removal had similar effects to cutting, although it was far less effective in increasing species diversity. In contrast, nitrogen enrichment strongly increased the living biomass while maintaining very low species diversity. Our results have indicated that semi-natural Mediterranean grasslands need specific management regimes for maintenance and restoration of species diversity. In the management of these grasslands, attention should be paid to the potential threat from nitrogen enrichment, especially when coupled with land abandonment.

1. Introduction

Most grasslands are composed of semi-natural vegetation and are a product of a long history of human use (Poschlod and WallisDeVries, 2002). Their continued existence depends upon correct management such as mowing or grazing (Köhler et al., 2005). These semi-natural communities are listed as a priority habitat for biodiversity conservation in European Union Habitats Directive (92/43/CEE). Despite this, these communities are now being threatened by rapid changes in agricultural practices, and specifically by the consequences of land abandonment (Bobbink and Willems, 1991) and phosphorus (Wassen et al., 2005) and nitrogen eutrophication (Berendse and Elberse, 1990; Stevens et al., 2004).

In Europe, grasslands have decreased considerably during the last century over a large part of their natural range, especially in Northern Europe (Poschlod and WallisDeVries, 2002), due to the loss of economic function as grazing or mown areas. Compared to Northern Europe (Bobbink and Willems, 1987; Poschlod and WallisDeVries, 2002) land abandonment

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started later in Central Italy in the early 1950s, although it is now widespread (Biondi, 2003). Abandonment of grasslands results in an early successional stage with progressive litter accumulation and increasing dominance of few perennial grasses. This is followed by shrub and woody encroachment with obvious changes in the structure and composition of the vegetation (Tilman, 1988).

A further threat to grassland diversity is nitrogen eutrophication. In particular, applications of nitrogen fertilizers (Foster and Gross, 1998) or enrichment by atmospheric nitrogen deposition (Stevens et al., 2004) have been shown to profoundly alter vegetation dynamics and reduce species diversity. For example, nitrogen enrichment can change species-rich calcareous grasslands into species-poor tall-sward communities that are dominated by a few highly productive grasses (Bobbink and Willems, 1991).

Undoubtedly, understanding the ecological mechanisms that regulate species diversity and productivity is a pivotal step towards conservation and restoration of semi-natural grasslands that are threatened by land abandonment and long-term eutrophication. The addition of growth-limiting nutrients to herbaceous plant communities frequently leads to an increase in plant production and a corresponding decline in plant species diversity as a consequence of increased competition for light (Tilman, 1988; Tilman and Pacala, 1993; Abrams, 1995). Although resource competition among living plants is certainly important in limiting the diversity of many plant communities, the accumulation of plant litter has an important role in reducing diversity at high levels of plant productivity. Several studies have demonstrated that a dense litter layer can limit the diversity of productive habitats by inhibiting species establishment through a variety of mechanisms such as shading (Bergelson, 1990; Facelli and Pickett, 1991), mechanical impediment (Wedin and Tilman, 1993) and the release of allelopathic compounds during litter decomposition (Rice, 1984; Bonanomi et al., 2006).

In more general terms, the restoration of abandoned species-poor grasslands is a target for conservation managers and management strategies that allow a rapid recovery from habitat degradation are urgently needed. With this in mind, many field experiments have been set up to investigate the influences of different management and nitrogen enrichment regimes on grassland diversity in Northern European countries (Bobbink and Willems, 1991). However, Mediterranean grasslands have received little attention from plant ecologists in comparison to Northern Europe, with existing studies mainly focused on the phytosociological classification of southern communities (Biondi et al., 1995). There is a lack of knowledge concerning the effects of nitrogen enrichment, plant litter accumulation and different cutting regimes on grassland productivity and plant species diversity. In the present study, we have conducted a 3-year field experiment in Central Italy species-poor grassland to investigate the effects on productivity and species diversity of addition of nitrogen fertilizer, coupled with removal of plant litter and artificial cutting. Our specific hypothesis was that nitrogen addition would increase living biomass and decrease species diversity, while disturbance by vegetation cutting and litter removal would produce opposite effects.

2. Methods

2.1. Study site description

This study was conducted in Central Italy (Fabriano; 43°20′N, 13°00′E) in an early-successional stage of an old field that represented a species-poor grassland that was dominated by *Brachypodium rupestre* (15–20 years post-agricultural abandonment). The previous agricultural use was for rotating crops, dominated by *Medicago sativa* and *Onobrychis viciifolia*. After abandonment, the vegetation rapidly became dominated by the perennial grass *B. rupestre*, which often forms an almost monospecific stand (Bonanomi and Allegrezza, 2004). Other common species included the grass *Dactylis glomerata*, the forbs *Clinopodium vulgare*, *Galium album*, *Tussilago farfara*, *Senecio erucifolius*, *Hypericum perforatum*, *Convolvulus arvensis* and *Daucus carota*, and the nitrogen fixer *Trifolium pratense*. The litter layer in this field was composed almost entirely of stem and leaf material from the grasses and mainly from *B. rupestre*.

The altitude of the field site is 350 m a.s.l., with an east-facing slope of about 10–15°. The underlying soil is a clay-loam with a pH of 8.4. Mean annual rainfall is 945 mm, with a moderate dry season during the summer months causing dry conditions. Mean monthly temperatures are between 21.9 °C (July) and 3.8 °C (January) (averaged over 46 years of observation; Fabriano, 357 m a.s.l. meteorological station, 7 km from the study site).

2.2. Experimental design and procedures

In April 2003, four randomized blocks with six permanent plots (200 × 200 cm) were established. Plots were arranged in three parallel columns, each separated by a 1 m buffer strip and protected from cattle and horse grazing by caging 140 cm in eight). Each plot was randomly selected for one of the following treatments: (1) untreated control; (2) cutting in early July at the end of the growing season; (3) litter removal in early July; (4) untreated with nitrogen application; (5) cutting in early July plus nitrogen application; and (6) litter removal in early July plus nitrogen application. Cutting was carried out each year with scissors to 2 cm above the soil surface to mimic mechanical mowing. The litter layer was removed from plots in April 2003, immediately before the beginning of the growing season and in July 2004 by gently lifting the litter mat by hand and without disturbance to the soil surface. Nitrogen was applied to the soil annually in early April in 2003, 2004 and 2005 as a commercial urea fertilizer at a rate of 3.5 g N m^{-2} $year^{-1}$. In soil, ureic nitrogen is readily transformed (hydrolyzed) in the presence of urease, an enzyme found in sufficient amounts in soil producing ammonia and carbon dioxide. The rate of nitrogen application was chosen to simulate a doubling of the nitrogen deposition through air pollution. The actual nitrogen deposition by air pollution in our study site is low, being less than

1.5 g N m^{-2} $year^{-1}$ (Ministero dell'Ambiente e della Tutela del Territorio, 2001). The experimental design thus provided the manipulation of litter levels and nitrogen fertilization and allowed the examination of the effects of litter and nitrogen on changes in community structure and species diversity.

2.3. Vegetation sampling and data analysis

In mid-July 2003–2005, aboveground living plant biomass was cut with scissors and litter harvested from each plot within three 20 × 20 cm community-sampling squares. The aboveground biomass collected from each plot was sorted into living plant material and litter for all of the phanerogamic species. The dry weight of each of the species was measured after drying at 50 °C for 7 days. Species diversity (Shannon index = H; Shannon and Weaver, 1949) was calculated based on the phytomass data. Furthermore, the species composition in each single plot for each year (2003–2005) was visually estimated to determine the level of cover of each single species by the Braun-Blanquet (1928) abundance-dominance scale: 5 = species with a 75–100% cover; 4 = species with a 50–75% cover; 3 = species with a 25–50% cover; 2 = species with a 10–25% cover; 1 = species with a 1–10% cover; + = species with < 1% cover; and *r* = species with a sporadic distribution.

Data were statistically analyzed by analysis of variance (Anova). Three-way Anova was used to test the effects of year, vegetation treatment (no treatment, litter removal and cutting) and nitrogen application (no nitrogen, nitrogen added) on the total aboveground living plant biomass and species diversity (H). The relationship between aboveground living plant biomass and species diversity (H) was estimated using linear regression. Significance was evaluated in all cases at $P < 0.05$.

3. Results

Total aboveground living plant biomass was significantly affected by year, cutting treatment and nitrogen application (Anova, $P < 0.001$, in all cases). Nitrogen addition significantly increased the living plant biomass in 2004 and 2005 (Fig. 1). In contrast, vegetation cutting, but not litter removal, reduced plant community biomass (Fig. 1). Nitrogen application and cutting treatments showed a statistically significant interaction (Anova, $P < 0.05$). Thus, nitrogen increased total plant biomass when applied alone and in the presence of litter removal in 2005, while a more pronounced biomass reduction was recorded with cutting in N-treated plots compared to non-fertilized plots (Fig. 1).

In 2003, grasses were dominant under all of the treatments (> 90% of total living biomass). In 2004 and 2005, vegetation cutting reduced the relative abundance of grasses (ranging from 70% to 75%) independent of the application of nitrogen and increased the abundance of both the forbs and the N_2-fixing species (Fig. 2). In contrast, litter removal was much less effective in reducing dominance of the grasses (Fig. 2).

The application of the different treatments for 3 consecutive years affected the cover of plant species in specific ways

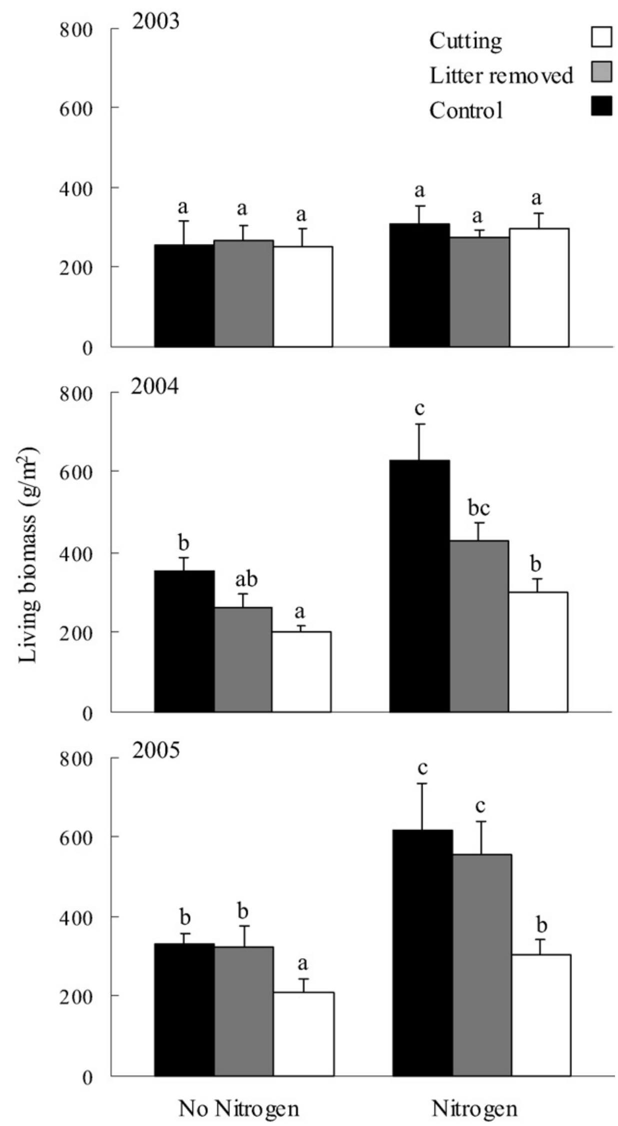

Fig. 1 – Effect of litter removal and cutting in the presence or absence of nitrogen fertilization on total aboveground living biomass after 3 consecutive years of treatment. The data are given as mean ± standard error of four replications, and the different letters indicate significant differences (Duncan test; $P < 0.05$).

(Table 1). Cutting positively affected the cover of almost all of the annual species, but in particular *Odontites lutea*, *Linum strictum* ssp. *corymbulosum* and *Medicago lupulina* (Table 1), with a limited, less-positive effect on the biennial species (e.g. *Anthemis tinctoria* and *Inula conyza*). For the perennial species, the effects of cutting were generally negative, but with some exceptions (e.g. *M. sativa* and *Linaria vulgaris*). However, both of the dominant perennial grasses *B. rupestre* and *D. glomerata* were strongly and negatively affected.

Litter removal affected species cover in a similar way to cutting, but with reduced intensity (Table 1).

In general, nitrogen enrichment decreased the positive effects of cutting on annual species (10 species instead of 11) and litter removal (only *O. lutea* instead of three species),

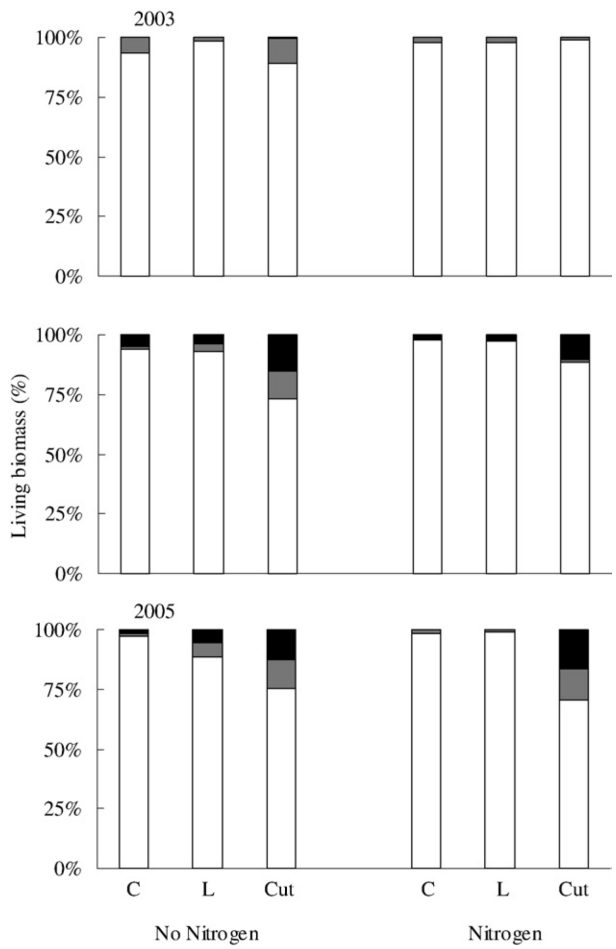

Fig. 2 – Effect on litter removal (L) and cutting (CUT) in the presence or absence of nitrogen fertilization on aboveground living biomass relative to untreated controls (C) of three different growth forms (grasses, open columns; forbs, gray columns; N_2-fixing species, black columns) after 3 consecutive years of treatment.

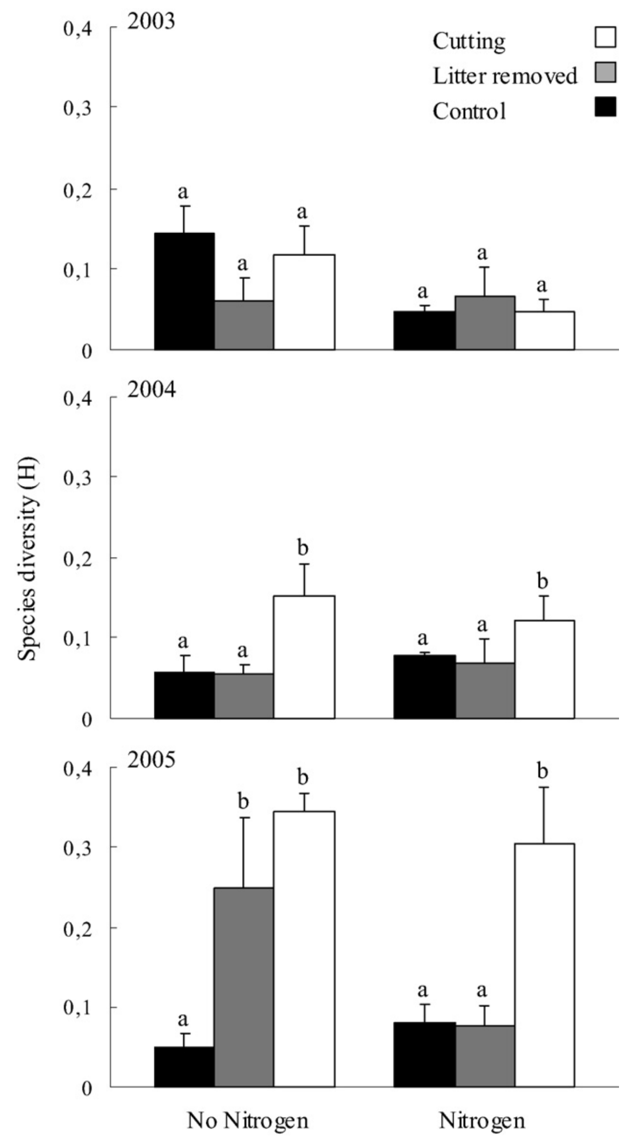

Fig. 3 – Effect of litter removal and cutting in the presence or absence of nitrogen fertilization on species diversity (H) after 3 consecutive years of treatment. The data are given as mean ± standard error of four replications, and the different letters indicate significant differences (Duncan test; $P < 0.05$).

and the same trend was obvious for biennial species (Table 1). In contrast, nitrogen enrichment reduced the negative effects of litter removal and vegetation cutting for some of the perennial species (e.g. *B. rupestre* for litter removal, and *G. album*, *C. vulgare* and *Centaurea nigrescens* ssp. *neapolitana* for vegetation cutting).

Species diversity was significantly affected by year and cutting treatment (Anova, $P < 0.001$, in both cases) but not by nitrogen application (Anova, $P = 0.11$). Moreover, the interactions between year and cutting treatment were statistically significant (Anova, $P = 0.011$). Cutting in summer increased the species diversity slightly in 2004 and this increase became more evident in 2005 (Fig. 3). Furthermore, cutting increased species diversity independently of the application of nitrogen fertilizer (Fig. 3). Litter removal increased species diversity only in 2005. However, the combination of litter removal and nitrogen application did not affect species diversity (Fig. 3).

Species diversity was strongly and negatively related to total biomass of grasses especially that of *B. rupestre* (Fig. 4). In contrast, species diversity was positively related to living biomass of the forbs and N_2-fixing species (Fig. 4). Furthermore, species diversity was negatively related to total living biomass and litter (r^2 0.02 and 0.15, respectively; $P < 0.05$ and $P < 0.01$, respectively), although this relationship was weak in both cases.

4. Discussion

In this study, both nitrogen fertilization and vegetation cutting significantly affected aboveground biomass and species diversity of a grassland community. Cutting obviously

Table 1 – Effect on litter removal and cutting in the presence or absence of nitrogen fertilization on cover of all species recorded in experimental plots after 3 consecutive years of treatment

Biological form	Species	No nitrogen		Nitrogen	
		Litter removal	Cutting	Litter removal	Cutting
Annual	*Anthemis arvensis* L.	0	+	0	+
	Blackstonia perfoliata (L.) Hudson	0	+	0	+
	Lathyrus aphaca L.	0	+	0	+
	Linum strictum L. ssp. *corymbulosum* (Rchb.) Rouy	+	+	0	+
	Medicago lupulina L.	+	+	0	+
	Odontites lutea (L.) Clairv	+	++	+	+
	Sherardia arvensis L.	0	+	0	+
	Sonchus asper (L.) Hill	0	+	0	0
	Trifolium campestre Schreber	0	+	0	+
	Veronica persica Poiret	0	+	0	+
	Vicia sativa L.	0	+	0	+
Biennial	*Anthemis tinctoria* L.	+	+	+	+
	Daucus carota L.	0	0	0	0
	Inula conyza DC.	+	+	0	0
	Melilotus officinalis (L.) Pallas	0	+	0	0
Perennial herbaceous and bulbous	*Brachypodium rupestre* (Host) R. and S.	–	—	0	—
	Dactylis glomerata L.	0	–	–	–
	Centaurea nigrescens Willd. ssp. *neapolitana* (Boiss.) Dostal	–	–	0	0
	Clinopodium vulgare L.	0	–	0	0
	Galium album Miller	0	–	+	0
	Hypericum perforatum L.	0	0	0	+
	Linaria vulgaris L.	+	+	0	+
	Medicago sativa L.	+	+	+	+
	Senecio erucifolius L.	0	0	0	0
	Trifolium pratense L.	0	–	–	–
	Tussilago farfara L.	–	–	0	0
Shrubs and trees	*Rosa canina* L. sensu Bouleng.	0	0	–	–
	Quercus pubescens Willd.	–	–	–	–

+, ++: relative increases in species cover. 0: no effect on species cover. –, —: relative decreases in species cover.

reduced aboveground biomass but increased species diversity, whereas nitrogen enrichment markedly increased aboveground biomass but maintained low species diversity.

The application of nitrogen fertilizer over three growing seasons led to a well-documented increase in plant biomass (Tilman, 1988). However, in many other studies the amount of nitrogen applied is high in comparison to the present study (Wedin and Tilman, 1993; Foster and Gross, 1998). Thus, the strong growth response using a relatively low level of nitrogen enrichment clearly indicates the nitrogen limitation of this community. In contrast, litter removal alone did not affect community biomass, but instead it decreases the effect of nitrogen enrichment on biomass. There was a decrease in aboveground biomass with vegetation cutting alone but when coupled with nitrogen addition it reduced the increase in growth. The decrease in biomass production after cutting may be due to the limited ability of the dominant species *B. rupestre* to compensate for this stress. McNaughton (1991) reported that the ability of perennial grasses to compensate for defoliation is species-specific and regulated by nitrogen availability. Litter removal and cutting had strong negative effects on many of the perennial species especially the dominant grass *B. rupestre*, where the negative effects of litter removal were reduced by nitrogen application. In contrast, the annual and biennial species generally showed positive responses to both litter removal and vegetation cutting.

Species diversity increased with summer cutting, irrespective of the application of nitrogen fertilizer. Species diversity increased progressively in each year to maximum values in 2005. A similar effect of cutting on species diversity has been reported for calcareous grassland dominated by *Brachypodium pinnatum* (Bobbink and Willems, 1991). This probably occurred as a result of the combined effects of the reduced dominance of the grasses, especially of *B. rupestre*, and the positive response of the annual and biennial species to vegetation cutting.

B. rupestre is know for its competitive ability in the absence of grazing and mowing which can result in rapid competitive exclusion in an invaded grassland (Bonanomi and Allegrezza, 2004). Indeed, in the present study species diversity was negatively related to aboveground biomass of *B. rupestre* and this pattern has been shown for *B. pinnatum* (Bobbink and Willems, 1987). Reduced dominance of *B. rupestre* after summer cutting may be in part due to lower non-structural carbohydrate content in the rhizomes, which can reduce the vigor of the shoots in the following autumn and spring (Bobbink and Willems, 1991). In contrast, the positive response of the annual and biennial species could result from an increase in the light availability at the soil surface (Bobbink and Willems, 1991; Foster and Gross, 1998), and/or a reduction in the intensity of root competition after elimination of the aboveground biomass of the dominant grass species (Casper and Jackson, 1997). Certain annual and biennial species may be able to rapidly colonize the empty space provided by the elimination of the dominant species following summer cutting, as has been suggested by previous empirical (Platt, 1975) and modeling (Tilman, 1994) studies. However, further investigations are needed to fully elucidate the specific mechanisms involved and their relative roles.

Litter removal did not increase species diversity over the first 2 years, but there was a significant increase in species

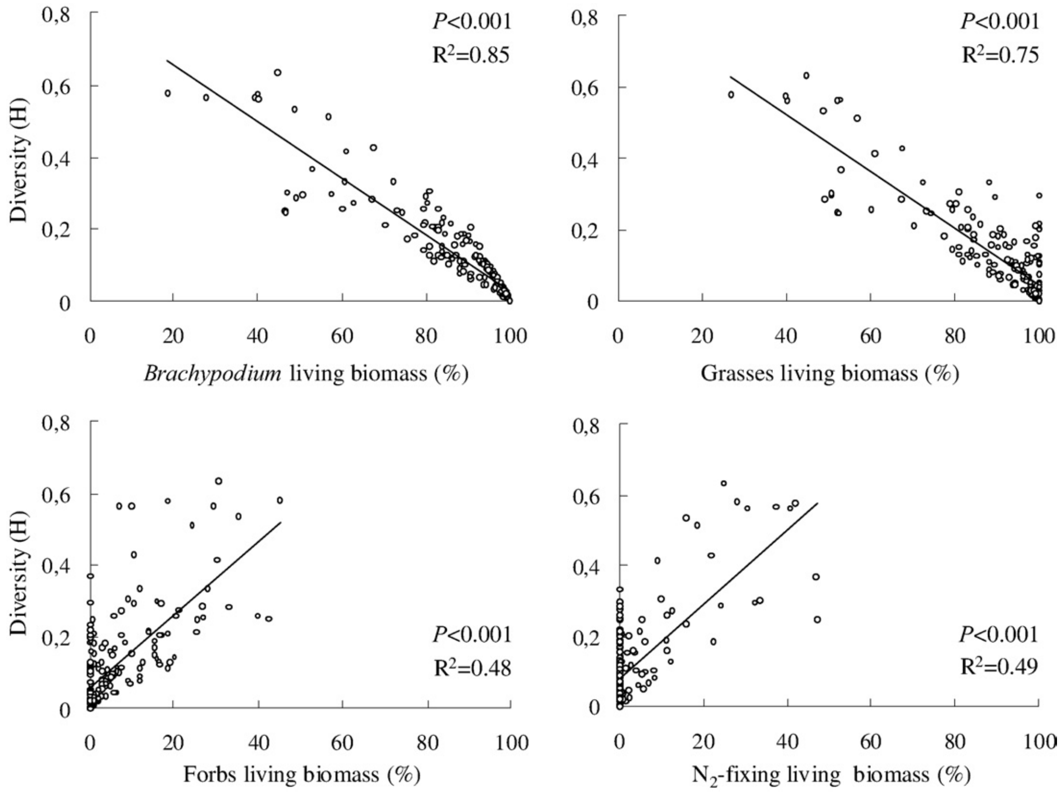

Fig. 4 – Regression analysis for species diversity (H) and relative living biomass abundance of: (1) *B. rupestre*; (2) total grasses; (3) total forbs; and (4) total N_2-fixing species. The levels of statistical significant are indicated on each graph.

diversity in the non-fertilized plots in the third year. Although the underlying mechanisms for this are unknown, greater light availability and changes in the red/far-red ratio at ground level (Schimpf and Danz, 1999), reduction in allelopathic effects (Blum et al., 1999; Bonanomi et al., 2005; Bonanomi et al., 2006) and in the mechanical impediments for seedling emergence (Wedin and Tilman, 1993; Xiang and Nilsson, 1999) may well be involved. Large amount of additional litter accumulate in response to nitrogen enrichment and this is likely to be at least one of the causes of suppression of species richness in the fertilized plots (Tilman, 1987; Carson and Barrett, 1988). The negative relationship between litter and species diversity supports this hypothesis. From this, we would argue that low species diversity in highly productive old fields and the reduction in species diversity that is often seen upon nutrient enrichment are caused by a combination of both inhibition by litter and resource competition associated with living plants. Finally, it should be noted that the response to cutting, litter removal and nitrogen addition in our Mediterranean grassland is consistent with results obtained in Dutch chalk grassland (Bobbink and Willems, 1991) and mid-successional old-field in Michigan (Foster and Gross, 1998).

Semi-natural grasslands need specific management regimes to prevent natural succession towards scrubland and woodland and to maintain high species diversity. Over the last few decades, grassland management regimes have been dramatically changed in Central Italy particularly with progressive land abandonment. In this context, our results indicate that annual summer cutting clearly decreases the dominance of *B. rupestre* and within 3 years this can allow a rapid restoration of higher species diversity. In contrast, litter removal is far less effective in restoring species diversity while nitrogen enrichment decreases species diversity, and also hampers the positive effects of litter removal. While nitrogen deposition remains low in the mountain of central Italy in comparison to Northern Europe (Ministero dell'Ambiente e della Tutela del Territorio, 2001; Stevens et al., 2004), it has the potential to lead to rapid reductions in species diversity in grasslands, particularly when coupled with land abandonment.

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