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BRIEF COMMUNICATION

Effects of competition and N and P supply on carbon isotope discrimination and ^{15}N-natural abundance in four grassland species

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Abstract

The effect of interspecific competition and element additions (N and P) on four grassland species (*Poa pratensis*, *Lolium perenne*, *Festuca valida*, *Taraxacum officinale*) grown under field conditions was studied. Two grasses (*L. perenne*, *F. valida*) grown in monoculture (absence of competition) showed lower carbon isotope discrimination ($\Delta^{13}C$) and enriched $\delta^{15}N$ values. Nitrogen addition (as urea) had inconsistent effects on species $\Delta^{13}C$ while caused enrichment of $\delta^{15}N$ of *P. pratensis* and *F. valida* but strong depletion of $\delta^{15}N$ of *T. officinale*. Phosphorous had no significant effect on $\Delta^{13}C$ but depleted $\delta^{15}N$ of all species.

Additional key words: fertilization, *Festuca valida*, grasses, *Lolium perenne*, *Poa pratensis*, *Taraxacum officinale*, water use efficiency.

The natural abundances of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes have been widely used in agricultural and ecological research over the last two decades. Carbon isotope discrimination ($\Delta^{13}C = \delta^{13}C_{air} - \delta^{13}C_{plant}$), is negatively related to the potential water use efficiency in C_3 species (Farquhar *et al.* 1989, Turner 1997). In ecological research, $\Delta^{13}C$ is a useful tool for relating plants' ecophysiologies to their environmental histories in terms of water relations, nutrition and competition for these resources (Sparks and Ehleringer 1997, Raeini-Sarjaz *et al.* 1998, Tsialtas *et al.* 2001). On the other hand, it is widely accepted that $\delta^{15}N$ in nature could not serve as a quantitatively reliable tracer of nitrogen sources (Evans 2001, Robinson 2001) but it is a qualitative integrator and indicator of nitrogen related processes, the interpretation of which is still an active area of research (Handley *et al.* 1998). Plant tissue $\delta^{15}N$ has been successfully used to predict site history (Chang and Handley 2000), identify N_2-fixing species and to quantify N_2-fixation (Roggy *et al.* 1999, Biggs *et al.* 2002), and to identify mycorrhizal symbiosis (Hobbie *et al.* 2000).

The simultaneous measurement of $\delta^{13}C$ and $\delta^{15}N$ in field-grown plants may be an important ecophysiological tool because water status largely affects plant $\delta^{13}C$ and also controls the plant availability of dissolved nitrogen in the rhizosphere (Peñuelas *et al.* 1999, 2000).

The aim of this work was to study the effect of interspecific competition and N and P additions on $\Delta^{13}C$ values and $\delta^{15}N$ of four species (*Festuca valida*, *Lolium perenne*, *Poa pratensis* and *Taraxacum officinale*) in a Mediterranean grassland.

The study site is a Mediterranean grassland (1115 m a.s.l., 40°26′N, 22°00'E) dominated by C_3 perennials. A detailed description of the site is given by Tsialtas *et al.* (2001). Three grasses (*Poa pratensis* L., *Lolium*

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Abbreviations: ANOVA - analysis of variance; $\Delta^{13}C$ - carbon isotope discrimination; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase.

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perenne L., *Festuca valida* L.) and the dicot *Taraxacum officinale* Weber ex Wigg constituted over 70 % of the above-ground biomass of the community between 1997 and 1999 (Tsialtas *et al.* 2001).

An area 15 × 25 m, representative of the grassland, was enclosed in early May 1996. Four treatments (control, grasses monocultures, N and P additions) were defined in a Completely Randomised Design. In the control treatment, species were grown under interspecific competition. Grasses monocultures were established, in three replications per species, during October 1996 by ploughing and incorporating the existing biomass, killing the seed bank by methyl bromide and sowing 1 m^2 plots with grasses seeds. Element additions (N and P) took place in six 1 m^2 plots (three for each element) where species grown under interspecific competition. Nitrogen was applied at 15 g(N) m^{-2} (in urea form) and phosphorus was given at 10 g(P) m^{-2} as superphosphate in October 1996 and 1997.

During 1998 growing season, four leaf samplings with three week intervals took place, beginning on 15 April. Leaf biomass was dried at 75 ºC for 48 h and ground to fine powder. Isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) were determined in three subsamples for each species, treatment and sampling occasion at Scottish Crop Research Institute, Dundee, UK, using a continuous flow isotope-ratio mass spectrometer *Europa Model 20-20* coupled to a *Roboprep* elemental analyzer (*PDZ Europa*, Crewe, UK) where $\delta X = [(R_{sample} - R_{standard})/ R_{standard}] \times 10^3$, R_{sample} and $R_{standard}$ are the ratios $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of leaf sample and standard, respectively. The universally accepted standard for $\delta^{13}C$ is the PDB limestone and for $\delta^{15}N$ is the atmospheric N_2. The biomass of *L. perenne* in the N addition plots at the third sampling (27 May) was insufficient for analyses.

Concurrently with leaf samplings, four soil samples were taken from control treatment and grasses monocultures from two soil depths (5 and 15 cm). Soil was treated with 2 M KCl and inorganic N determined by micro-Kjeldahl method. Soil water content [%(d.m.)] was determined gravimetrically.

Data of soil water and inorganic N contents were subjected to *ANOVA* as a three factor Completely Randomized Design with treatments (control and grasses monocultures), samplings and soil depths as main factors. Leaf $\Delta^{13}C$, $\delta^{15}N$ and leaf nitrogen concentration [%(d.m.)], averaged over treatments, were compared by Duncan's multiple range test for treatments (control, grasses monocultures, N and P additions) and species.

Soil water [%] and (N) [μg g^{-1}(d.m.)] contents were affected by all the main factors (treatments, leaf samplings, soil depth) and their interaction (Table 1). Water and N content were highest in monoculture plots of *L. perenne*. For both parameters, no significant differences were found between control and monocultures of *P. pratensis* and *F. valida*. Soil water and N contents were higher at 5 cm than 15 cm.

Table 1. Analysis of variance (*ANOVA*) of soil water content [%(d.m.)] and soil inorganic N [μg(N) g^{-1}(d.m.)] for the control and grasses (*Poa pratensis*, *Lolium perenne*, *Festuca valida*) monocultures, for the four sampling occasions and the two soil depths (5 and 15 cm). *** - $P < 0.001$, * - $P < 0.01$.

		Soil water content		Soil inorganic N	
	df	*F*	*P*	*F*	*P*
Treatments (T)	3	6.37	***	18.29	***
Samplings (S)	3	258.41	***	248.08	***
T×S	9	4.28	***	11.31	***
Depths (D)	1	104.25	***	133.17	***
T×D	3	3.92	*	1.70	0.17
S×D	3	19.83	***	21.21	***
T×S×D	9	1.38	0.21	3.57	***
CV		9.35 %		18.68 %	

When grasses grown under no interspecific competition (monocultures) $\Delta^{13}C$ values were unaffected (*P. pratensis*) or reduced (*L. perenne*, *F. valida*). Nitrogen addition had no effect on $\Delta^{13}C$ of *L. perenne* and *T. officinale* but decreased or increased the $\Delta^{13}C$ values of *P. pratensis* and *F. valida*, respectively (Fig. 1). Phosphorus caused a significant decrease of $\Delta^{13}C$ in *L. perenne* but had no effect on the rest species.

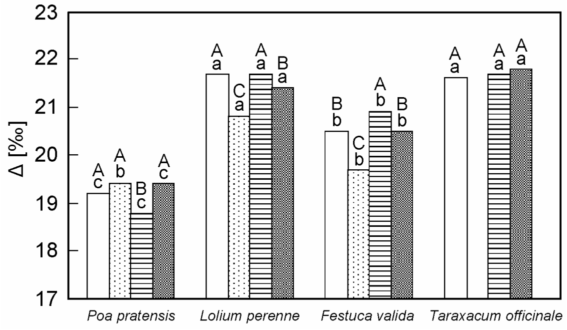

Fig. 1. Seasonal means of $\Delta^{13}C$ values for the four treatments of grasses (*Poa pratensis*, *Lolium perenne*, *Festuca valida*) and the three treatments of *Taraxacum officinale* (control - *empty columns*, monoculture - *dotted columns*, +N - *stripped columns*, +P - *closed columns*). Each bar represents the mean of 4 harvesting occasions (3 replications in each harvest). Capital letters were used to compare treatment means within species while minor letters to compare species means between each treatment. Bars with the same letter do not differ at $P < 0.05$ (Duncan's multiple range test).

Monoculture caused significant increases of $\delta^{15}N$ and leaf (N) concentrations of the grasses (Table 2). Nitrogen addition increased significantly leaf (N) concentration in all species and increased $\delta^{15}N$ of *P. pratensis* and *F. valida* but depleted $\delta^{15}N$ of *T. officinale*. The main effect of P addition was the strong depletion of $\delta^{15}N$ of all species (Table 2).

A positive correlation between $\delta^{15}N$ and leaf N was

Table 2. Comparisons of seasonal means of ^{15}N natural abundances (δ^{15}N) and N concentration in leaves of the four species studied in the different treatments. For the same column, means superscripted with the same letter do not differ significantly at $P < 0.05$.

Treatment	*Poa pratensis*		*Lolium perenne*		*Festuca valida*		*Taraxacum officinale*	
	δ^{15}N [‰]	N [% (d.m.)]	δ^{15}N [‰]	N [% (d.m.)]	δ^{15}N [‰]	N [% (d.m.)]	δ^{15}N [‰]	N [% (d.m.)]
Control	-0.37^c	2.23^b	0.51^b	2.46^b	0.38^c	2.45^b	1.92^a	3.36^b
Monoculture	5.09^a	3.83^a	5.65^a	3.54^a	6.50^a	3.64^a		
+ N	3.68^b	3.82^a	0.38^b	3.57^a	2.60^b	3.93^a	-1.62^c	3.91^a
+ P	-1.61^d	2.11^b	-1.38^c	2.32^b	-1.74^d	2.60^b	-0.48^b	3.10^c

Species	Monoculture δ^{15}N [‰]	N [% (d.m.)]	+N δ^{15}N [‰]	N [% (d.m.)]	+P δ^{15}N [‰]	N [% (d.m.)]
Poa pratensis	5.09^b	3.83^a	3.68^a	3.82^a	-1.61^{bc}	2.11^b
Lolium perenne	5.65^b	3.54^a	0.38^c	3.57^a	-1.38^b	2.32^b
Festuca valida	6.50^a	3.64^a	2.60^b	3.93^a	-1.74^c	2.60^b
Taraxacum officinale			-1.62^d	3.91^a	-0.48^a	3.10^a

found for *P. pratensis* ($r = 0.81$, $P < 0.001$, $n = 15$) and *F. valida* ($r = 0.57$, $P < 0.05$, $n = 15$) for pooled data. When data combined over species, treatments and samplings, a positive correlation ($r = 0.52$, $P < 0.001$, $n = 59$) was evident. Also, a negative relationship ($r = -0.32$, $P < 0.05$, n = 59) was found between Δ^{13}C and δ^{15}N.

Few attempts have been done to use stable carbon isotopes to study the effect of the interspecific competition on plant water use efficiency. In grasses monocultures (*i.e.* in absence of interspecific competition), Δ^{13}C values were decreased in two (*L. perenne*, *F. valida*) out of three species, in agreement with previous reports (Williams *et al.* 1991, Theodose and Bowman 1997). The reduction of Δ^{13}C in monocultures was between 0.8 to 0.9 ‰ (for *F. valida* and *L. perenne*, respectively) and laid within the range (0.5 - 0.9 ‰) given by Williams *et al.* (1991). These researchers ascribed the reduction of Δ^{13}C to the increase of soil N availability or the depletion of soil water content. In our work, Δ^{13}C of grasses in monocultures indicates the differences of the intrinsic water use efficiency of the species. Monoculture resulted in the narrowing of the differences of Δ^{13}C between species (2.5 ‰ in control, 1.4 ‰ in monocultures).

Nitrogen decreased Δ^{13}C values of *P. pratensis* but increased or had no effect on Δ^{13}C of the rest species. Analogous, diverse effects have been reported by other researchers (Condon *et al.* 1992, Gordon *et al.* 1999). A decrease of Δ^{13}C at increased leaf N concentrations could be the effect of lower stomatal conductance of the fertilized plants which synthesize more Rubisco (Field and Mooney 1986). However, this negative relationship between Δ^{13}C and leaf N is not consistent (Handley *et al.* 1994, Shangguan *et al.* 2000).

Phosphorus addition decreased Δ^{13}C of *L. perenne* but had no effect on Δ^{13}C of the other species. Brück *et al.* (2000) reported decreased Δ^{13}C when P was applied on sorghum but Ebdon *et al.* (1999) found no effects of P fertilization on field-grown *P. pratensis*.

The deep, tap-rooting *T. officinale* showed the smallest changes (0.2 ‰) of Δ^{13}C between treatments (control, N and P additions) indicating a stability of species water economy.

In grasses monocultures, δ^{15}N values were significant higher compared to control as a result of soil N enrichment in ^{15}N through the processes of N cycling (inorganisation and nitrification) imposed on plant biomass incorporated in the soil (Högberg 1997). Nitrogen addition increased leaf N concentration but its effect on δ^{15}N was species specific. Although urea is strongly δ^{15}N depleted (Högberg 1990, Högberg and Johanisson 1993), its application is related with enriched foliar δ^{15}N values due to the discrimination against ^{15}N of the processes (nitrification, leaching and losses as volatile NH_3) causing losses of N during N cycling (Högberg *et al.* 1995). The depleted δ^{15}N of *T. officinale* could be attributed to the uptake of urea NH_4^+-N which was not subjected to δ^{15}N enrichment of the N cycling processes (*e.g.* nitrification). Phosphorus addition caused a strong depletion of δ^{15}N of all species but had a minor effect on leaf N concentration. This finding is on contrary with previous reports where P addition enriched δ^{15}N in forest species in Scandinavia (Högberg *et al.* 1992).

Limitation of soil water leads to leaf δ^{15}N enrichment (Austin and Sala 1999) and to lower Δ^{13}C values due to stomata closure (Picon-Cochard *et al.* 2001). Thus, the negative relationship found between Δ^{13}C and δ^{15}N is causal and in agreement with previous reports (Peñuelas *et al.* 2000).

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