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Nitrogen Use Efficiency in a Semi-arid Community and its Relationship with Water Use Efficiency and Soil Water and Nitrogen

[1,2]*Ioannis T. Tsialtas and* [1]*Demetrios S. Veresoglou*

[1]Laboratory of Ecology and Environmental Protection,
School of Agriculture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Hellas
[2]Department of Experimentation, Hellenic Sugar Industry SA, Larissa factory, 411 10 Larissa, Hellas

Abstract: The aim of this work was to explore any relationship between Nitrogen Use Efficiency (NUE, as assessed by C/N ratio) with Water Use Efficiency [WUE, as assessed by carbon isotope discrimination, (Δ)] and soil water or N content in six grassland species (*Poa pratensis*, *Lolium perenne*, *Festuca valida*, *Taraxacum officinale*, *Plantago lanceolata*, *Achillea millefolium*). A negative relationship between C/N ratio and Δ was evident for *P. pratensis*, *L. perenne*, *F. valida* and *P. lanceolata* and thus, a strong, negative relationship was found for these traits at community level ($r = -0.60$, $p<0.001$). For all species, C/N ratio was negatively related with soil total N but a significant, negative relationship between C/N ratio and soil water content was evident for four (*P. pratensis*, *T. officinale*, *P. lanceolata*, *A. millefolium*) out of six species. Indirect assessments (C/N, Δ) could be successfully used in studying resources use efficiency changes under field conditions.

Key words: Carbon isotope discrimination · C/N ratio · grassland · soil resources

INTRODUCTION

Poor environments impose restrictions to plant productivity due to limited supply of resources. In resource-poor habitats, high resource use efficiency gives a competitive advantage to species and thus, traits contributing in use efficiency seems to affect community structure [1-3]. Semi-arid areas like Mediterranean are characterized by water deficit conditions occurring gradually from spring to summer and high Water Use Efficiency (WUE, the ratio of dry matter produced per kg water consumed for its production) was found to contribute in species dominance and community structure formation [3]. In N-poor soils, species with high Nitrogen Use Efficiency (NUE) were found to dominate in community [2]. In semi-arid regions, soil water availability plays a crucial role on soil N availability [4-6] and thus it is interesting to define any relationship between species WUE and NUE.

In last two decades, carbon isotope discrimination (Δ, a measure of the $^{13}C/^{12}C$ ratio in plant tissues compared to the air), determined mainly in leaves, is used as a useful tool for studying integrated plant ecophysiology in field [7]. Leaf Δ was found to be inversely related with long term water use efficiency (WUE, the ratio of the biomass produced to the water consumed to produce it) in C_3 species [8]. Also, the C/N ratio in plant tissues was used to estimate long-term NUE [9]. These two indirect indices could provide powerful information rather conveniently on resource use efficiency under field conditions.

Since few studies on species resource use efficiency in semi-arid regions and especially in semi-arid grasslands have been conducted [10], it is interesting to study the seasonal changes of NUE of community species and its relationship with soil water and N availability.

MATERIALS AND METHODS

The study was conducted at old, natural grassland (40° 26′ N, 22° 00′ E, 1115 m a.s.l.) dominated by C_3 perennial grasses [*Poa pratensis* L., *Lolium perenne* L., *Festuca valida* (Uechtr.) Penzes] and forbs (*Trifolium repens* L., *Taraxacum officinale* Weber ex Wigg, *Plantago lanceolata* L., *Achillea millefolium* L.) located

Corresponding Author: Dr. J.T. Tsialtas, Department of Experimentation, Hellenic Sugar Industry SA, Larissa Factory, 411 10 Larissa, Hellas

at Mount Vermion, western Macedonia, Greece. The seven species account for over 80% of the total above-ground biomass [3]. About 60% of annual precipitation (526 mm) occurs in winter and water availability is the main limiting factor of community productivity from mid-spring onward. The soil is a Pleistocene loam with pH 6.1, CEC 29.2 $cmol_c\ kg^{-1}$, free $CaCO_3$ 3.08%, total N 70 $\mu g\ kg^{-1}$ and P-Olsen 4.88 $mg\ kg^{-1}$. From the beginning of May till mid-July and every two weeks, six vegetation samplings took place in order community productivity and species composition to be determined. In each sampling occasion 12 quadrats (25 × 25 cm) were randomly collected, sorted to component species, dried at 75°C for 48 h and ground to fine powder. Six randomly selected sub-samples for each species sent for % N, % C and $\delta^{13}C$ determinations at Munich Technical University, Germany. Analyses were conducted using a Delta Plus Isotope Ratio Mass Spectrometer, (Finnigan MAT®, Bremen, Germany) coupled to an elemental analyser (EA 1110, Carlo Erba Instruments®, Milan, Italy). $\delta^{13}C$ in (‰) was calculated as:

$$[(R_{sample} - R_{standard})/R_{standard}] \times 10^3$$

where R_{sample} and $R_{standard}$ are the ratios $^{13}C/^{12}C$ of leaf sample and standard, respectively. The universally accepted standard is PDB limestone. Carbon isotope discrimination (Δ) was calculated as:

$$\frac{\delta_{air} - \delta_{plant}}{1 + \delta_{plant}/1000}$$

Where δ_{air} is the $\delta^{13}C$ of air CO_2 and is ca.-8‰.

At each sampling date, four soil samples were taken from two depths (0-5 cm and 5-15 cm). After air-drying, soil was extracted with 2M KCl and inorganic nitrogen ($NO_3^- + NO_2^- + NH_4^+$) concentration determined by a micro-Kjeldahl method. Soil water content was estimated by comparing wet and dry weights.

Data of Δ and C/N ratio were subjected to Analysis of Variance (ANOVA) as a Completely Randomized design with species and samplings as main factors. *T. repens* was excluded from analysis firstly for its N_2-fixing ability, which bias the results and secondly due to its absence from collected samples at last sampling. Means were compared with LSD test at $p<0.05$. Statistical analysis was carried out with M-STAT statistical package (MSTAT-C, version 1.41, Crop and Soil Sciences Department, Michigan State University, USA).

RESULTS AND DISCUSSION

Both Δ and C/N were significantly affected by species, samplings and their interaction. *P. pratensis* showed the lowest Δ values while *L. perenne* and *T. officinale* had the highest ones. A gradual decline of Δ with the progress of the growing season was evident when data combined over species (Fig. 1a). *P. pratensis* and *L. perenne* had the highest C/N ratio and *T. officinale* showed the minimum values. Combined over species, C/N ratio increased toward mid-summer (Fig. 1b). Since Mediterranean-type areas are characterized by increasing temperatures and decreasing rainfall toward summer, species are enforced to utilize more efficiently soil limited (water and nitrogen) resources [2, 3] and this was supported in our work by decreasing Δ (WUE) and increasing C/N ratio (NUE), respectively. Analogous trend of Δ was reported by Smedley *et al.* [11] for a grassland community.

At community level, a significant negative relationship ($r = -0.60$, $p<0.001$) between C/N and Δ was evident (Fig. 2) meaning that WUE and NUE were simultaneously increased. As regards individual species, the relationship between Δ and C/N ratio was significant for *P. pratensis*, *L. perenne*, *F. valida* and *P. lanceolata* (Fig. 2). Chen *et al.* [12] also reported species dependent relationship between these two parameters. However, they found a positive relationship between NUE (C/N ratio) and WUE (Δ) when N was applied to two grasses. Commonly, increased leaf N is related to decreased Δ [13, 14], due to reductions in C_i/C_a ratio of the mesophyll caused by the higher photosynthetic capacity of high-N leaves [15] and that could be an explanation for the results of Chen *et al.* [12]. In our case, the positive relationship seems to be the

Table 1: Correlation coefficients and significance level of the relationships between C/N ratio and soil water content and soil total N. Where Pp = *P. pratensis*, Lp = *L. perenne*, Fv = *F. valida*, To = *T. officinale*, Pl = *P. lanceolata*, Am = *A. millefolium*. ns = not significant, *p<0.05, **p<0.01, ***p<0.001

	Water content					
(cm)	Pp	Lp	Fv	To	Pl	Am
5	-0.83*	ns	ns	-0.87*	-0.84*	-0.88*
15	ns	ns	ns	-0.91*	ns	-0.85*
(cm)	Total N					
5	-0.96**	-0.99***	-0.97***	-0.96**	-0.86*	-0.94**
15	ns	ns	-0.82*	ns	-0.83*	ns

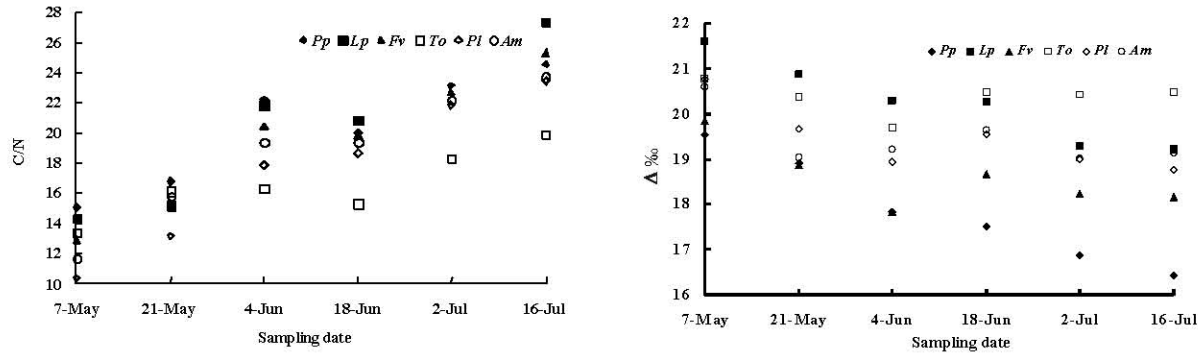

Fig. 1: Species trends of Δ (WUE) and C/N ratio (NUE) for the six samplings conducted. Where Pp = *P. pratensis*, Lp = *L. perenne*, Fv = *F. valida*, To = *T. officinale*, Pl = *P. lanceolata*, Am = *A. millefolium*

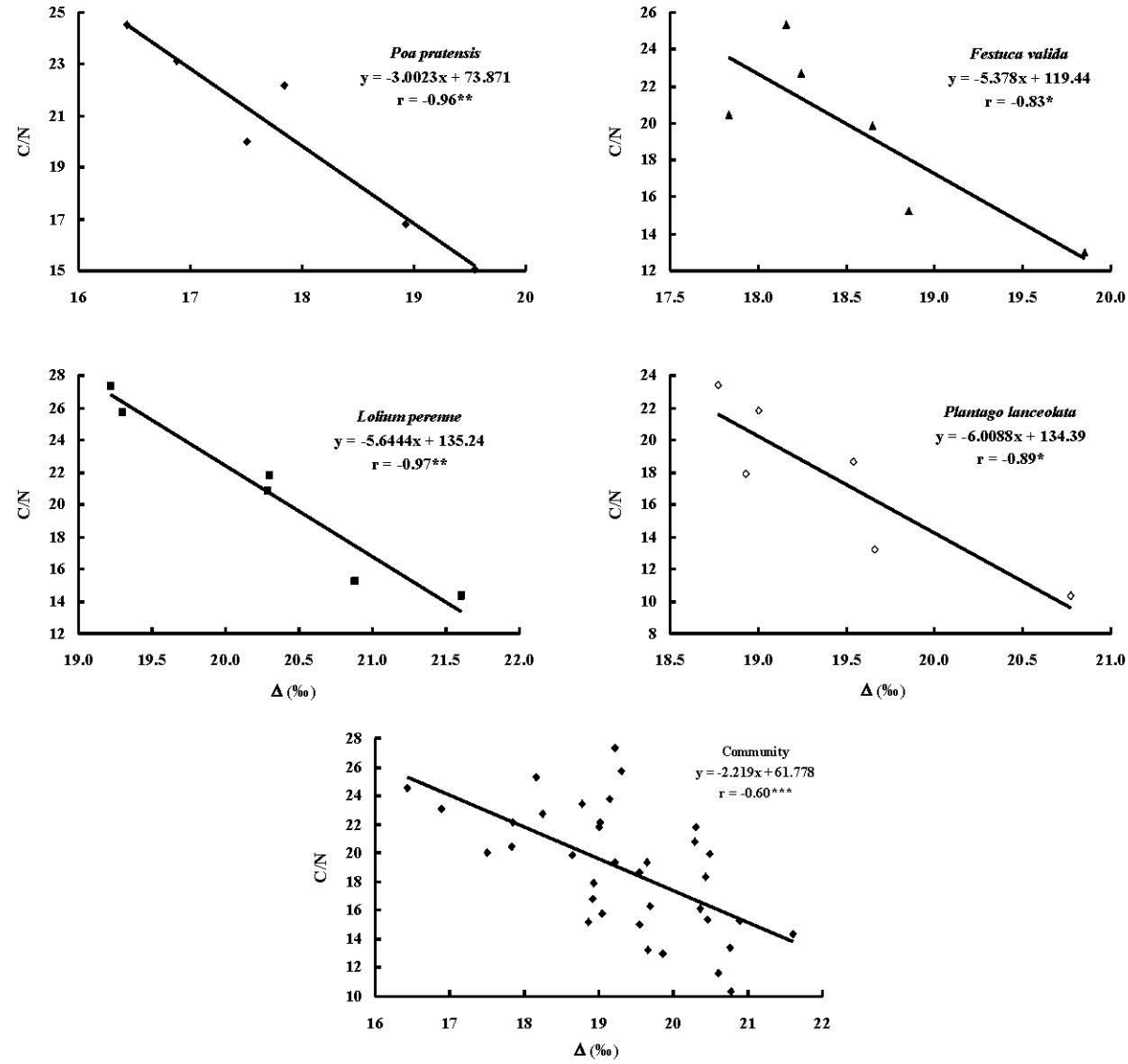

Fig. 2: Significant relationships between Δ (WUE) and C/N ratio (NUE) for specific species (*P. pratensis*, *L. perenne*, *F. valida*, *P. lanceolata*) and community (the total of six species) *p<0.05, **p<0.01, ***p<0.001

impact of soil water availability on Δ since under semi-arid environments like Mediterranean, N uptake is a function of soil water pulses [4].

NUE (C/N ratio) was strongly and negatively related with soil total N for all species. For *F. valida* and *P. lanceolata*, this relationship was also significant for the deeper (15 cm) soil layer (Table 1). An analogous negative relationship was also evident between C/N and soil water content for four (*P. pratensis*, *T. officinale*, *P. lanceolata*, *A. millefolium*) out of six species. For *T. officinale* and *A. millefolium*, this relationship was significant for both soil depths. It is noticeable that for the deep-rooted *T. officinale* [3], correlation coefficient was higher for the deeper soil layer (15 cm) than for the surficial one (Table 1). Our findings are in accordance with Yuan *et al.* [16] who reported a species specific, monotonical increase of NUE with the decrease of soil resource availability in semi-arid grassland in northern China.

Concluding, increase of utilization efficiency with depletion of soil resources was evident at both community and species specific level. C/N was negatively related with Δ meaning that species in this semi-arid grassland increased simultaneously the use efficiency of limiting resources. Indirect assessments (C/N, Δ) could become a powerful and non-laborious tool for studying the effects of exogenous factors on resource use efficiency under field conditions.

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