LETTER

Mechanisms of positive biodiversity–production relationships: insights provided by δ^{13}C analysis in experimental Mediterranean grassland plots

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Abstract

We investigated the role of water use in a Mediterranean grassland, in which diversity was experimentally manipulated, and a positive relationship was observed between plant species richness and productivity. Soil moisture patterns and stable carbon isotope ratios (δ^{13}C) in leaves indicated greater water use by plants growing in species-rich mixtures compared to monocultures. These results suggest that complementarity or facilitation may be the mechanism responsible for the positive relationship between plant diversity and ecosystem processes.

Keywords

Biodiversity mechanisms, δ^{13}C, mixtures, species diversity, stable carbon isotopes.

INTRODUCTION

A positive relationship between plant species richness and productivity has been shown in intercropping (Trenbath 1974; Vandermeer 1989) and biodiversity (Naeem *et al.* 1994; Tilman *et al.* 1996) experiments. Recently, the same relationship has been shown across Europe (Hector *et al.* 1999) for a variety of plant communities. The ecological mechanisms underlying this positive association, however, are not well understood, but could result from complementarity in resource use (Naeem *et al.* 1994; Tilman *et al.* 1996) and facilitation (Hooper & Vitousek 1998; Hector *et al.* 1999). Alternative mechanisms, based on a "sampling effect" (Huston 1997; Tilman *et al.* 1997), were shown not to be the dominant factor responsible for the higher productivity of these species-rich experimental communities (Hector *et al.* 1999, 2000).

Water stress is one of the main factors constraining productivity in Mediterranean plant communities (Espigares & Peco 1995). Determining how these communities use water may elucidate some of the underlying mechanisms associated with the positive relationship between species richness and productivity. To this end, we used stable carbon isotope analysis (δ^{13}C) as an indicator of long-term plant water use in experimental plant communities of different species richness. The leaf carbon isotope ratio serves as an index of the ratio of intercellular to ambient CO_2 concentrations (c_i/c_a) when carbon in the leaf is assimilated, and can be related to stomatal behaviour (Farquhar & Richards 1984; Rundel *et al.* 1988) and hence to water use by plants.

To test this possible water stress mechanism, we measured both δ^{13}C and soil water availability during a brief rainy period in a Mediterranean grassland in Portugal. Our results support this mechanism, and we discuss the implications of these findings.

MATERIALS AND METHODS

Our site, a Mediterranean grassland, is located in central Portugal (38°46′N, 8°38′W) in a flat meadow with sandy loam soil, with low water-holding capacity, prone to water deficits. In autumn 1996, a total of 36 plots of 2 × 2 m were sown, including monocultures of all the sown species and species-rich mixtures of 8 and 14 herbaceous species, and two plots were left bare. Mixtures were sown with randomly chosen species, and each level of species richness had different assemblages of species (Hector *et al.* 1999). Each plant assemblage was duplicated.

All existing vegetation was removed and the seed bank was eliminated by heating soil using black plastic covers placed over the soil between 1 July and 15 September 1996.

The sowing density in each plot was 2000 seeds/m^2 (corrected by germination tests) divided equally among the sown species (graminoids: *Avena sativa, Phalaris brachystachys, Holcus lanatus, Dactylis glomerata, Lolium multiflorum;* nitrogen-fixing legumes: *Ornithopus compressus, Trifolium subterraneum, Vicia sativa;* other herbaceous species: *Plantago lanceolata, Rumex pulcher, Conyza albida, Silene gallica, Misopates orontium, Torilis arvensis*). Periodically, all plots were weeded.

We used the standardized protocols of BIODEPTH to measure above-ground plant biomass and root biomass (Hector *et al.* 1999). Plot cover was first estimated visually in January 1997 and November 1998. The realized species richness was the number of species present in the quadrats (20 × 50 cm) sampled for biomass each year.

For δ^{13}C analyses, we sampled functionally different plant species with C$_3$ physiology: *T. subterraneum, O. compressus* (nitrogen-fixing legumes), *D. glomerata, H. lanatus* (graminoids) and *P. lanceolata* (forb). These species, which co-occur in natural grasslands, were the most dominant in at least one plot of the species-rich communities (*P. lanceolata,* four plots; *O. compressus,* one plot; *H. lanatus,* four plots; *D. glomerata,* one plot; *T. subterraneum,* second dominant, one plot). Four fully matured leaves exposed to full sunlight from at least four plants of each species per plot were collected at harvest time. Dry leaves were ball-milled and subsamples of 1 mg were analysed for δ^{13}C using an ANCA-SL Stable Isotope Analysis System (Europa Scientific Ltd, U.K.). ^{13}C/^{12}C ratios were calculated against the P. D. belemmite PBD standard (precision of ± 0.2‰). To check for strong gradients of CO$_2$ within plant canopies that could affect inferences about stomatal conductance (Buchmann & Ehleringer 1998), we measured the concentration profiles of CO$_2$ in the canopy air from 20 cm above the soil surface to *c.* 60–80 cm (above the canopy) in four unmanipulated reference plots (leaf area index: 0.7, 1, 2.6, 5) during peak photosynthetic activity using an infra-red gas analyser (EGM-1, PP-systems, U.K.). Also, to evaluate potential differences in evaporative demand (Ehleringer *et al.* 1992), leaf temperatures were measured (leaf temperature thermocouple, Li-Cor 1600, Li-Cor, U.S.A.) during the peak growing period for three species (*O. compressus, H. lanatus, P. lanceolata*) growing in monocultures and mixtures of 14 sown species. Leaf nitrogen concentration was measured in the same samples analysed for δ^{13}C in 1998 (ANCA-SL).

To determine if the measured differences in δ^{13}C were related to greater water availability, we measured the soil water content at two depths over 16–27 March 1998. Soil water content was measured at 0–15 cm and 15–30 cm in bare plots, monocultures and mixtures by time-domain reflectometry (TDR, soil moisture equipment), during an intermittent rainy period in March 1998. Much of the rainfall during the study occurred in intermittent rainy

periods from February to May, i.e. during peak growth of vegetation.

Total biomass and cover differences between monocultures and mixtures were tested using an independent sample *t*-test. A two-way ANOVA (TW) using species and species richness (monocultures or mixtures) as fixed factors was used to test for differences in leaf δ^{13}C and leaf nitrogen concentration. Leaf δ^{13}C values of each species for the two years were tested for differences in species richness with repeated measures ANOVA (RM) using species richness and year as fixed factors and plots nested in species richness. The inverse relationship between the leaf δ^{13}C value of each species and the number of realized species richness in both years was tested by multiple regression. Analysis of covariance (ANCOVA) was performed using leaf δ^{13}C as the dependent variable, species richness as the independent variable and above-ground biomass as the covariate. Soil water content at 0–15 cm and 15–30 cm was tested for differences in species-rich and bare plots with RM using species richness and sampling dates as fixed factors and plots nested in species richness. Variables were tested for normality and homogeneity of variances.

RESULTS

Realized diversities in 1997 and 1998, used as explanatory variables in our analyses, were strongly correlated with sown diversities (linear regression: 1997: $R^2 = 0.99$, d.f. = 1.36; 1998: $R^2 = 0.87$, d.f. = 1.33; $P < 0.001$). In both years, the total biomass in species-rich mixtures was significantly higher than that in monocultures (*t*-test: 1997: $t = -4.86$, d.f. = 34, $P < 0.001$; 1998: $t = -3.94$, d.f. = 26, $P < 0.01$). In the early season, the total cover in mixtures was already higher than that in monocultures (*t*-test: 1997: $t = -3.32$, d.f. = 32, $P < 0.01$; 1998: $t = -3.576$, d.f. = 25, $P < 0.01$).

Leaf δ^{13}C (Fig. 1) was significantly different in both years between monocultures and mixtures (TW: 1997: $F_{1,34} = 14.16$, $P < 0.001$; 1998: $F_{1,34} = 10.43$, $P < 0.01$) and among species in both years (1997: $F_{4,34} = 17.14$, $P < 0.001$; 1998: $F_{4,34} = 29.32$, $P < 0.001$). When monocultures and mixtures were compared, leaf δ^{13}C was higher in monocultures for all species, and significant for three (RM: *T. subterraneum,* $F_{1,6} = 7.09$; *P. lanceolata,* $F_{1,7} = 7.28$; *H. lanatus,* $F_{1,9} = 7.03$; $P < 0.05$). When related to realized species richness, leaf δ^{13}C of all species decreased with increasing species richness, a significant relationship for four species (*P. lanceolata, D. glomerata, T. subterraneum, O. compressus;* multiple regression, $P < 0.05$, $n = 15$–19). As biomass increased significantly in both years with realized species richness, we performed an ANCOVA and found that above-ground biomass was significantly related to the differences in leaf δ^{13}C (ln-transformed biomass:

$F_{1,70} = 19.98$, $P < 0.001$). CO_2 gradients were small (*c.* 4 p.p.m.) and leaf temperatures varied little (*c.* 1°C), and thus neither CO_2 gradients nor strong differences in evaporative potential played any significant role in these results. Additional analyses indicated no significant differences in leaf nitrogen concentration (TW: $F_{1,30} = 0.361$,

$P > 0.05$) between leaves of the same species growing in monocultures or in mixtures.

Higher soil water content was observed in species-rich plots, which suggests that $\delta^{13}C$ results indicate less water stress in the more species-rich, more productive plots. Prior to light rain (two first dates), no significant differences (RM: $P > 0.05$) in soil water content at the two depths were observed between bare, monocultures and species-rich plots (Fig. 2). During the subsequent 4 days with light intermittent rain, the water content at 0–15 cm (root fraction in all plots averaged 73% in 1998) was significantly higher in mixtures than in monocultures (RM: $F_{1,16} = 6.14$, $P = 0.02$), while less water was found in the bare plots (Fig. 2A). During the same period, the soil water content at 15–30 cm was significantly lower ($F_{1,16} = 6.95$, $P < 0.05$) in mixtures than in monocultures (Fig. 2B), perhaps due to reduced stomatal limitations in response to greater water availability in the upper soil layer.
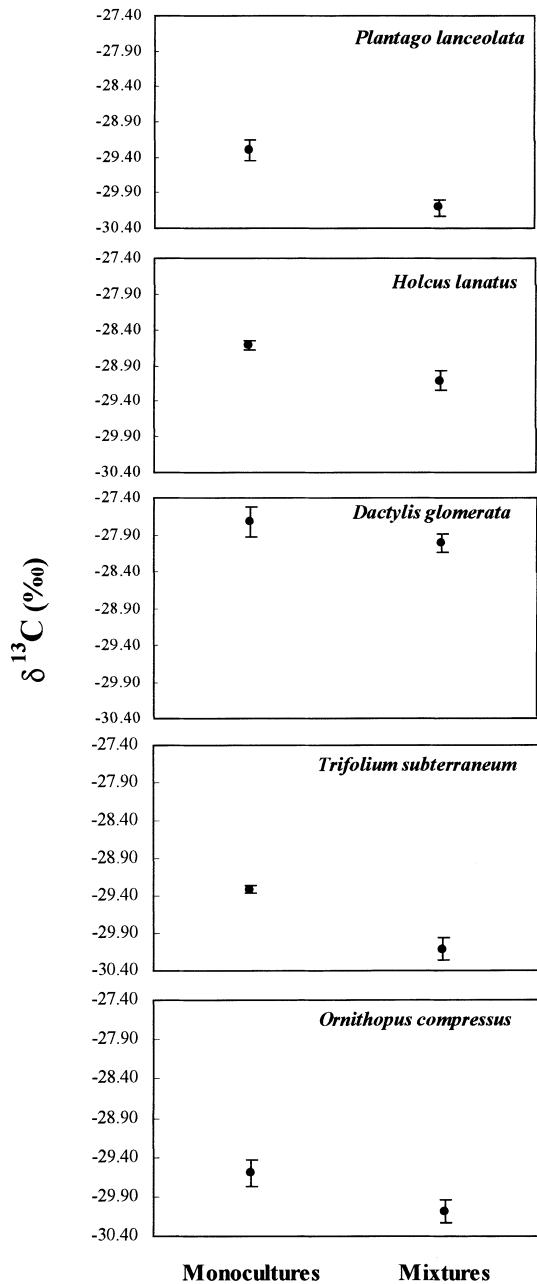
DISCUSSION

Lower leaf $\delta^{13}C$ indicates higher c_i/c_a ratios, resulting from either lower chloroplast demand for CO_2 or greater stomatal apertures affecting the supply rate of CO_2 and transpiration, and hence plant water use (Ehleringer & Cooper 1988; Farquhar *et al.* 1989). As the leaf nitrogen concentration was

Figure 1 Leaf carbon isotope ratio ($\delta^{13}C$) of *Plantago lanceolata, Holcus lanatus, Dactylis glomerata, Trifolium subterraneum* and *Ornithopus compressus* in monocultures and in species-rich mixtures (8 and 14 sown species). Plots are means of 1997 and 1998 measurements and bars are one standard error.
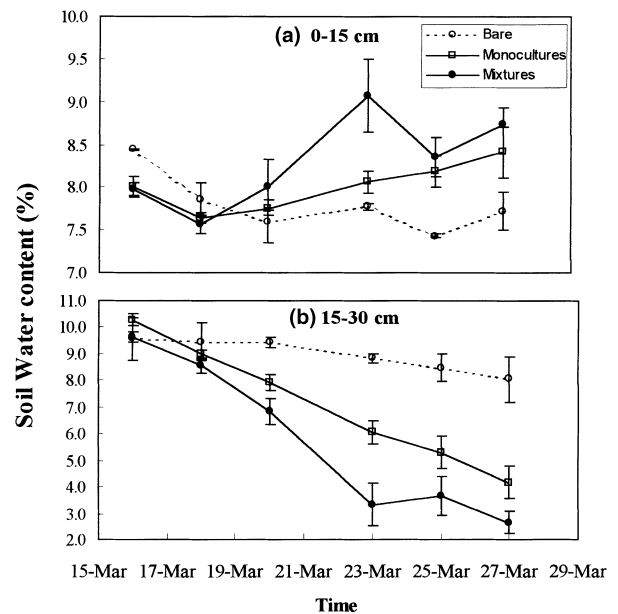

Figure 2 Volumetric soil water content at (A) 0–15 cm and (B) 15–30 cm. Time-domain reflectometry (TDR) measurements were performed during a period with intermittent rain on bare plots and plots with monocultures and species-rich mixtures (8 and 14 sown species). Note differences in scales.

not different between species growing in monocultures or mixtures, the lower leaf $\delta^{13}C$ for plants in mixtures (and the higher c_i/c_a ratio) probably resulted from decreased stomatal diffusion limitations to photosynthesis (Hubick *et al.* 1986; Virgona *et al.* 1990), and not to differences in photosynthetic capacity (Farquhar *et al.* 1982; Davies & Pereira 1992).

The low soil moisture in bare plots suggests that plant cover could have been important in reducing evaporation and percolation losses. Also, the higher biomass in mixtures may have collected dew during this period of higher humidity. These results agree with previous work (Ehleringer & Cooper 1988), where a decrease in $\delta^{13}C$ reflected an increase in intercellular carbon dioxide with increases in soil moisture and plant cover. As water uptake depends on root activity where moisture is available (Lauenroth & Aguilera 1998), leaf $\delta^{13}C$ was consistent with plants in the species-rich communities having more water available in the upper soil where roots were concentrated.

In water-limited systems, facilitation and/or complementarity in water use has been shown for the tree–herbaceous system (Joffre & Rambal 1988), but not within the herbaceous layer. Our experimental results are consistent with complementarity and/or facilitation as attributes of biodiversity, and are consistent with the contemporary theory of partitioning resources in species-rich communities (Silvertown *et al.* 1999). Our experimental design used co-occurring species of different structural and functional attributes. Such differences probably relate to facilitation and differential water use (and possibly nutrients) and partitioning. This can explain the early season differences, with higher total cover in species-rich mixtures than in monocultures. Higher growth rates from more complete use of the available water, and the resulting greater cover, would then facilitate reduced water loss to evaporation after rain events (or dew collection), as was observed for the upper soil layer. The increased water availability would then feed back to higher growth, increased cover and improved soil water availability.

In addition to the presence of disturbance (Huston 1994) and temporal variability in resource availability (Huisman & Weissing 1999), the differential partitioning of resources by species, and/or facilitation, is important in the establishment and maintenance of species diversity. Some degree of separation in resource utilization (niche separation) and of facilitation reduces the competition among species that would be more prevalent within monocultures. In the context of alternative hypotheses, our water relations results, consistent with the measured positive relationship between biomass and plant species richness, support the contention that this relationship arises in part from complementarity and/or facilitation and not simply from a few productive species dominating diverse communities (sampling hypothesis).

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BIOSKETCH

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