RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. **13**, 1320–1324 (1999)

Plant δ^{15}N Associated with Arbuscular Mycorrhization, Drought and Nitrogen Deficiency

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It has long been evident that plant δ^{15}N chiefly reflects the processes which fractionate ^{15}N/^{14}N rather than the δ^{15}N of plant N source(s). It has emerged recently that one of the most important fractionating processes contributing to the whole plant δ^{15}N is the presence/absence, type or species of mycorrhiza, especially when interacting with nutrient deficiency. Ecto- and ericoid mycorrhizas are frequently associated with ^{15}N-depleted foliar δ^{15}N, commonly as low as −12‰. As shown by the present study, plants having no mycorrhiza, or those infected with various species of arbuscular mycorrhiza (AM)-forming fungi, interact with varying concentrations of soil nitrogen [N] and moisture to enrich plant ^{15}N by as much as 3.5‰. Hence the lack of a mycorrhiza, or variation in the species of AM-forming fungal associations, can account for about 25% of the usually reported variations of foliar δ^{15}N found in field situations and do so by ^{15}N enrichment rather than depletion.

Received 26 January 1999; Revised 2 March 1999; Accepted 14 April 1999

There are few papers experimentally describing the effects of arbuscular mycorrhization on plant δ^{15}N. Handley *et al.*[1] showed for *Ricinus communis* that non-inoculated, whole plants were more ^{15}N-enriched (0.7‰) than inoculated ones. This result was consistent with later field-based studies[2] which showed that the rank order of δ^{15}N for plants having different mycorrhizal associations was: none/AM > ecto > ericoid. Azcón *et al.*[3] extended the experimental basis of this work to lettuce and barley, comparing the δ^{15}N of well-watered plants, given one of two concentrations of N and either non-inoculated or inoculated with one of two species of AM-forming fungi. The greatest effect on whole plant δ^{15}N (about +3‰) was related to concentrations of supplied N interacting with fungal species. The present experiment, repeating the experimental conditions used by Azcón *et al.*,[3] contrasts the plant δ^{15}N and δ^{13}C values obtained from non-inoculated or AM-inoculated lettuces given one of two external concentrations of N, and either sufficient water or drought.

EXPERIMENTAL

The experiment and analyses of plant P concentrations were done at the Estación Experimental del Zaidín in Granada, Spain. Other elemental and isotopic analyses were conducted at the Scottish Crop Research Institute. Whole plant tissues and soils were analysed for %N, δ^{13}C and δ^{15}N by continuous-flow isotope ratio mass spectrometry (ANCA-SL Model 20–20 and Europa Tracer Mass, Europa Scientific, Crewe, England).[1] δ^{13}C and δ^{15}N are expressed as parts per mil (‰) and calculated as $\delta^{n}X = [(R_{sample} - R_{standard})/R_{standard}]\ 10^3$, where R is the ratio of heavy-to-light isotope. Data analyses and graphs were done using Statistica software (Statsoft, Norman, Oklahoma, USA).

The δ^{13}C of shoots are reported, because the theory underpinning interpretation of plant δ^{13}C is based on whole leaves or shoots;[4] additionally, the δ^{13}C of fungal C, thought to be ^{13}C-enriched relative to bulk substrate[5] could confound interpretation of the whole plant or root values. Whole plant values are reported for δ^{15}N and nitrogen (N) and phosphorus (P) content.

The growth conditions were largely as described by Azcón *et al.*[3] except that two levels of supplied water were contrasted with presence/absence of AM and concentrations of externally supplied nitrogen [N]. The appropriate plants (Table 1) were inoculated, as previously described,[3] with *Glomus deserticola*. Briefly, plants (5 per treatment) were germinated and grown on to harvest (Table 1) with weekly applications of either 2.5 mmol (N level 1) or 5 mmol (N level 2) $Ca(NO_3)_2$ in 5 mL of solution once per week; K^+ was added as K_2SO_4 to provide 1:1 N and K. Phosphorus, as K_2HPO_4, was given to appropriate plants throughout the experiment, totalling 100 mg kg^{-1} pot^{-1}. After two weeks the seedlings were transplanted into pots containing 500 g of steam-sterilised 5:2 (v/v) soil + quartz sand.[3] After four weeks, the plants were placed on a watering regime of either 120% (well-watered) or 60% (droughted) of field capacity. After three months growth the plants and soils were harvested.

Of the five replicates in each treatment, two were consumed by phosphorus (P) analyses, and the bulk value used in conjunction with the appropriate treatment, hence the lack of data for variation of P content within treatments.

The δ^{15}N comparisons are among treatments given the same isotopic source of N. No attempt was made to relate plant δ^{15}N to external source δ^{15}N. The δ^{15}N of bulk soil

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Contract/grant sponsor: Scottish Office of Agriculture, Environment and Fisheries Department.

CCC 0951–4198/99/131320–05 $17.50

Table 1. Experimental design

Well-watered					
N level 1			N level 2		
C	+P	+F	C	+P	+F
Droughted					
N level 1			N level 2		
C	+P	+F	C	+P	+F

C = Controls, no added phosphorus no inoculation.
+P = phosphorus added, no inoculation.
+F = fungal inoculation, no added phosphorus beyond that in soil.

Table 2. Significance levels associated with variables and treatments and significant interactions. Analysis of variance followed by least significant difference (LSD)

		Treatment		
Plant part	Variable	Water	[N]	Fungal treatment
Plant	Dry weight	<0.0001	0.07	0.14
Shoot	δ^{13}C	<0.0001	0.70	<0.0001
Plant	δ^{15}N	<0.0001	<0.0001	<0.0001
Plant	N (mg/plant)	<0.0001	<0.0001	0.40
Plant	P (mg/plant)	—	—	—

was not measured before beginning the experiment because there was too little N in this soil and quartz sand mixture for an isotopic analysis to be done.[3] We assumed, on the basis of the results of the previous experiment[3] using the same potting mixture, that all but a trivial amount of the N in the harvested plants and soils originated from the applied fertiliser (δ^{15}N = +4.6‰). The N of the original lettuce fruits was trivialised in the course of plant growth.

RESULTS

Plant dry weight (Table 2, Fig. 1(a)) responded significantly to the amount of water supplied and secondarily (non-significantly, $p = 0.07$) to the supplied N concentration ([N]); there was no significant effect of inoculation (+Fungus treatment) or supplemental P on whole plant dry weight. All well-watered plants accumulated more dry weight biomass than droughted ones (Fig. 1(a)) [N] was significantly related ($P < 0.05$) to plant dry weight within the well-watered controls and P-amended treatments, but not the inoculated ones; among the droughted plants no other treatment had a significant effect on plant dry weight.

Shoot δ^{13}C was also significantly related to fungal treatment (Control, +P or +Fungus) and, as expected, was significantly related to the amount of water supplied (Table 2, Fig. 1(b) so that well-watered plants were less ^{13}C-enriched than the droughted ones. Whole plant dry weight (Fig. 1(a) generally increased as shoot δ^{13}C became more negative. δ^{13}C was significantly related to [N] only in the well-watered, +Fungus treatment, in which, as expected,[6] more N was associated with a less negative δ^{13}C (about 1‰ difference between droughted and well-watered). Inoculated plants accumulated no more dry weight biomass (Fig. 1(a) than did P-amended ones.

The results of soil analyses validated that the major source of N for the plants was the applied fertiliser N. The bulk soil δ^{15}N became more ^{15}N-enriched than fertiliser N during the course of the experiment (averaging $+9.8 \pm 3$‰). However, there was very little of it. Total soil N averaged 0.016 ± 0.02 % (dry weight) across all treatments (data not shown), indicating that plant-available N was even less. This result is consistent with the findings of a previous experiment[3] using the same experimental design and materials. In that experiment amounts of total N were similarly small, and water extracts showed that plant-available N (mineral N) were at the lower limit of detection. It should also be noted that the analytical precision for δ^{15}N diminishes at such low N concentration.

All plant δ^{15}N values were positive, and all treatments (Table 2) were associated with highly significant differences of whole plant δ^{15}N. With one exception (Fig. 2(a)), low [N] plants were more ^{15}N-enriched than ones receiving the higher concentration of N. The δ^{15}N of droughted plants (at high and low [N]) was generally higher than that of well-watered ones (Fig. 2(a)). The two exceptions were: (1) Control + high [N] and (2) +Fungus + low [N]. The largest plant δ^{15}N (+5.3‰) was for inoculated, droughted and high [N].

The range of means of δ^{15}N for non-inoculated plants was 2.2‰; for inoculated plants it was 3.2; and the total range for all treatments was 3.5‰. For all but one treatment, shoot-root difference of δ^{15}N was small (-0.3 ± 0.2‰ standard deviation (s.d.)). The shoot-root difference for well-watered controls (no added P; no inoculation) was significantly different from other treatments ($p < 0.001$) and larger (-2.3 ± 0.9‰ s.d.).

Plant N content (mg/plant, Fig. 2(b)) responded as expected: with well-watered, high [N] plants > droughted, high [N] plants > well-watered, low [N] > droughted, low [N]. There was no obvious relationship between amount of plant N (mg/plant) and plant δ^{15}N. Plant N concentration (%N), however, was significantly correlated with whole plant (but not shoot or root) $\delta^{15}\text{N} : \delta^{15}\text{N}_{\text{plant}} = 4.9 - 2.1$ (%N), $r = 0.69$ and $P_{\text{slope}} = 0.0016$. The well-watered, P-amended plants (Fig. 2(c)) accumulated the most P per plant at both N concentrations; of the inoculated plants, the well-watered ones accumulated the most P.

DISCUSSION AND CONCLUSIONS

Under the most stressed conditions (drought and/or low [N]) fungal inoculation conferred a potential water use efficiency advantage in terms of ^{13}C discrimination. Under well-watered conditions, this advantage disappeared, and [N] appeared to control shoot δ^{13}C. Among well-watered plants, inoculation overcame the effects of N deficiency so that both [N] yielded similar dry weight biomasses. Under drought and low [N], the δ^{15}N values of inoculated plants were larger than in any other treatments and corresponded to relatively low amounts of plant N and P. It is possibly useful to speculate whether seasonal soil drying and, thus, increasingly limited N supply, could explain the growing-season-related changes in AM plants documented in some studies.[7,8]

In an experiment parallel to the present one[3] a cultivar of barley (Betzes) exhibited a range of 2.3‰ in high versus low N treatments when given one of two species of AM-forming fungi and a corresponding range of 1.5‰ when non-inoculated. In both cases, low [N] was associated with whole plant ^{15}N enrichment. In the present study, there was a highly significant correlation between plant N concentration (auto-correlated with [N]) and plant δ^{15}N, such that

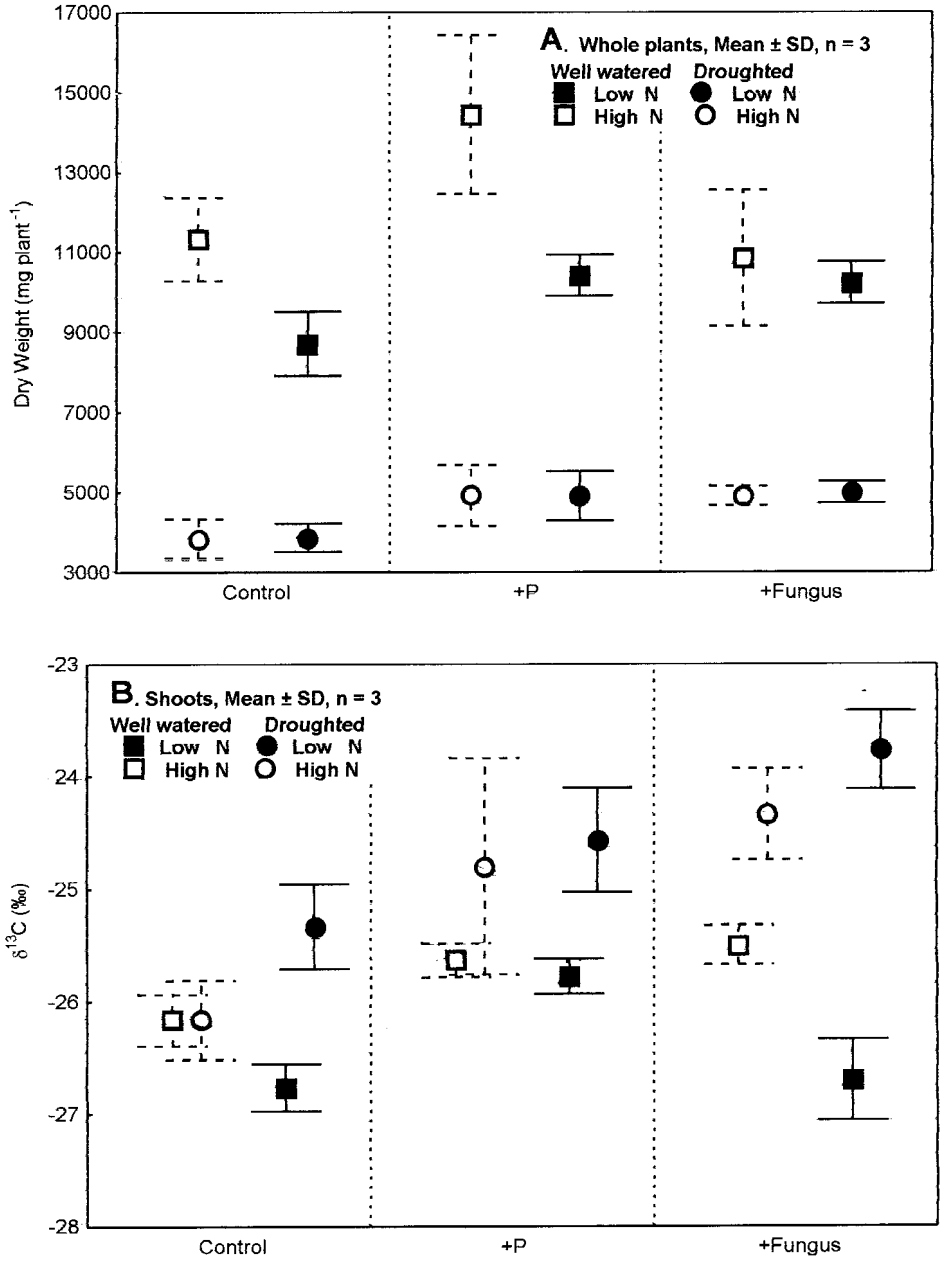

Figure 1. Dry weights of whole plants (a) and δ^{13}C of shoots (b) in response to treatments.

greater plant N concentration was associated with lower plant δ^{15}N. The decrease in plant δ^{15}N under higher external N concentrations has been previously interpreted as greater discrimination against ^{15}N at the first assimilatory enzyme.[9,10] No such experiments, however, extended the range of external N supply into that of N deficiency. The finding for diverse genotypes of wild barley,[11] where N deficiency was found to predominantly incur lower shoot and whole plant δ^{15}N than controls, leads to questioning whether assimilatory fractionations can wholly explain the observed δ^{15}N values. In this experiment, no external N was supplied during treatments,[11] hence losses, rather than assimilatory fractionations, must be called upon to explain the resulting, lower plant δ^{15}N. In this same study, one genotype became ^{15}N-enriched (rather than depleted) relative to controls, as occurred in the present study of lettuce, illustrating that the response to a range of N supplies is partially controlled, even within one plant species, by genotypic differences. This leads intuitively to the supposition that different plant taxa may exhibit varying responses to stresses such as drought, salt[11] impaired mycorrhizal association or nutrient deficiency.

In most cases, it has been found that plant shoots are slightly ^{15}N-depleted relative to bulk soil.[7] So far, in all but the very simplest systems, no satisfactory means exist for measuring the δ^{15}N of plant N sources in soil.[12] The plants in this study ranged from ^{15}N-depleted (+1.8‰) relative to the initial, source value of +4.6‰ to slightly ^{15}N-enriched (+5.3‰). We have no way of knowing how much that initial source value changed during plant uptake between applications and, therefore, how much the observed variation in whole plant δ^{15}N is related to assimilatory discrimination, post-assimilation losses or varying amounts of N assimilation over the course of source value changes.

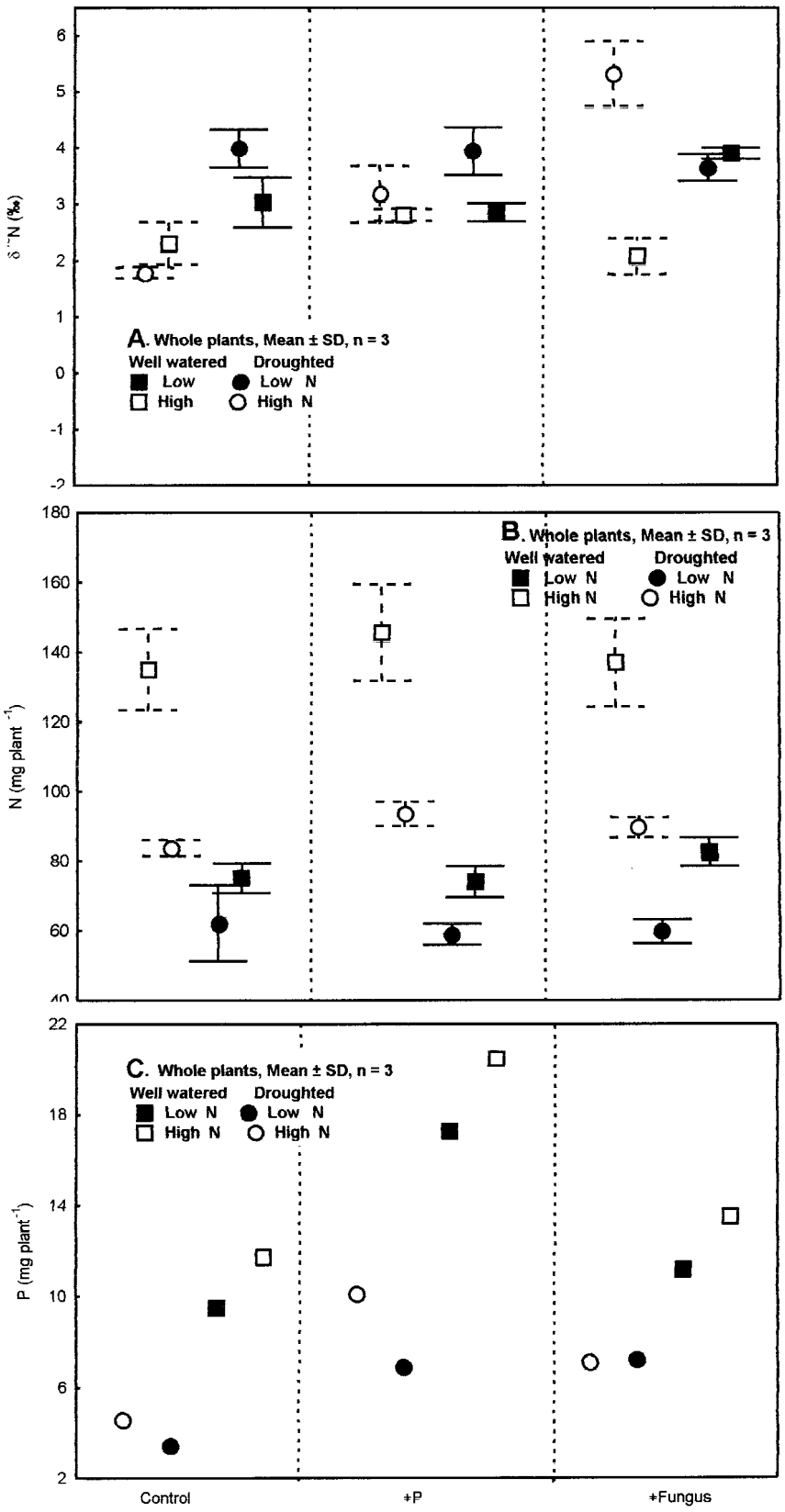

Figure 2. Whole plants: (a) δ^{15}N, (b) N content and (c) P content in response to treatments.

Depending on nutrient availability, the presence/absence and type of mycorrhiza can have effects on plant δ^{15}N ranging from nil to very large. The now-frequently reported ranking of foliar δ^{15}N values related to mycorrhizal type, is: non-mycorrhizal/AM > ecto > ericoid.[2,13] It has become evident more recently that within this ranking, the range of values increases as does external nutrient deficiency.[14,15] Conversely, under nutrient sufficient conditions,[15] the foliar

δ^{15}N values of plants were statistically indistinguishable when one group had no/AM and the other had ecto-and ericoid mycorrhizas.

For relating δ^{15}N values to plant nutrition, it would be useful to put limits on the contribution which each type of mycorrhiza makes to the range of foliar δ^{15}N values found in the field. Plants having ecto- or ericoid mycorrhizas can have very negative foliar δ^{15}N values, commonly as low as -12‰[7] and becoming more negative as nutrients become more deficient,[14] and less negative as nutrient supply improves, suggesting that the effects on plant δ^{15}N of ecto- and ericoid mycorrhizas can account for a large part of the variation in range of foliar δ^{15}N found at unfertilised sites. There is less known about the contributions which arbuscular mycorrhizas and the absence of mycorrhizas make to observed ranges of plant δ^{15}N values.

The results of the present work and its foregoing, parallel study[3] confirm that drought, N deficiency and mycorrhizal status (none or varying species of AM-forming fungus) can lead to plant ^{15}N enrichment. If we take -12‰ as an extreme plant δ^{15}N value, and the $+5.3$‰ of the present study as another, the above conditions relating to no/AM, drought and N deficiency can account, potentially, for about one-quarter of the usually reported range of plant δ^{15}N values. The results of work done with diverse genotypes of non-mycorrhizal barley[16] show that the direction and magnitude of plant δ^{15}N response to these variables is dependent on genetic factors.

Acknowledgements

Scottish Crop Research Institute is grant-aided by the Scottish Office of Agriculture, Environment and Fisheries Department.

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