Use of isotopic discrimination (d13C and d15N) for screening drought tolerance of legume grassland plants

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Objectives of the study As a consequence of Global Changes, perennial pastures have to face more and more extreme climatic conditions such as longer drought periods which impact forage production and, as a consequence, reduce the sustainability of livestock systems. In that context it is important to evaluate the capacity of grassland species to tolerate water shortage. This point is of major interest for *Fabaceae*, the most frequent family after *Poaceae* in grassland ecosystems, since they allow application of N fertilizer to be reduced while maintaining acceptable production levels.

Methodology

In that purpose, we set 2 experiments which aimed at screening the drought tolerance of grassland species

(i) in 2012 we grew, on soil columns, 14 *Fabaceae* and 6 grasses under controlled environment in a greenhouse under four growth conditions, by crossing two levels of both Phosphorus and water availability;

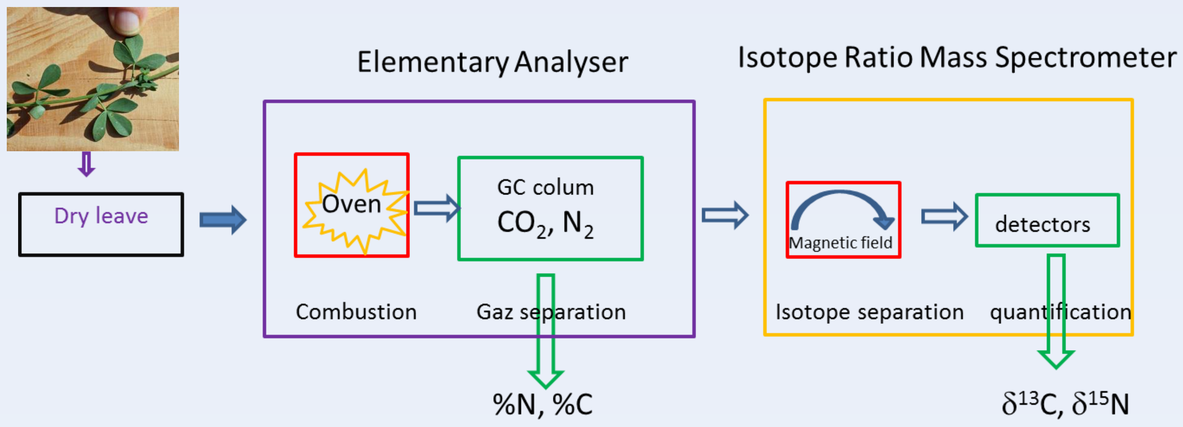
(ii) (ii) in 2013, 24 *Fabaceae* and 4 *Poaceae* were seeded in a common garden on 2 m² plots and grown under full water availability; in both experiments, species were randomly distributed in 4 repetitions blocks.

The 2012 greenhouse experiment view and the 14 first *Fabaceae* species

1	*Anthyllis vulneraria L.*
2	*Coronilla Minima L.*
3	*Lathyrus pratensis L.*
4	*Lotus corniculatus L.*
5	*Medicago sativa L.*
6	*Melilotus albus Medik.*
7	*Medicago lupulina L.*
8	*Onobrychis sativa Lam*
9	*Trifolium repens L.*
10	*Trifolium fragiferum*
11	*Trifolium campestris Schreb.*
12	*Trifolium pratense L.*
13	*Vicia cracca L.*
14	*Vicia sepium L.*

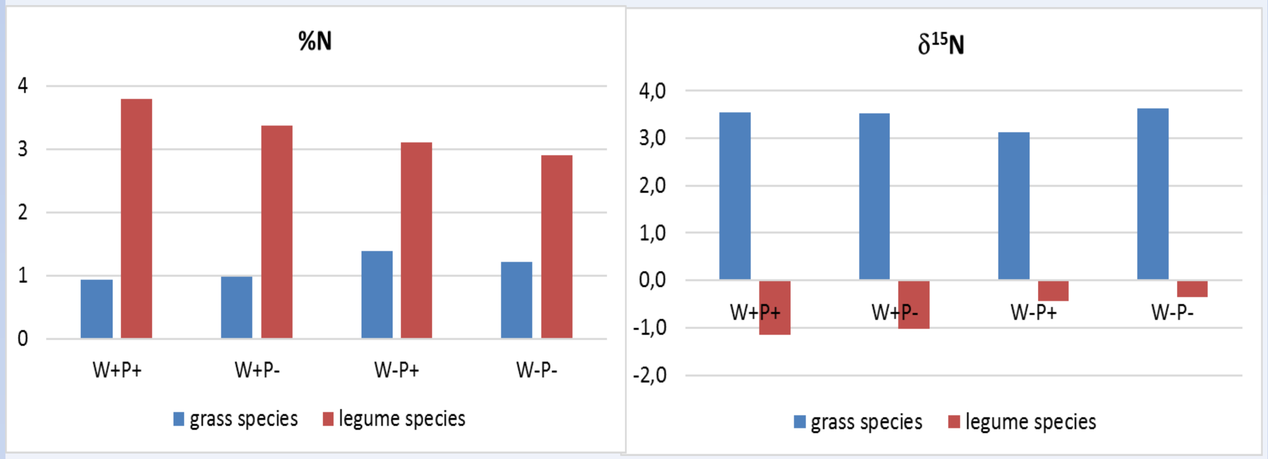
Stable isotopes analysis

Stable isotopes as $\delta^{13}C$ values are good indicators for drought tolerance which could information to select plant species in function of their water use efficiency, productivity and photosynthetic yield. As we screen *Fabaceae* species, the $\delta^{15}N$ values have been used to monitor their productivity and needs.
At harvest shoots were sampled and dried at least 48H (60°C) to determine above ground biomass. Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ abundances were measured subsequently on ground material



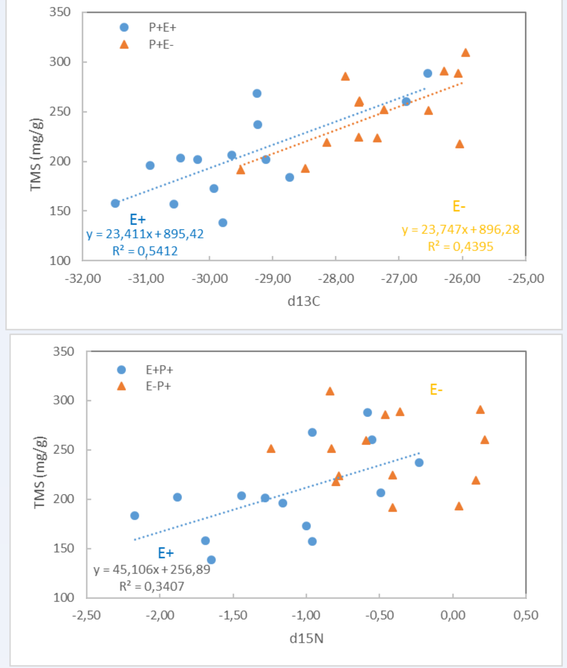
The %C, %N, $\delta^{13}C$ and $\delta^{15}N$ were measured on a Isoprim 100 IRMS coupled with a elementar analyser (Microcube).

Some results of the green house in 2012



We find a significant impact of the growth conditions on the N% and $\delta^{15}N$ (mean values from -1.15 to -0.36 ‰), but no on the grass specie (mean value range from $\delta^{15}N$ = +3.12 to +3.62 ‰). Water was a higher limitation factor compared to Phosphorus.

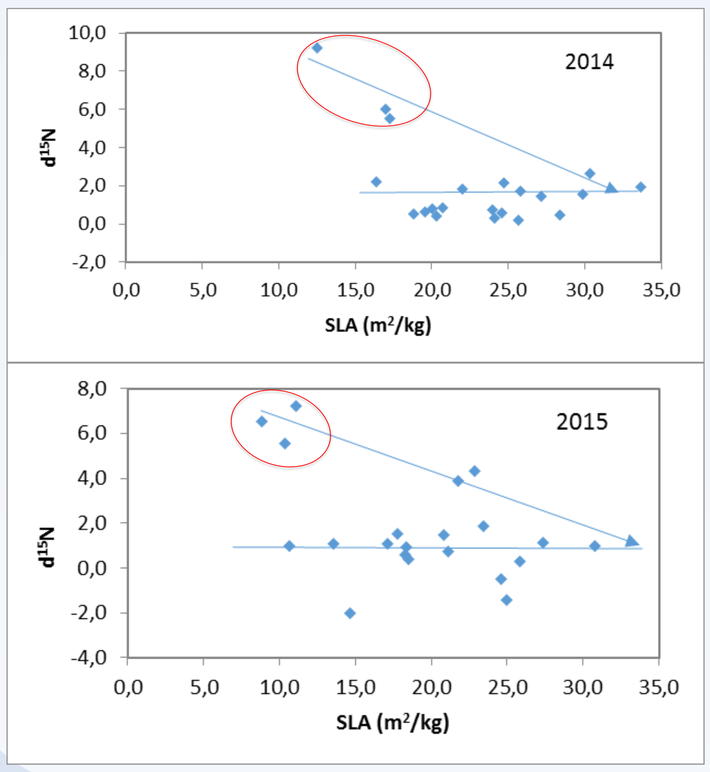
Isotopic results influence of water stress



In the Leaf Dry Matter Content (LDMC) plot versus the leaf isotopic $\delta^{13}C$ content, we can clearly seen the effect of the water stress given less negative values, i.e. showing a lesser photosynthetic activity.

For the $\delta^{15}N$, the same tendancy is observed, that could suggest a worse efficiency of rhizobiome nodules.

Results plot 2014-2015

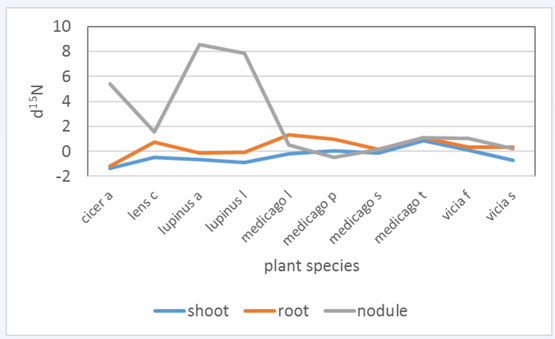


On the field site, with no limitation of water, the surprise was that same Mediterranean species, like *Anthyllis montana, Astragalus monspess* and *Coronilla minina*, present highly positive $\delta^{15}N$ values (from +5.54 to +9.24 ‰).

This result was find for both 2014 and 2015. These plants also displayed the higher C/N values (>15).

Conclusion

There is very few investigations about the use of nitrogen isotopes for the study of grass land plants.
M. Unkovich (New Phytologist 2013) has shown that isotope discrimination of $^{14}N/^{15}N$ provides new insight into biological nitrogen fixation.



In our case, the positive $\delta^{15}N$ values find for the Mediterranean species could indicate that these plants, in these growing conditions, are no more able to fix correctly the Air N_2. Further measurements will be made on roots and nodules

Reference:

- Fort, F., Cruz, P., Catrice, O., Delbrut, A. Luzarreta, M., Stroia, C. & Jouany, C. (2015): Root functional trait syndromes and plasticity drive the ability of grassand Fabaceae to tolerate water and phosphorus shortage. Env. Ecp. Botany, 110: 62-72.

Aknowlegements:
Legumip program
(2014-2016)

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