Can Shading Affect Nitrogen Fixation Of Forage Legume Swards? An Assessment Of B-Value Through The ^{15}N Natural Abundance Method

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Introduction

Tree-based intercropping systems are gaining pace as a land-use strategy to cope with climate change and provide environmental, economic, and social benefits (Kay et al., 2019). The integration of nitrogen-fixing crops between trees can be a solution to increase the land productivity and reduce the reliance on external inputs by increasing nitrogen (N) availability and then both tree and crop growth (Querné et al., 2017). Intercropping perennial legumes with trees can also reduce nitrogen losses, due to the higher amount of N accumulated in stable forms in the soil due to biological N_2-fixation and N root compartimentation (Hernandez-Esteban et al., 2019). On the other hand tree competition for light (Mantino et al., 2021), water (Nasielski et al., 2015) and nutrients (Isaac et al., 2014) eventually could limit legume growth and N_2-fixation (Querné et al., 2017). The isotopic method based on ^{15}N natural abundance is one of the most used methods to assess Biological Nitrogen Fixation (BNF) (Unkovich et al, 2008). The B-value, that is defined as the $\delta^{15}N$ value of a legume when completely dependent on N_2-fixation for satisfying its N demand, is of primary importance for BNF estimations (Nebiyu et al., 2014). The B-value may vary with species, plant age at harvest and growing conditions, e.g. light availability (Unkovich and Pate, 2000). Therefore, the B-value found in literature could not be representative for all legumes and environments, in particular for legumes grown intercropped with trees and thus subject to shading conditions. In this pot experiment we assessed the B-value of several forage legumes, as affected by different levels of simulated shading and grown in N-free medium.

Materials and Methods

A greenhouse pot experiment was established in March 2021 at the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa to determine the B-value for ^{15}N calculations about two forage legume species grown in a field trial located at the Center of Agri-Envirnomental Research "Enrico Avanzi" of the University ofPisa, San Piero a Grado (Pisa) (43°41'6.97"N 10°20'29.22"E), using the same shade treatments.The experimental layout complies with a two-factor randomized complete block (RCB) with three replicates (24 x 22 x 20 cm sizing each pot). The first factor (FORAGE SPECIES) includes three different swards: i) sulla (*Hedysarum coronarium* L.) cv. Silvan, (ii) alfalfa (*Medicago sativa* L.) cv. Messe (iii) ryegrass (*Lolium multiflorum* L.). cv. Teanna, as a non-legume control for natural levels of ^{15}N in the medium. The second factor (SHADE) had three increasing shading levels: s0) the control, representing full light availability, s25) and s50), corresponding to a reduction of potential light availability of 25 and 50% respectively. Pots were filled with river sand, with the aim to avoid interactions between the substrate and N_2-fixation, and before the sowing, sulla and alfalfa seeds were inoculated with their own rhizobacteria. According to Varella et al. (2011), shading was simulated by woody slats, with the same frame of the field trial (0.10 m wide, with a distance between each slat of 0.10 m for s50 and 0.20 m for s25). Slats were placed at 80 cm above bench level after cropsowing. To simulate field conditions, the PAR (Photosynthetic Active Radiation) was supplied using a proper LED-lamps system (https://www.c-led.it) during the day. Sulla and ryegrass failed because they were sown too late, thus their sowing was replicated in November 2021. To assess the level of N_2-fixation of legume crops under the different levels of shade, aboveground biomass was manually mowed four times (from July to October) during the growing season 2021 for alfalfa, and three times (from February to May) during the growing season 2022 for sulla and ryegrass. At the end of the season also the digged roots were collected. The biomass was oven-dried at 60°C, milled and inserted in tin capsules will be analyzed with an elemental CHN analyzer and mass-spectrometer to estimate ^{15}N natural abundance ($\delta\,^{15}N$) (Peoples et al., 2015).

Results

The preliminary results of alfalfa $\delta\,^{15}N$ showed not significant shading effect at each mowing time to shoots (tab. 1) and roots, respectively . Only at the second mowing, s25 resulted in significantly higher $\delta\,^{15}N$ than s50 and s0 respectively (- 0.585 vs -1.161 and -1.182 $\delta\,^{15}N$), possibly meaning reduced N_2-fixation but it wasn't possible to

explain this difference with the shading treatment. Overall, for alfalfa grown in controlled environment, nitrogen fixation wasn't affected by shading.

Table 1. Mean value ± standard error (SE) of alfalfa shoot $\delta^{15}N$ at each mowing time during the season 2020/21.

	mowing			
treatment	**1**	**2**	**3**	**4**
s0	-0.52 ± 0.06 a	-1.18 ± 0.17 b	-1.09 ± 0.14 a	-0.71 ± 0.03 a
s25	-0.71 ± 0.14 a	-0.59 ± 0.11 a	-1.02 ± 0.09 a	-0.82 ± 0.06 a
s50	-0.54 ± 0.10 a	-1.16 ± 0.35 b	-1.28 ± 0.15 a	-0.97 ± 0.07 a

Different lowercase letters indicate significantly different $\delta^{15}N$ shoot values for the Tukey HSD test ($P<0.05$).

Conclusions

The greenhouse pot trial in controlled conditions allowed to get on-station data to use with BNF calculations. Using of the same shading treatment applied in the field it was needed to obtain thorough B-values according to the parallel field experiment conditions. For alfalfa, grown in N-free medium and under controlled conditions, shading did not affect δ 15N, thus, B-value data collected on this pot trial, could be useful to assess BNF for legume swards cropped in Tuscany costal plain, also under tree-based intercropping systems.

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