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RESEARCH ARTICLE

Underestimated role of legume roots for soil N fertility

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Abstract Nitrogen (N) is a major fertilizing element for plants. The distribution of N in legumes is influencing the efficiency of the next crop. Nitrogen storage in legumes is actually estimated by N fixation in shoots, whereas there is little knowledge on the contribution of roots and nodules to legume N and soil N. Here, we studied the contribution of roots and nodules of grain and pasture legumes to plant N and soil N in Mediterranean fields. Experiments were run under rainfed conditions for a 2-year period in three regions of Portugal. Entire plants including top plant and visible roots and nodules were sampled at the end of the growing seasons for grain legumes, sweet and yellow lupine, and over two harvests in case of pastures. N_2 fixation was measured for grain legumes and pasture legumes using ^{15}N tracing. Our results show that aboveground N concentration did not vary among legumes, but differed in the belowground tissues. Field studies show that 7–11 % of total legume N was associated with roots and nodules. Data also show an allocation of 11–14 kg N fixed t^{-1} belowground dry matter in indeterminate legumes, which represents half the amount of total aboveground plant. This finding demonstrates that investigation relying only on shoot N underestimates the role of legumes for soil N fertility.

Keywords Aboveground material · Mixed permanent pasture · ^{15}N techniques · Pasture type · Sweet lupine · Visible root and nodule · Yellow lupine

1 Introduction

The distribution of nitrogen (N) in shoots, pods and seeds, and belowground tissues of grain, pasture, forage legumes has important implications for the N harvest index and fertilizer N value of legume residue for the subsequent crop in a rotation or for the neighboring non-legume in intercropping systems. Plant N from symbiotic fixation of atmospheric N_2 is added to the ecosystem mainly via the decomposition of legume parts or via urine and feces from grazing animals in pastures. In addition, rhizodeposition, i.e., exudates and products of root and nodule necrosis, may also add N to the soil (Fustec et al. 2010; Herridge et al. 2008; Huss-Danell et al. 2007; Høgh-Jensen and Schjoerring 2000; Walley et al. 2007). Thus, N_2-fixing plants can make important contributions to increase the sustainability of agricultural ecosystems by reducing the use of energetically costly mineral N fertilizers.

Nevertheless, most estimates of symbiotic N_2 fixation rely on shoot N only (Carranca et al. 1999a, b; Herridge et al. 2008; Muhammad et al. 2006; Peoples et al. 2001). Limited knowledge on the contribution of belowground tissues to the N harvest index and fertilizer N value is partly due to difficulties in undertaking quantitative studies on belowground inputs. Estimates of N balance based on standard root harvesting data, i.e., visible roots and nodules, may be confounded by neglecting the decayed roots, detached nodules, and rhizodeposits (Fustec

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et al. 2010). However, the contribution of root N and rhizodeposited N to the soil N pool is difficult to measure, particularly in the field, and most studies for this purpose have been conducted in soil cores or pots. Most methods of quantifying belowground biomass require a considerable labor and time investment. Recently, Walley et al. (2007) assumed that root and rhizodeposited N was respectively 14 and 10 % of total plant N when calculating N_2 fixation by pulse legumes in the Northern Great Plains of North America.

In this context, a study was developed under rainfed Mediterranean conditions for a 2-year period to provide an actual quantitative estimate of the contribution of harvested roots and nodules of grain and pasture legumes (Fig. 1) for total plant N and soil N benefit. It was hypothesized that belowground of legumes may have a significant contribution for the total N_2 fixation. The study may contribute for the awareness regarding legume role for a consistent sustainability of Mediterranean ecosystems.

2 Material and methods

2.1 Site description

Three experiments, A, B, and C, were conducted in three regions of Portugal: (A) south west (Pegões, 38° 24′ N, 8° 35′ W), (B) central west (Óbidos, 39° 20′ N, 9° 13′ W), and (C) south inland (Estremoz, 38° 51′42.66 N, 7° 32′32.83″ W and Vaiamonte, 39° 07′–39° 08′ N, 7° 29′–7° 30′ W).

All experimental sites were generally characterized by a Mediterranean-type climate with mild autumns and winters and hot and dry summers. Average minimum and maximum air temperatures did not differ significantly during both growth periods at location A in 2001/2002 and 2003/2004 and ranged from a minimum of 6 °C in December and January to a maximum of 23–26 °C in April, May, and October (Carranca et al. 2013). Monthly rainfall in 2001/2002 varied from a maximum value of 126 mm in January 2002 to the lowest amount of 20 mm in February 2002, and in 2003/2004, it ranged from 179 mm in October 2003 to 29 mm in April 2004; for the 30-year period, the mean annual rainfall in the region was 708 mm and the mean annual temperature was 15.5 °C. Taking into account data from a meteorological station which existed in the neighborhood at site B (Caldas da Rainha), the mean annual air temperature in 2004/2005 and 2005/2006 was 15 °C (Carranca et al. 2013) and ranged from a monthly average of 10.4 °C in January to 19.8 °C in August. The 30-year mean annual rainfall was about 610 mm, but less than 10 % occurred between May and September. In 2010/2011 and 2012/2013 at site C, the mean monthly temperature did not differ significantly between Estremoz and Vaiamonte and ranged from 10 to 17 °C during the growth period. Mean annual rainfall in Vaiamonte was about 700 mm whereas in Estremoz was about 670 mm.

Fig. 1 View of Portuguese experimental sites with sweet lupine at Pegões (**a**), yellow lupine at Óbidos (**b**), and improved pasture at Vaiamonte (**c**)

In location A, soils are sandy and correspond to Haplic Podzols (IUSS 2006); they are moderately acidic, i.e., $pH_{(H2O)}$=5.5–6.0, and show total organic C and N contents of 7 and 0.6 g kg^{-1}, respectively at 0–20 cm depth. In location B, soils are Haplic Podzols associated with Haplic Arenosols (IUSS 2006) and show neutral reaction, i.e., $pH_{(H2O)}$=6.9, and contents of total organic C and N of 3 and 0.2 g kg^{-1}, respectively at 0–20-cm depth. In location C, soils correspond mostly to Dystric Cambisols and Eutric Luvisols overlying granitic bedrock (IUSS 2006), respectively at Vaiamonte and Estremoz, and show a sandy loam texture at 0–20-cm depth; they are moderately acidic, with $pH_{(H2O)}$ of 5.5–6.0, and the contents of organic C and N are 15 and 1.3 g kg^{-1}, respectively, and do not differ in both soils.

2.2 Layout of the experiments

In 2001/2002 and 2003/2004, at location A, a 2-year experiment was run in a completely randomized block design with eight replicates. The sweet lupine (*Lupinus albus* L.) was sown at a rate of 60 seeds m^{-2} in plots of 5 m^2 each. Root inoculation was by native *Rhizobium* sp.

In 2004/2005 and 2005/2006, at location B, a 2-year experiment was also run in a completely randomized block design with eight replicates. The yellow lupine (*Lupinus luteus* L.) was sown at a seeding rate of 12 g seeds m^{-2} in plots of 5 m^2 each. Similarly to experiment A, in this experiment, root infection was also by natural N_2-fixing bacteria in soil.

At location C, permanent mixed swards were chosen in four agro-forestry systems located at south inland Portugal: at Estremoz, two improved pastures older than 5 and 12 years and one natural pasture older than 25 years and, at Vaiamonte, one introduced pasture older than 30 years. In 2010/2011 and 2012/2013, a 2-year experiment was run under completely randomized blocks with three replicates. In each pasture, plots were randomly distributed outside the cork oak tree (*Quercus suber* L.) canopy influence. These 1.2-m^2 plots were moved to adjacent sites after each plant harvest. Subclover (*Trifolium subterraneum* L.) was the dominant legume in all pastures and represented more than 90 % of legumes. During the experimental period, root nodulation in all pasture legumes was by native soil fixing bacteria. The experimental plots were protected with fences and cages to restrict the access of cows, sheep, and pigs for grazing the herbage during the study period.

Oat (*Avena sativa* L.) was sown at a rate of 12 g seeds m^{-2} as a reference crop for both grain legumes. Ammonium nitrate fertilizer doubly enriched with 5 atoms% ^{15}N was applied annually in the liquid form to all plots, 1 week after plants emergence and at rates of 10 and 20 kg N ha^{-1}, respectively for both sweet and yellow lupines and for oat by using the *A*-value technique. In pastures of experiment C, the associated non-legumes, mostly *Phalaris*, *Lolium*, *Plantago*, and composite plants were used as reference crops. ^{15}N fertilizer was applied to each plot using the ^{15}N dilution technique (Carranca et al. 1999a, b) in the same form and enrichment as reported for experiments A and B, and the rate of 3 kg N ha^{-1} applied 1 week after plant emergence and repeated in new plots after the winter cut, after discarding the aerial biomass produced during autumn-winter. New plots were arranged in adjacent areas in 2012/2013, the second year of experiment.

Phosphorus (P) and potassium (K) were annually applied as basal dressing at a rate of 60 kg ha^{-1} for grain legumes. For pastures, P was only incorporated into the soil in 2010 at basal dressing at a rate of 40 kg ha^{-1}, whereas K was annually applied as topdressing using a rate of 50 kg ha^{-1}.

2.3 Plant sampling and analysis

In experiments A and B, complete plant legume and oat, i.e., above- and belowground material, were harvested from ^{15}N-labeled areas at physiological maturity, to establish the symbiotic N_2 fixation capacity by legumes. In experiment C, complete pasture plants were excavated from each plot in winter, at the end of February, and in spring, at the end of April, recovering as much visible roots and nodules as possible, either attached to the plants or spread in the soil surrounding the roots. We emphasize that rhizodeposits were not taken into account.

In the laboratory, pasture legumes were separated from non-legumes. In all experiments, plant material was divided into aboveground material and visible roots, plus nodules in case of legumes. Plant samples were washed with deionized water with care to avoid losing plant material and were dried at about 75 °C for 24 h to determine the dry matter yield (g DM m^{-2}) and ground lower than 0.5 mm, avoiding any cross contamination, and analyzed to evaluate the N concentration (g N kg^{-1} DM) and %^{15}N enrichment, respectively by dry combustion (LECO) and automatic N analyzer-mass spectrometer at the Laboratorio de Isotopos Estables, at Universidad Autonoma, in Madrid, Spain. Plant N yield (g N m^{-2}) was calculated as the product of DM yield and N concentration. Percent N derived from the atmosphere (%Nda) by legumes was estimated using the reference value of 0.3663 % for air natural ^{15}N abundance, and the non-legumes were used as reference crops (Carranca 1996; Carranca et al. 2013). The corresponding plant tissue in the non-legumes was used as reference. Fixed N_2 (g N m^{-2}) was calculated as the product of %Nda and N yield (g N m^{-2}).

2.4 Evaluation of symbiotic N_2 fixation

The *A*-value technique (Carranca 1996; IAEA 1983) was used to evaluate the symbiotic N_2 fixation in A and B experiments. The *A*-value is based on the concept similar to that for ^{15}N isotopic dilution that is when a plant is confronted with two or more different sources of a given nutrient, it will absorb from each source an amount which is in direct proportion to the respective amount available for the crop. This approach is applied when fixing and non-fixing crops receive different amounts of fertilizer N. This is the case of poor soils, when a higher amount of N is added to the non-fixing crop to ensure its adequate growth. Using the *A*-value, %Nda was estimated in each plant tissue according to the expression (Carranca 1996; IAEA 1983):

$$\text{Nda}(\%) = A_{(\text{fix})} \times \%\text{Ndf}_{(\text{fix. leg.})}/A_{\text{fertilizer(fix. leg.)}} \tag{1}$$

where,

fix. = fixed
fix. leg. = fixing legume

$$A_{(\text{fix})} = A_{\text{soil+fix.}} - A_{\text{soil(non–fix. leg.)}} \tag{2}$$

where,

$$A_{\text{soil+fix.}} = \left(100–\%\text{Ndf}_{(\text{fix. leg.})}\right)/\left(\%\text{Ndf} \times \text{amount of fertilizer N}_{(\text{fix leg})}\left(\text{kg ha}^{-1}\right)\right) \tag{3}$$

$$\text{Ndf}\ (\%) = \text{N derived from fertilizer}\ (\%) = \left(\%\text{atom}^{15}\text{N exc.}_{(\text{plant})}/\%\text{atom}^{15}\text{N exc.}_{(\text{fertilizer})}\right) \times 100 \tag{4}$$

$A_{\text{fertilizer (fix. leg.)}}$=amount of labeled fertilizer applied to fixing legume (kg N ha^{-1})

$$\text{N}_2\text{fixed}\left(\text{kg N ha}^{-1}\right) = (\%\text{N in legume}/100) \times \left(A_{\text{soil(fix. leg.)}} - A_{\text{soil(non-fix. leg.)}}\right) \tag{5}$$

Using the ^{15}N dilution technique, %Nda was estimated in each plant tissue according to the expression (Carranca et al. 1999b):

$$\text{Nda}(\%) = \left[1–\left(\%\ \text{at.}\ ^{15}\text{N exc}_{(\text{fix. leg.})}/\%\ \text{at.}\ ^{15}\text{N exc.}_{(\text{non-fix. crop})}\right)\right] \times 100 \tag{6}$$

where,

at. exc. = atom excess
fix. leg. = fixing legume
non-fix. crop = non-fixing crop

2.5 Statistical analysis

In each experiment, results were analyzed by ANOVA (Statistics 6.0) to evaluate effects of factors such as years of study, plant tissue, pasture type and cuts on legumes biomass, N concentration, legume N, Nda, and N_2 fixation. Mean separation was performed for significant differences with plant organs using the Bonferroni's test.

3 Results and discussion

Improved N management is needed to optimize economic returns to farmers and minimize environmental concerns associated with the N use. Symbiotically N_2 fixed is of particular significance in sustainable agriculture as it allows reducing the use of chemical N in the production of field crops. It contributes to productivity both directly, where the fixed N_2 is harvested in grain or pasture/forage aboveground material, or indirectly by contributing to the maintenance or enhancement of soil fertility in the agricultural system. The majority of values for legume N_2 fixation reported in the literature are based on shoots, because root harvesting under the field condition is extremely difficult and time consuming.

The hypothesis of the present study was confirmed, as it was observed that the belowground tissues, i.e., roots and nodules, of both grain and pasture legumes showed a significant contribution for the total N_2 fixed in the plant and for soil N benefit.

In experiments A and B, harvested roots and nodules biomass of sweet and yellow lupines cultivated in the sandy soils represented 15–16 % of the aerial plant dry weight (356 and 288 g DM m^{-2}, respectively for sweet and yellow lupines) (Table 2). In experiment C, recovered belowground tissues of pasture legumes represented 21 % of aboveground plant biomass, i.e., 173 g DM m^{-2}, a greater percentage than for grain legumes (Table 1). Pasture legumes exhibited abundant fine roots with nodules, whereas the tested grain legumes had taproots where nodules were attached, but most of the adventitious lateral roots were not nodulated.

The N concentration in plant tops in experiments A, B, and C was quite similar and averaged 24 g N kg^{-1} DM, but in visible roots and nodules, the total N varied from 12.2, 25.8, and 19.5 g N kg^{-1} DM, respectively in the sweet lupine, yellow lupine, and subclover in the mixed stands (Table 1). In case of pasture legumes, the N concentration in harvested roots and nodules was 19.5 g N kg^{-1} DM, quite similar to 19.3 g N kg^{-1} DM determined by Huss-Danell et al. (2007) for red clover (*Trifolium pratense* L.) cultivated in Sweden.

Fixation rate was above 90 % Nda in both grain legumes and did not differ significantly between above- and belowground plant tissues in both cases (Table 1). Under the stress environmental conditions, i.e., sub-humid dry climate and

Table 1 N concentration in belowground tissue varied among legume species but did not differ in the aboveground material. Means of above- and belowground biomass (g DM m^{-2}), N concentration (g N kg^{-1} DM), legume N (g N m^{-2}), N derived from atmosphere (%Nda), and N_2 fixed (g N m^{-2}) in experiments A, B, and C assuming the mean effects of factors (years of study, pasture type, and cuts) and for the significant interactions of these factors with plant tissues in case of pasture legumes

Source of variation		Legume biomass (g DM m^{-2})	N concentration (g N kg^{-1} DM)	Legume N (g N m^{-2})	Nda (%)	N_2 fixed (g N m^{-2})
Sweet lupine						
Aboveground	356a	26.2a	8.9a	94a	8.9a	
Belowground	58b	12.2b	0.7b	92a	0.7b	
Yellow lupine						
Aboveground	288a	22.2a	6.4a	99a	6.3a	
Belowground	42b	25.8a	0.6b	98a	0.6b	
Pasture legumes[a]						
Aboveground	173a	23.5a	2.2.7a	72a	3.5a	
Belowground	37b	19.5b	0.50b	51b	0.4b	
Significant interactions for pasture legumes						
Years of study[b] × plant tissue[c]						
1	1	116.5a	23.8a	2.8a	77.4a	2.1a
1	2	25.4c	21.0b	0.5c	50.0b	0.2c
2	1	56.8b	22.9ab	1.6b	68.4a	1.4b
2	2	11.7c	18.0b	0.3c	53.3b	0.2c
Pasture type[d] × plant tissue						
1	1	52.9bc	27.5a	1.4bc	81.4ab	1.1b
1	2	16.4c	21.0c	0.3 cd	62.5abc	0.2c
2	1	49.1bc	16.8d	1.0bcd	60.3bc	0.8bc
2	2	9.3c	14.2d	0.2d	46.6c	0.1c
3	1	179.7a	28.6a	4.8a	86.2a	4.2a
3	2	25.7bc	25.1ab	0.6bcd	45.0b	0.3bc
4	1	65.0b	22.8bc	1.4b	63.8abc	1.0b
4	2	23.0bc	20.1 cd	0.4bcd	50.4c	0.2c
Cut[e] × plant tissue						
1	1	65.5b	26.3a	1.9a	70.5ab	1.6a
1	2	24.5c	20.7b	0.6b	45.4c	0.3b
2	1	107.8a	20.4bc	2.4a	75.3a	1.9a
2	2	12.7c	18.3c	0.2b	56.8bc	0.1b

For each legume species, means with different letters in each column were significantly different ($p<0.05$), according to Bonferroni's test

[a] Annual production (cut 1+cut 2)

[b] 1=2010/2011, 2=2012/2013

[c] 1=aboveground, 2=belowground

[d] 1=natural, older than 25-years; 2=sown, older than 5 years; 3=sown, older than 12 years, 4=sown, older than 30 years

[e] 1=first cut in winter, 2=second cut in spring

poor soils, the symbiotic N_2 fixation capacity of both grain legumes with native fixing bacteria was very high and fully replenished plants N requirements. Visible roots and nodules contributed to 7 and 9 % of total plant fixed N_2, respectively, by sweet and yellow lupines (Table 1, Fig. 2).

Pasture legumes, mostly subclover, presented a lower fixation rate than grain legumes (Table 1). However, the rate of fixation was moderate to high in all pastures and similar to that reported in earlier studies (Carranca et al. 1999b). These results indicate that indigenous rhizobial population in all sites was efficient in the symbiosis with the pasture legumes, even in the case of natural pasture at Estremoz (72 % Nda). Belowground tissues in pasture legumes fixed 51 % Nda, which represented 71 % of nitrogen derived from atmosphere in the aerial plant material (72 % Nda), and accounted for 11 % of total N_2 fixed in the plant (4.0 g N m^{-2}).

ANOVA was applied to data obtained in the three experiments (A, B, C), and results are included in Table 2. Although

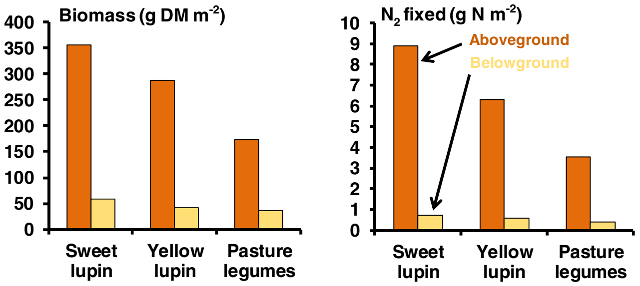

Fig. 2 The contribution of N_2 fixed by belowground tissues of grain and pasture legumes is relevant. Biomass (g DM m^{-2}) and N_2 fixed (g N m^{-2}) in above- and belowground tissues of lupines and pasture legumes

factors, i.e., years of study and plant tissue in case of experiments A and B and years of study, pasture type, cut and plant tissue in case of experiment C, significantly affected most legume characteristics, in general, interactions with plant tissues were not significant ($p \geq 0.05$), except for pasture legumes (Table 2). However, interactions in pasture legumes showed that only the aboveground material was significantly affected by years of study and pasture type (Table 1). The improved pasture older than 12 years at Estremoz, i.e., pasture three in Table 1, fixed 8.3 g N m^{-2} in the top plant, about four times higher than the amount of N_2 fixed by other pasture legumes, which did not differ from each other. That value was greater than the amount of N_2 fixed by belowground tissues in all pastures, i.e., 0.4 g N m^{-2} (Table 1). A significant effect ($p < 0.05$) on fixed N_2 was also observed for the interaction between cuts and plant tissues in pasture legumes (Table 2), where the average top plant material fixed a higher amount of N_2 during the spring, i.e., 1.9 g N m^{-2} at cut 2, but not significantly different from 1.6 g N m^{-2} at cut 1 at the end of winter (Table 1). The lowest value was found in the belowground tissues for both cuts, which did not vary significantly from each other and corresponded to 0.2 g N m^{-2}. Years of study did not affect the belowground characteristics (Table 1).

The impact of belowground N contribution on estimated N_2 fixed by tested grain and pasture legumes ranged from 7 to 11 %. Estimates for both lupine species (7–9 %) were smaller than the values (13–14 %) quantified by Unkovich et al. (1997) and Walley et al. (2007) for lupine roots under the field condition. However, they were in the range of values (9–12 %) reported by Fillery and McNeill (2001) for *Lupinus angustifolius* L. The value determined in the present pasture legume (11 %) is also within the last range, but is smaller than the estimate of 12.6 % reported by Huss-Danell et al. (2007) for the red clover in a mixed stand under rainy conditions and even smaller than the range of values (17–24 %) reported by McNeill et al. (1997) for subclover grown in intact cores of soil-plant systems. Present data were determined under sub-humid dry climate in rainfed conditions whereas most data in the literature were obtained under soil moist and controlled air temperature or under higher rainfall and lower temperature. High air temperature increases root and nodule decomposition

Table 2 N_2 fixation did not vary in the belowground tissue. ANOVA results for sweet and yellow lupines and pasture legumes biomass, Nda, N concentration, legume N, and N_2 fixed

Source of variation	Legume biomass	Nda	N concentration	Legume N	N_2 fixed
Sweet lupine					
Years of study (Y)	***	***	***	***	***
Plant tissue (T)	***	***	***	***	***
Significant interaction					
Y×T	*	ns	ns	ns	ns
Yellow lupine					
Years of study (Y)	***	ns	**	***	***
Plant tissue (T)	***	***	ns	***	ns
Interaction					
Y×T	ns	ns	ns	ns	ns
Pasture legumes					
Years of study (Y)	***	ns	**	***	**
Pasture age (P)	***	**	***	***	***
Cut (C)	*	***	***	***	ns
Plant tissue (T)	***	*	***	***	***
Significant interactions					
Y×P	*	**	ns	ns	**
Y×C	ns	ns	**	***	*
Y×T	**	ns	ns	***	**
P×C	**	***	***	ns	ns
P×T	***	*	ns	ns	***
C×T	***	ns	***	ns	*
Y×C×P	*	***	***	***	**
Y×C×T	ns	*	ns	ns	*
P×C×T	ns	*	**	ns	ns

ns non-significant ($p \geq 0.05$, probability level for *F*-values)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (probability level for *F*-values)

and mineralization rate in soil; then, the recovered material at harvest was probably lesser under the present field conditions than in controlled environments, since the rhizodeposition was not taken into account. We emphasize that our values are much lower than those 22–68 % reported in the studies of Walley et al. (2007), Fuhrer (2004), Fustec et al. (2010), Mathieu et al. (2007), McNeill and Fillery (2008), Peoples and Griffiths (2009), and Yasmin et al. (2006) which included the rhizodeposits that represented more than 7 %.

For all legume species, the content of plant N per unit area was highly dependent on legume biomass. This was particularly relevant in indeterminate legumes with indeterminate nodules as it is the case of tested grain and pasture legumes. On average, 20–25 kg of aboveground N was fixed for average ton of aboveground DM by tested grain and pasture legumes. These data confirmed the range of 20–25 kg N fixed t^{-1} aboveground DM proposed by Peoples et al. (2001) in Eastern Australia, for *L. angustifolius* and clover. Nevertheless, those values were higher than the range 5–16 kg N fixed t^{-1} DM estimated by Carranca (2013) for shoots of indeterminate pea, faba bean, chickpea, and subclover in similar semi-humid dry climate conditions. This shows the high N harvest indices of these legumes. Using the same reasoning, present data showed an allocation of 11–14 kg N fixed t^{-1} visible belowground DM, corresponding to about half the amount of top plant DM and denoting the relevant potential contribution of legumes belowground to the sustainability of agricultural systems. Total legume N input via N_2 fixation by grain and pasture legumes ranged 31–39 kg N fixed t^{-1} DM.

Present data proved that studies relying only on the shoot N greatly underestimate the role of legumes in maintaining the N fertility in soils. However, whereas the aboveground measurements give net cumulative seasonal values, root measurements rather give point-in-time values and do not account for the turnover of fine roots and nodules and the leakage of root cells content. Formowitz et al. (2009) and Urquiaga et al. (1998) supported that the positive effects of legume root residues in soil are small in a short term due to the slow mineralization rate, but this fact depends on the type of roots and climate. Mycorrhizal hyphae in thicker pasture roots is formed by chitin, a recalcitrant material which can account significantly for the stable organic matter pool, lasting for years in the soil (Norby and Jackson 2000), whereas non-mycorrhized roots as in grain legumes and fine roots in pasture legumes are fast-decomposing material. Depending on the climate characteristics, that fact may be positive in order to restrict the potential N losses out of the ecosystem. Caddish et al. (2002) reinforced this statement and referred that belowground N inputs may become protected from mineralization then playing a more important role in building of soil structure rather than in soil N supply by its high C content. Our results undoubtedly show that the contribution of grain and pasture legumes for the improvement of soil fertility and stability enhancement of Mediterranean ecosystems is in high proportion associated with the amount and functions of the respective belowground.

4 Conclusion

Present results highlight the valuable inputs of biomass and N of visible belowground material for the ecosystems. Under the present Mediterranean conditions, aboveground N did not vary among grain and permanent pasture legumes with different compositions and ages, but differed in the belowground organ. The contribution of visible roots and nodules to the accumulated N by the sweet and yellow lupines and subclover in mixed stands, either improved or natural pastures with different ages, represented 7–11 % of total N_2 fixed. Present data showed an allocation of 11–14 kg N fixed t^{-1} of belowground dry matter, i.e., the half amount of total aboveground plant material which reveals the relevant contribution of legume harvested roots and nodules to the sustainability of agricultural systems even though the rhizodeposits were not accounted for. This proves that N balance studies relying only on the shoot N greatly underestimate the role of legumes in maintaining the soil N fertility. Further long-term studies on the contribution of belowground tissues to the soil N fertility and soil structure are fully encouraged to confirm present data and statements.

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