

ROOT-INDUCED NITROGEN MINERALIZATION AND ITS AVAILABILITY TO PLANTS

SHAGUFTA GILL¹, MUHAMMAD ABID¹ FAROOQ-E-AZAM² AND NIAZ AHMED¹

¹Department of Soil Science, University College of Agriculture, B.Z. University, Multan, Pakistan

²Nuclear Institutes for Agriculture and Biology, P.O. Box 128, Jhang Road, Faisalabad, Pakistan

*Corresponding author e-mail: kharalabid@yahoo.com

Abstract

A greenhouse experiment was conducted to compare maize (*Zea mays* L.) and dhaincha (*Sesbania aculeata* L.) in terms of biomass accumulation (especially root proliferation) and efficiency of N uptake from potentially mineralizable pool with emphasis on root-induced N mineralization. Pool of potentially mineralizable N was developed by incubating the clay loam soil with sucrose and ¹⁵N-labelled ammonium sulphate with the objective to differentiate between the sources of plant N. After 6 weeks of incubation, 55% of the mineralizable N was derived from the added ammonium sulphate and 45% from the native soil N. Plants grown in ¹⁵N-labelled potted soil were harvested at 3, 4, and 5 weeks after sowing and studied for water content and partitioning of biomass, N and ¹⁵N into root and shoot portions. Accumulation of dry matter in root and shoot portions was 7 and 28 times higher, respectively, in maize as compared to *Sesbania*. Water content of the shoot portions was almost similar in both crops, while roots of *Sesbania* contained significantly higher water content. Higher root proliferation in maize led to significantly more accumulation of dry matter and N as compared to *Sesbania*. Efficiency of N utilization (biomass produced per unit N in plants) was also higher in maize as compared to *Sesbania*. However, roots of the latter were more efficient in extracting N from soil as determined by amount of N taken up per unit root mass. Since, roots of *Sesbania* did not show nodules, entire plant N was ascribed to native soil N or from the recently developed pool of potentially mineralizable N. The differences in the uptake of N by the two crop types were attributed to i) root proliferation and thus the access to available N and ii) root-induced changes in the N mineralization potential that were assumed to result from differences in quality and quantity of rhizodeposits.

Introduction

Agricultural crops meet more than 50% of their nitrogen (N) requirements from soil organic matter (SOM). This variation can partially be attributed to the root proliferation and the soil volume being explored for nutrient acquisition. Plant types with more extensive root system will take up more N from the soil matrix irrespective of the origin of N (Anil *et al.*, 1998; Hougaard-Nielsen *et al.*, 2001). It is known that mineralization of soil N has positive impact on the availability of carbonaceous materials (Azam *et al.*, 1993; Woods *et al.*, 1987). Root exudates (rhizodeposits) can enhance the rate of N mineralization through the so-called "priming" action (Jenkinson *et al.*, 1985). Increase in N mineralization depends on the type of plants grown and the quantity of rhizodeposits; 30-50% of the plant carbon (C) gathered during life cycle of plants being transported below-ground (Sanchez *et al.*, 2002). Hence, plants receive not only directly from the mineral N in soil but also indirectly by inducing N mineralization of rhizodeposits.

Differences in N acquisition between leguminous and non-leguminous crops can be significant in view of the intensity of root proliferation and the soil volume being explored; rooting system of cereals being more extensive (Anil *et al.*, 1998). However, differences in the quality and quantity of rhizodeposits and the resultant changes in microbial population responsible for mineralization-immobilization of N may vary with the type of plants grown (Sanchez *et al.*, 2002). Based on a long-term crop rotation experiment, Vanotti & Bundy (1995) speculated that growth enhancement of maize after soybean was due to the stimulation of soil N mineralizing microbes by soybean. Alfalfa was also reported to enhance soil N mineralization (Radke *et al.*, 1988). In the light of above

discussion, the objectives of present studies were to compare a leguminous *Sesbania aculeata* (dhaincha) and a non-leguminous *Zea mays* (maize) crops in terms of Biomass accumulation especially root proliferation during short-term growth in the pots; efficiency of N uptake from potentially mineralizable pool and efficiency of N acquisition by roots. Potentially mineralizable soil N pool was labeled with ¹⁵N to facilitate differentiation between sources of plant N.

Materials and Methods

Experimental site: Soil used in this study was collected from the top 0-15 cm of an experimental field (under wheat-rice rotation with no leguminous crop in between) at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Air-dried and sieved (< 2 mm) clay loam soil had the following characteristics: pH (1:1, soil:water suspension), 7.6; electrical conductivity (EC), 0.78 dS m⁻¹; organic carbon, 0.58%; total nitrogen, 0.065%; NH₄⁺-N, 3.1 mg kg⁻¹ soil; NO₃⁻-N, 8.9 mg kg⁻¹ soil; sand, 30%; silt, 31%; clay, 39%; water-holding capacity, 30%.

¹⁵N enrichment of soil: A labeled pool of potentially mineralizable N was created in soil to facilitate the determination of root-induced mineralization and plant uptake of N. Sixty kilogram of air-dried and sieved (< 2 mm) soil was placed in six plastic tubs (10 kg tub⁻¹) and moistened to 15% (w/w) with a solution of sucrose and ¹⁵N-labelled ammonium sulphate (4.123 atom % ¹⁵N). Concentration of both ingredients in the solution was adjusted such that 1500 mg C and 50 mg N were added kg⁻¹ soil (C/N ratio of the added material being 30). The treated soil was placed at 25°C and the water content was

maintained at field capacity by regularly weighing and making up the weight loss with distilled water. At weekly intervals during five weeks of incubation, the soil samples were given 0.1% sucrose in aqueous solution to maintain mineralization-immobilization cycling of N. During the 5th week, aliquots of soil were analyzed daily for the determination of mineral N. Only traces of mineral N were recorded. The soil was air-dried for 30 h and sub-samples were analyzed for i) total N, ii) potentially mineralizable, and iii) isotopic composition of both the N pools. The bulk of soil was used in the plant growth experiment.

Plant experiment: Three kg portions of the air-dried and sieved (< 2 mm) soil were placed in 18 plastic pots (12 cm diameter and 25 cm depth). Five seeds of each crop type dhaincha/jamtar (*S. aculeata* L.) and maize (*Zea mays* L.) were sown at a depth of 15 mm in the dry soil of 9 pots. Solution of potassium dihydrogen phosphate was added to bring the soil moisture content to 15% (w/w) and P addition level of 25 mg kg⁻¹ soil. The pots were placed in a greenhouse and the plant stand thinned out to 3 after 5 days of sowing. During 5 weeks of the experiment, temperature in the greenhouse varied from 33 to 42°C and relative humidity from 30 to 40%; photosynthetically active radiation (PAR) as measured by CI-340 (CID, USA) was 850 µmol cm⁻¹ sec⁻¹. Triplicate pots were taken at 3, 4, and 5 weeks after sowing. Plants were removed from the pots together with soil and the latter was washed away using a stream of water. Plants were immediately blotted, root and shoot portions separated and subjected to the measurement of fresh and dry weight (at 65°C in a hot air oven). Water content was calculated as percent of the dry matter in root or shoot portions.

Laboratory analysis: For pH, EC_e, textural components, and water-holding capacity; the methods outlined by U.S. Salinity Lab. Staff (1954) were followed. Organic C was

determined using a modified wet oxidation method (Azam & Sajjad, 2005). Total N and mineral N were determined using methods described by Bremner (1996) and Keeney & Nelson (1982), respectively. Potentially mineralizable N (NH₄-N released during 2-weeks of incubation under anaerobic conditions) was determined as described by Stanford (1982). For the determination of isotopic composition of N, samples resulting from steam distillation were analyzed on a mass spectrometer as described by Buresh *et al.*, (1982) and Mulvaney *et al.*, (1997).

Statistical analysis: Analysis of variance (ANOVA) technique was used and standard deviation of means and coefficient of correlations were calculated (Steel & Torrie, 1980) using Microsoft Excel software. Standard deviation of means was used to test the significance of differences between treatment means using the SAS statistical package (Anon., 1998).

Results

Growth attributes and nitrogen uptake: Data in Table 1 presents total and mineralizable N as well as the ¹⁵N abundance of these pools in soil incubated for 6 weeks with sucrose and ¹⁵N-labelled ammonium sulphate. Of the initially added AS-N, 92.5% was recovered in the soil after 6 weeks of incubation indicating a loss of 7.5%. A greater proportion (79.6%) of the AS-N added to the soil was transformed into potentially mineralizable forms, i.e., 86.2% of that remaining determined in soil, while 13.8% was assumed to be present in relatively recalcitrant forms. Mineralizable N had 2.077 % more ¹⁵N as compared to 0.252 % in the total soil N. During incubation of soil with ammonium sulphate and sucrose, substantial quantities of soil N were also transformed into potentially mineralizable forms; the contribution of soil-N and AS-N being 45 and 55%, respectively.

Table 1. Origin and amounts of total and mineralizable N derived from soil and applied ammonium sulphate.

Type of N	mg kg ⁻¹	¹⁵ N-ex	%Ndfam	%Ndfs	mgNdfam	mgNdfs	% of AS-N
Total	689.1	0.252	6.7	93.3	46.2	642.9	92.47
Mineralizable	72.0	2.077	55.3	44.7	39.8	32.2	79.64

Ndfam: N derived from ammonium sulphate (AS); Ndfs: N derived from soil

Accumulation of dry matter in root and shoot portions was 7 and 28 times higher, respectively, in maize as compared to *Sesbania* (Fig. 1a). The differences in dry matter accumulation narrowed with time suggesting a decrease in the growth rate of maize relative to that of *Sesbania* and/or inherent differences in the growth pattern of the two plant types. As a result, maize plants showed a significantly wider root/shoot ratio; the average for 3 harvests being 0.97 and 0.23 in maize and *Sesbania*, respectively (data not shown). Maize showed an increase in root/shoot ratio from 0.80 at H1 to 1.15 at H3, while no significant change was observed in *Sesbania* where the ratio was 0.23 and 0.22 at the two harvests, respectively. However, the gain in dry matter between harvests followed a similar pattern in both crops with significantly

higher values for maize than in *Sesbania* (Fig. 1b). Roots of *Sesbania* showed negligible nodulation probably due to lack of rhizobia in the soil or due to higher amounts of mineral/mineralizable N in soil.

Trends in N content of the root and shoot portions of maize and *Sesbania* (Fig. 2a) were similar to those observed for dry matter yield as the two parameters showed a significant positive correlation ($r = 0.98$, $p=0.05$, $n = 18$). Maize plants accumulated about 8 times more N compared to *Sesbania* due to more extensive root system that explored a greater soil volume for nutrient acquisition. Uptake of N in maize was significantly reduced between H2 and H3 as compared to that between H1 and H2 (Fig. 2b); a reverse was true for *Sesbania* that showed a consistent increase in N

uptake over the entire growth period. In both the crops, a higher proportion of N was partitioned to shoot than for root but the difference was more pronounced in *Sesbania* where shoot portion contained several times higher N as compared to that in roots. Percent N content of the root and shoot portions was significantly lower for maize than in *Sesbania* (average of 3 harvests was 2.0 and 3.4% for shoot and 1.6 and 2.8% for root,

respectively) but decreased in both the cases with the advancement of growth from H1 and to H3 (data not shown). The efficiency of N utilization (expressed as the ratio of biomass produced to N taken up) was at the 3 harvests was >40% better in maize than *Sesbania* (Fig. 3a). It was several times higher between H2 and H3 as compared to that between H1 and H2 in maize but showed only small increase in *Sesbania* (Fig. 3b).

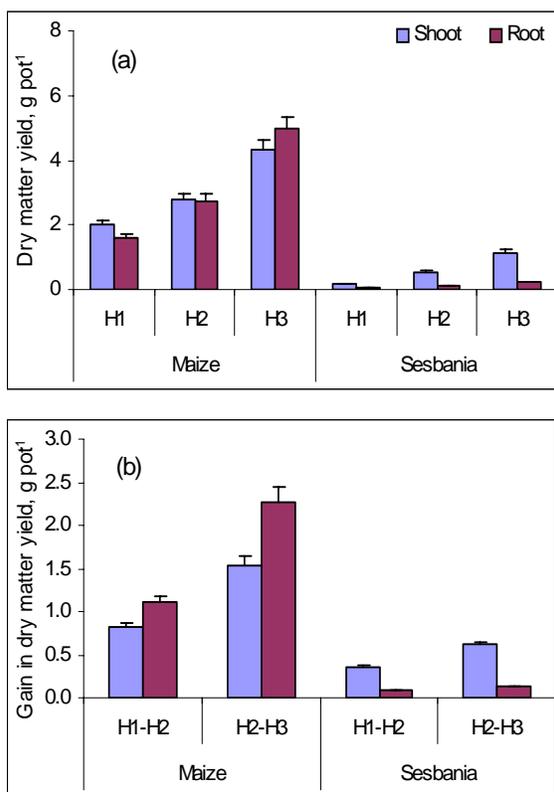


Fig. 1. Dry matter of maize and *Sesbania* and its distribution in shoot and root portions at and between harvests (H1, H2 and H3). The bars show \pm SD.

Uptake of N from newly applied ¹⁵N-labelled ammonium sulphate (that was immobilized in the presence of sucrose) by the two crop types and its distribution in root and shoot portions (Fig. 4a) showed a pattern similar to that observed for the total N (Fig. 2a); the two parameters showed a significant correlation ($r = 0.99$, $n = 18$, $p=0.05$). Similarly, significant correlations ($r = 0.99$, $n = 18$, $p=0.05$) were also obtained between total N content of the root and shoot portions of the two crops and N derived from soil (Fig. 4b). Close correlation ($r = 0.97$, $n = 18$, $p=0.05$) was also found between N derived from soil and ammonium sulphate (Fig. 4a and 4b). However, due to poor root proliferation of *Sesbania* compared to maize (Fig. 1a), it received significantly lower percentage of N originating from ammonium sulphate, i.e., 37.6 and 8.1% in the two crop types, respectively (the values are average of 3 harvests). Since no nodules were formed on *Sesbania* roots as mentioned earlier, the entire N in *Sesbania* as

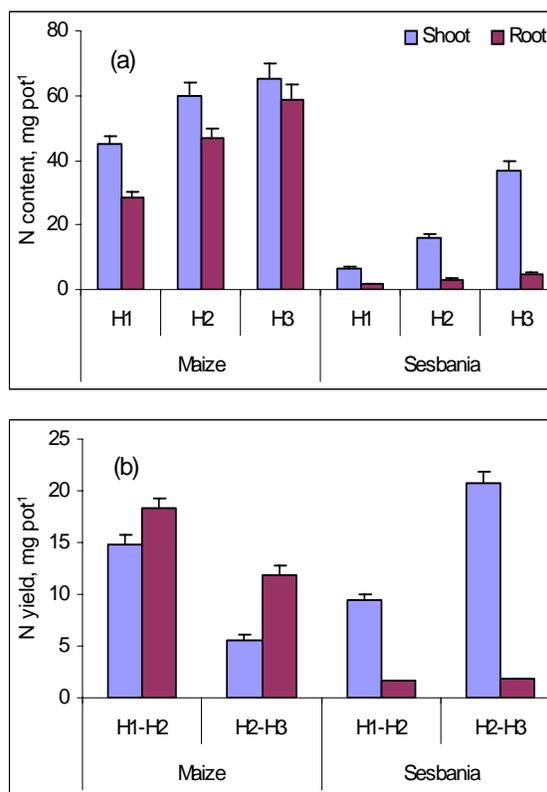


Fig. 2. Nitrogen content/yield of maize and *Sesbania* and its distribution in shoot and root portions at and between harvests (H1, H2 and H3). The bars show \pm SD.

well as maize was assumed to come from the soil. Hence any isotopic dilution observed was attributable to the mineralization and uptake of native soil N. The maize plants showed a significantly higher isotopic dilution at H3. On an average (all harvests), however, the two plant types had essentially similar ¹⁵N abundance.

In spite of the fact that *Sesbania* had a lower root biomass as compared to maize, efficiency of N uptake per unit root mass was much higher in the former, the average of 3 harvests 178 and 37, respectively (Fig. 5). The root efficiency of N uptake decreased with time in both the cases, i.e. from 46 at H1 to 25 at H3 in maize and from 206 to 167 in the case of *Sesbania*. Nitrogen derived from the applied ammonium sulphate appeared to be more easily available than that derived from soil as the average values of efficiency for the 3 harvests were 19 and 17 for the two sources, respectively in maize and 97 and 81 in *Sesbania*.

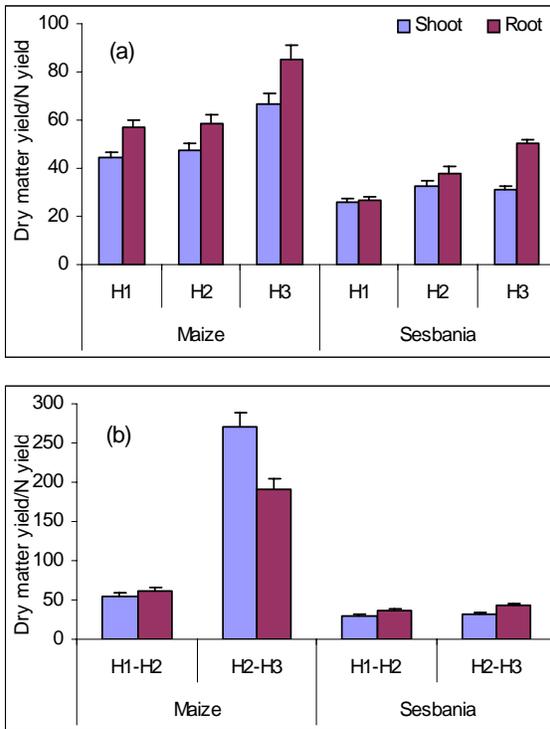


Fig. 3. Efficiency of N utilization for biomass production (dry matter yield/N yield) at and between harvests (H1, H2 and H3). The bars show \pm SD.

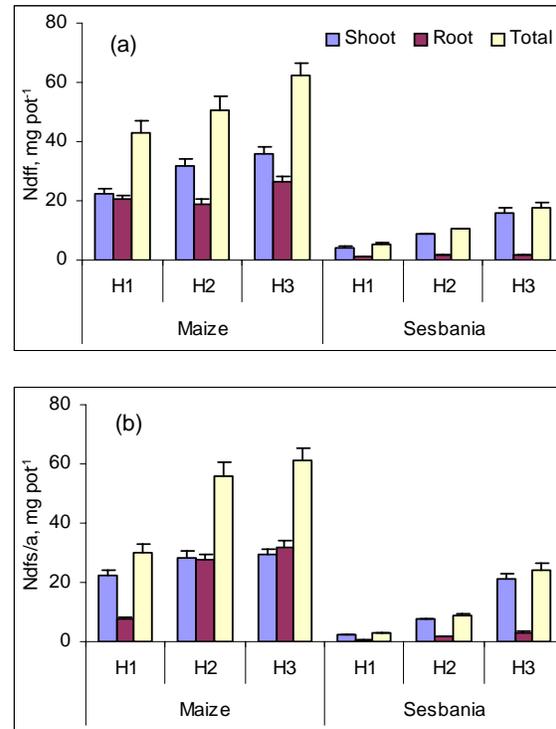


Fig. 4. Plant N (shoot and root portions) at different harvests derived from fertilizer (applied as ammonium sulphate to build pool of potentially mineralizable N in soil) and soil/atmosphere i.e., NDFS/a [since *Sesbania* roots did not have the nodules, all N was assumed to be derived from soil]. The bars show \pm SD.

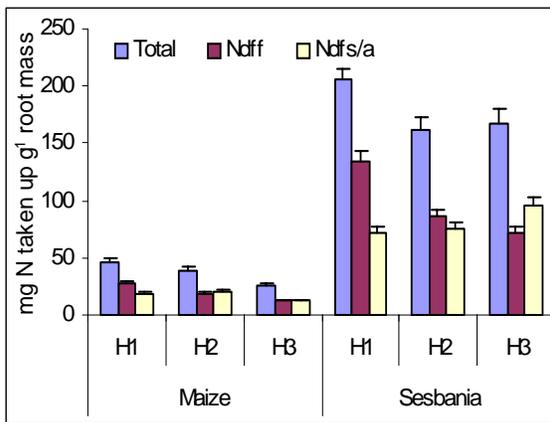


Fig. 5. Efficiency of roots to acquire N from soil expressed as mg N g⁻¹ root mass. The bars show \pm SD.

Discussion

Maize and *Sesbania* differed significantly in root biomass and consequently the shoot biomass yield (Fig. 1a). Such differences in biomass accumulation by leguminous and non-leguminous crops have been reported (Martin *et al.*, 1991; Breland & Bakken, 1991; Hawkes *et al.*, 2005). Differences in dry matter accumulation narrowed with time suggesting a decrease in the growth rate of maize relative to that of *Sesbania*. It would have

been mainly due to genetic difference in the growth pattern of the two plant species. This was possibly also by an early exhaustion of readily available nutrients by maize that showed a much proliferated root system as compared to *Sesbania*. As a result, maize plants showed a significantly wider root/shoot ratio; the average for 3 harvests being 0.97 and 0.23 in maize and *Sesbania*, respectively (data not shown). Maize showed an increase in root/shoot ratio from 0.80 at H1 to 1.15 at H3, while no significant change was observed in *Sesbania* where the ratio was 0.23 and 0.22 at the two harvests, respectively. These results also suggested that with the passage of time, maize plants absorbed the nutrients at a faster pace than *Sesbania* as a cause of difference in root biomass. It was also reflected by a difference in root/shoot ratios. However, the gain in dry matter yield between harvests followed a similar pattern in the two crops with significantly higher values for maize than *Sesbania* (Fig. 1b).

Roots of *Sesbania* showed negligible nodulation probably due to the lack of rhizobia in the soil or to higher amounts of mineral/mineralizable N in soil. Although dynamics of potentially mineralizable N were not monitored, a rapid net mineralization of N could be expected following the depletion of readily oxidizable C sources (Ahmed *et al.*, 1973). During the growth period, mineralized and accumulated N in the soil could be sufficient to inhibit nodule formation. Our earlier studies suggests significant enhancement of nitrification in the rhizosphere of leguminous plants

(Gill *et al.*, 2006), while an inhibitory effect of nitrate on nodule formation and subsequent N₂ fixation has been reported by Butler (1988).

Change in N content of the root and shoot portions of maize and *Sesbania* (Fig. 2a) were similar to that observed for dry matter yield. These two parameters also showed a significant positive correlation ($r = 0.98$, $p=0.05$, $n = 18$) suggesting that N uptake is the single most important factor in determining the biomass yield. Maize plants accumulated about 8 times more N compared to *Sesbania* due to more extensive root system that explored a greater soil volume for nutrient acquisition. Similar observations have been recorded by others (Ledgard *et al.*, 1985). Thus the mere difference in the rate of growth led maize plants exploit the soil nutrients at a faster pace and in higher quantities as compared to *Sesbania* during the short span of the study. Uptake of N in maize was significantly reduced between H2 and H3 as compared to that between H1 and H2 (Fig. 2b). It suggested an early exhaustion of available N; a reverse was true for *Sesbania* that showed a consistent increase in N uptake over the entire growth period. In both the crops, a higher proportion of N was partitioned to shoot rather than root but the difference was more pronounced in *Sesbania* where shoot portion contained several times higher N as compared to roots. The differences widened with time suggesting a more active N transport to the aerial parts and/or higher shoot mass relative to root mass. Percent N content of the root and shoot portions was significantly lower for maize than *Sesbania* but decreased in both the cases with the advancement of growth from H1 and to H3. This observation is in line with reports that show a wider C/N for non-leguminous than leguminous plants and is attributable mainly to the differences in C accumulation. The amount of N available for plant uptake does not differ significantly except for plant-specific influences on the mineralization processes as discussed later. The differences in biomass accumulation by maize and *Sesbania* are more pronounced as the former being a C₄ plant would have higher photosynthetic efficiency in addition to having higher leaf area. This difference was also obvious when efficiency of N utilization (expressed as the ratio of biomass produced to N taken up) was calculated at 3 harvests; being >40% higher in maize than in *Sesbania* (Fig. 3a). It was several times higher between H2 and H3 as compared to that between H1 and H2 in maize but showed only small increase in *Sesbania* (Fig. 3b).

Uptake of N from applied ¹⁵N-labelled ammonium sulphate (that was immobilized in the presence of sucrose) by both crops and its distribution in root and shoot portions (Fig. 4a) showed a pattern similar to that observed for the total N (Fig. 2a). Both parameters showed a significant correlation ($r = 0.99$, $n = 18$, $p=0.05$). Similarly, significant correlations ($r = 0.99$, $n = 18$, $p=0.05$) were also obtained between total N content of the root and shoot portions of the two crops and N derived from soil (Fig. 4b). These correlations suggest that the potentially mineralizable pool was uniformly labeled and contained almost similar proportion of N derived from soil organic matter and ¹⁵N-labelled ammonium sulphate. This contention is also supported by a close correlation ($r = 0.97$, $n = 18$, $p=0.05$) between N derived from soil and

ammonium sulphate (Fig. 4a and 4b). However, due to poor root proliferation compared to maize (Fig. 1a), *Sesbania* had access to a relatively smaller proportion of ¹⁵N-labelled pool. Thus, opposite to maize, *Sesbania* obtained a significantly lower percentage of N originating from ammonium sulphate, viz., 37.6 and 8.1% in the two crops, respectively. Since no nodules were formed on *Sesbania* roots, N contribution from biological N fixation did not exist. Therefore, the entire N in *Sesbania* as well as maize can be assumed to come from the soil. Hence, any isotopic dilution observed was attributable to the mineralization and uptake of native soil N that was relatively recalcitrant compared to more heavily labeled mineralizable fraction. Maize plants showed significantly higher isotopic dilution at H3 indicating that easily mineralizable component was exhausted earlier leading to release of N from more recalcitrant fractions and its uptake by plants. On an average (all harvests), however, the two plant types had essentially similar ¹⁵N abundance. Martin *et al.*, (1991) reported similar results for maize and non-nodulating soybean.

In spite of the fact that *Sesbania* had a lower root biomass as that of maize, efficiency of N uptake per unit root mass was much higher in the former, the average of 3 harvests being 178 and 37 mg N g⁻¹ root matter, respectively (Fig. 5a). The root efficiency of N uptake decreased with time in both the cases viz. from 46 at H1 to 25 at H3 in maize and from 206 to 167 in case of *Sesbania*. Nitrogen derived from the applied ammonium sulphate appeared to be more easily available than that correspondingly derived from soil. The average values of N uptake efficiency in 3 harvests were 19 and 17 for the two sources in maize and 97 and 81 in *Sesbania*. Difference in the root efficiency of N mineralization/uptake could be attributed to the nature of rhizodeposits by both plant types. Jensen (1996) and Høgh-Jensen & Schjoering (2001) reported a higher N rhizodeposition in leguminous than in non-leguminous plants. According to Jensen (1996), N rhizodeposits from leguminous plants are more labile and higher in quantity than those from non-leguminous ones and could therefore cause a higher mineralization of soil N.

Conclusions

Data presented support to the conclusion that:

1. With N application, *Sesbania* and maize not only differed in accumulating biomass, but *Sesbania* contained significantly higher water content as compared to maize. However, higher root proliferation in maize led to significantly more accumulation of dry matter and N if compared with *Sesbania*.
2. Efficiency of N utilization was higher in *Sesbania* compared with maize. Nevertheless, roots of *Sesbania* were more efficient in extracting N from soil as determined by amount of N taken up per unit root mass. Since, roots of *Sesbania* did not show nodules, entire plant N was ascribed to native soil N or from the recently developed pool of potentially mineralizable N.

3. Differences in the uptake of N by both crops could be attributed to root proliferation pattern. Root-induced changes in the N mineralization potential were assumed to result from differences in quality and quantity of rhizodeposits.

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