

## NATURALLY ABUNDANT $^{15}\text{N}$ TO DETERMINE THE RATE OF NITRATE UPTAKE BY PLANTS

D. N. SIRISENA,

*Rice Research And Development Institute, Batalagoda, Ibbagamuwa  
and*

D. ATKINSON

*Scottish Agricultural Collage, University of Edinburgh, Edinburgh, UK.*

### ABSTRACT

Naturally abundant  $^{15}\text{N}$  is a parameter to assess the nitrogen transformations in the soil-plant system. Studies were conducted to find the accumulation of  $^{15}\text{N}$  in relation to rate of nitrate uptake by Mustard (*Sinapsis alba*), Phacelia (*Phacelia tanacetifolia*), Chenopodium (*Chenopodium album*) and Ryegrass (*Lolium perenne*). It was observed that different plant species absorbed nitrate at different rates. Uptake rates were highest in mustard followed by phacelia and ryegrass and the lowest in chenopodium. After a 8-week period amount of nitrate-N depletion (28mg pot<sup>-1</sup>) from the soil was similar among all plant species tested. Total nitrogen contents in plants were different among plant species but they were not related to the rate of nitrate up take by the plants. Total nitrogen accumulation was highest in ryegrass followed by phacelia and mustard and the lowest in chenopodium.  $^{15}\text{N}$  content in plants differed among plant species too. Ryegrass and chenopodium, which absorbed nitrate at a slower rate, had the highest amount of  $^{15}\text{N}$ . Further,  $^{15}\text{N}$  accumulation was higher in the plant parts where the total nitrogen content was higher. Therefore, from this study it can be suggested that plants, which absorbed nitrate faster at the late in the season have the highest concentration of naturally abundant  $^{15}\text{N}$  in the plants and  $^{15}\text{N}$  allocation of plants is related to the accumulation of N in plant parts.

**KEYWORDS:** Nitrate uptake,  $^{15}\text{N}$ ,

### INTRODUCTION

The natural abundance of  $^{15}\text{N}$  content of the atmosphere is constant, but there are differences in the  $^{15}\text{N}$  contents of natural products. Most organisms discriminate  $^{14}\text{N}$  against  $^{15}\text{N}$ , the heavier isotope, during biological and chemical processes (Bremner and Hauck, 1982). This discrimination therefore produces a product that is depleted in  $^{15}\text{N}$  and a substrate that is enriched in  $^{15}\text{N}$ . The microbes, which transform one form of nitrogen into another, prefer  $^{14}\text{N}$  to  $^{15}\text{N}$ . As such microbial products of soil nitrogen is depleted in  $^{15}\text{N}$  and the remaining substrate is enriched in  $^{15}\text{N}$ . In the nitrification process nitrogen transformation resulting the production of nitrate, which are depleted in  $^{15}\text{N}$  and the remaining ammonium are enriched in  $^{15}\text{N}$ . On the other hand in the denitrification process nitrate is transformed into nitrogen gasses where nitrate is enriched in  $^{15}\text{N}$  and nitrogen gasses are enriched in  $^{15}\text{N}$ . Therefore, enrichment of natural abundance of the  $^{15}\text{N}$  in the mineral nitrogen of the soil can be observed (Turner *et al.*, 1987). Nitrogen transformations are higher in the agricultural fields than in fallow fields

(Shearer and Kohl, 1990; Turner *et al.*, 1987) and as such  $^{15}\text{N}$  content of the soil must be higher at the later stage of the cultivation than in the early stage.

Nitrogen absorption differs from plant to plant. It is reported that some plants absorb more nitrates than ammonium and absorption rates also differ among plant species. Plants that absorb nitrate rapidly have an advantage of reducing nitrate leaching in farmer fields. When plants prefer ammonium to nitrate, application of ammonium fertilizer is much better than application of nitrate fertilizer to get better crop yields. Therefore, it is important to know the rate of nitrate up take by the plant species and the ability of plant species to absorb different form of nitrogen before making a fertilizer recommendation.

Still there is no criterion for selection of plant species on rate of absorption of nitrate or on preference of absorption of ammonium to nitrate. There are no differences in the absorption of  $^{14}\text{N}$  and  $^{15}\text{N}$  by plant species (Huffaker and Rains, 1978). Therefore, information about the accumulation of natural abundance of  $^{15}\text{N}$  by plant species can make use for selection of different plant species for different cropping rotation and to identify the nitrogen pool from which the plants absorbed nitrogen for their growth. Therefore, the present experiment was designed to find out the accumulation of natural abundance of  $^{15}\text{N}$  in different plant species in relation to rate of nitrate up take by plants.

## MATERIALS AND METHODS

Soils were collected after harvesting of the of the summer cereal crop from the field number T5/6 of Craibstone farm, in UK, were air dried, sieved through 4 mm sieve and packed into plastic pots (Capacity 10 litres). The pots were filled to within 2 cm of the top, tapping the pot several times during the process; to consolidate the soil. Soil samples were collected from the site to determine physical properties, total N and  $^{15}\text{N}$  (table 1).

Table 1. Properties of the soil used for the experiment.

<i>Parameter</i>	<i>Description</i>
Texture	Sandy loam
Bulk density g cm <sup>-3</sup>	0.95
pH (1:2.5 CaCl <sub>2</sub> )	6.01
Total N %	0.40
Natural Abundant $^{15}\text{N}$ %	0.3671 ( $\delta^{15}\text{N}=3.005$ )

The weight of dry soil per pot was 9.5kg. Rhizon soil moisture samplers were inserted in all the pots at 7.5 cm. from the top of the soil level to collect soil solution samples for nitrate determination. All pots were moistened to 35% (W/W) soil moisture (moisture content at field capacity) and this moisture level was maintained throughout the growing period. Rhizon soil moisture samplers were allowed to settle for one week before the first solution sample was collected for analysis. There were five treatments in the experiment comprising 4 crop species and the plant free bare soil. Each treatment was replicated three times. Pots were arranged in a glass house in accordance with Randomized Complete Block Design. Pots with bare soil were treated with herbicides to keep the pot free of weeds. Seeds of all the crop varieties, Mustard (*Sinapsis alba*), Phacelia (*Phacelia tanacetifolia*), Chenopodium (*Chenopodium album*) and Ryegrass (*Lolium perenne*), were sown at the rates of 85, 65, 50, and 92 g/pot respectively. Pots were weighed 3-4 times a week during the period of plant growth and watered with de-ionized water to bring back to the original moisture level (leaching did not take place at the moisture content). Soil solution samples were collected from each pot at 0, 2, 4, 6 and 8 weeks after seeding and analyzed for their nitrate contents. Plants were uprooted at 8 weeks after sowing, roots were washed in running water. Above and below ground plant parts were separated, dried at 65°C and weighed. Total nitrogen and  $^{15}\text{N}$  in the above and below ground plants were determined using mass-spectrometer.  $\delta^{15}\text{N}$  of the samples were calculated as follows:

$$\delta^{15}\text{N} = \frac{\text{Atom. \% N in the sample} - \text{Atom. \% }^{15}\text{N in the standard}}{\text{Atom. \% }^{15}\text{N in the standard}} \times 1000$$

## RESULTS AND DISCUSSION

Changes of the concentration of nitrate in the soil solution of the pots grown with different plant species are shown in the figure 1. In comparison to pots of bare soil the concentration of nitrate in the soil solution of planted pots decreased during the growing period of all plant species. Nitrate concentration of soil solution in the pots grown with mustard and phacelia had dropped by 58% and 40% respectively at the end of the second weeks after planting.

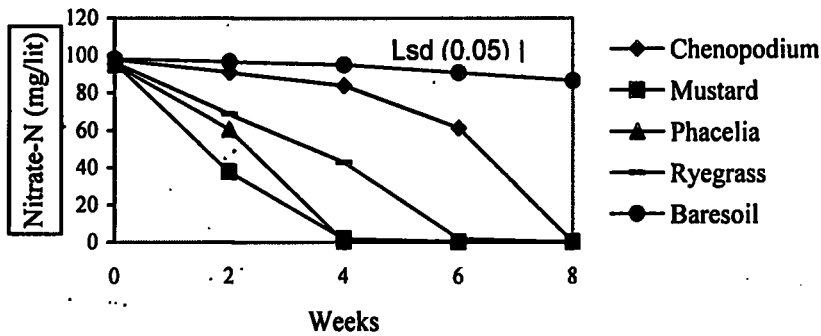


Figure 1. Nitrate-N concentration of soil solution at different intervals after sowing cover crop seeds.

Estimated average rate of daily nitrate depletion by mustard during this period was  $4 \text{ mg lit}^{-1} \text{ day}^{-1}$ . Respective rates for phacelia, ryegrass and chenopodium during the two weeks period were 2.9, 1.7 and  $0.4 \text{ mg lit}^{-1} \text{ day}^{-1}$  respectively. These values were significantly ( $p < 0.05$ ) lower compared to the rate of depletion of mustard (was  $4 \text{ mg lit}^{-1} \text{ day}^{-1}$ ). Within a period of 4 weeks nitrate concentration of soil solution in the mustard and phacelia grown soils depleted to below  $1 \text{ mg lit}^{-1}$ . The highest rate of depletion of nitrate in the soils grown with phacelia was observed during 2-4 weeks period after the seeds were sown. During this period the nitrate concentration of the soil solution has depleted at the rate of  $4 \text{ mg lit}^{-1} \text{ day}^{-1}$ . The rate of depletion of nitrate in the pots grown with ryegrass was more or less constant throughout the 6 weeks period. But at 6 weeks, nitrate concentration of the soil solution reached to its' minimum concentration. Nitrate concentration of the soil planted with chenopodium reduced slowly during the first 4-week period and reached minimum at the 8 weeks period. The maximum rate of depletion has occurred during the 6 to 8 week period.

Nitrate concentration of the KCl soil extract at the time of harvesting is shown in table 2. As seen in the figure 1 that nitrate concentration of the cropped soil was significantly lower in comparison to that of bare soil. The nitrate concentration of the soils of planted pots varied between  $3.24$  to  $2.82 \text{ mg kg}^{-1}$  depending on the species. That of the bare soil was as high as  $31 \text{ mg kg}^{-1}$ . Results indicated that nitrate lost from the soil is more or less equal under all plant species.

**Table 2. Nitrate concentration (KCl soil extraction) in the soil at the time of harvesting.**

<i>Treatment</i>	<i>NO<sub>3</sub> concentration (mg kg<sup>-1</sup>)</i>
Chenopodium	3.24
Mustard	2.98
Phacelia	2.90
Ryegrass	2.82
Control	31.0
LSD (p<0.05)	1.00

The amount of nitrogen stored differed among plant species (table 3). Amount of nitrogen in the plants was lower than the amount of nitrate lost from the soil when *mustard* and *chenopodium* were grown to the soil. Amount of nitrate lost and the amount of nitrogen in plants were almost similar when *phacelia* was grown. *Ryegrass* had the highest amount of nitrogen in plants and the values were higher than the amount of nitrate lost from the soil.

Amounts of <sup>15</sup>N in the plant parts are reported in the table 4. The <sup>15</sup>N content differed among plant species as well as plant parts of the same species. The highest <sup>15</sup>N contents in roots were recorded from *ryegrass* followed by *chenopodium* plants. <sup>15</sup>N in below ground plant parts are significantly higher in *ryegrass* followed by *phacelia*, *chenopodium* and *mustard*. <sup>15</sup>N in the above ground plant parts is higher in *chenopodium*, followed by *mustard*, *phacelia* and *ryegrass* respectively.

**Table 3. Total N content in above and below ground plant materials.**

<i>Treatment</i>	<i>N storage of plant (mg pot<sup>-1</sup>)</i>	
	<i>Above ground plant parts</i>	<i>Below ground plant parts</i>
Chenopodium	282	20
Mustard	185	33
Phacelia	248	20
Ryegrass	190	84
LSD (p<0.05)	9.52	6.72

Table 4.  $\delta^{15}\text{N}$  concentration in below and above ground plant parts.

<i>Treatment</i>	$\delta^{15}\text{N}$ in below ground plant parts (‰)	$\delta^{15}\text{N}$ in above ground plant parts (‰)
Chenopodium	3.96	6.90
Mustard	3.60	5.79
Phacelia	4.25	5.50
Ryegrass	6.18	5.68
LSD (p<0.05)	1.92	NS

It was observed in this study that two weeks after seeds were sown, nitrate content of the soil solution dropped in all treatments except in the pot with bare soil. Moisture content of the soil was kept constant by adding small amount of water equal to the amount of water lost, therefore, leaching or collection of water at the bottom of the pot was not observed. On the other hand soils were kept under aerobic conditions at field capacity. Therefore, heavy losses of nitrate through denitification cannot be expected (Kowalanko and Cameroon, 1976) At the same time immobilization of nitrogen is also low under cropped conditions (Jingguo and Bakken, 1989; Hart *et al.*, 1979). As such crop uptake is the only possible way of depleting nitrate from the soil solution. Nitrate uptake of the plants differed among plant species. The four plant species can therefore be ranked according to the rate of nitrate depletion of the soil as mustard> phacelia> ryegrass> chenopodium.

The amount of nitrogen stored also differed among plant species. Though *Ryegrass* took nitrate at slower rate, it had taken up large amount of nitrogen and most of them stored in the below ground plant parts. Ryegrass has the ability to absorb ammonium as well as nitrate (Clarkson and Warner, 1979) and as such it may be a reason for uptake of more N by ryegrass. On the other hand, chenopodium had taken up nitrate at slower rate and the amount of nitrogen taken up was also low but most of the nitrogen is stored in the above ground plant parts. The  $\delta^{15}\text{N}$  content of plant N was higher than that of soil N (Tables 1 and 4). It was suggested by Rennie and others (1976) that  $\delta^{15}\text{N}$  of plants should be lower than the that of soil because there was a discrimination against  $^{15}\text{N}$  in plant uptake of N. But later Turner ad others (1987) studied the  $^{15}\text{N}$  of soil N during the growing season and suggested that  $\delta^{15}\text{N}$  were higher because during the growing season  $\delta^{15}\text{N}$  of mineral N was increased. He observed that at the end of growing season  $\delta^{15}\text{N}$  of the plant samples were also higher because plant uptake nitrogen from the mineral N pool. According to his findings he proposed that there was no discrimination against the heavier  $^{15}\text{N}$  isotope when plant uptake N from the soil.

Results of this experiment are therefore in agreement with the findings of Turner and others. It was observed here that  $\delta^{15}\text{N}$  values differed among the different plant species and it has a negative relationship with the rate of nitrate uptake from the soil. Ryegrass and chenopodium that absorbed nitrate at a lower rate had the highest  $\delta^{15}\text{N}$  values while mustard and phacelia that absorbed nitrate at higher rates had the lower  $\delta^{15}\text{N}$  values. Therefore it can be suggested that during the growing season the  $\delta^{15}\text{N}$  content of mineral N had increased and as a result plants that actively absorbed N late in the season had the ability to absorb more  $^{15}\text{N}$  during the growing period.

During the nitrification process ammonium is enriched with  $^{15}\text{N}$  due to discriminate absorption of  $^{15}\text{N}$  by the soil microbes. Since ryegrass has the ability to absorb ammonium as well as nitrate (Clarkson and Warner, 1979) it can be suggested that uptake of  $^{15}\text{N}$  enriched ammonium ions by ryegrass may also enhances the  $^{15}\text{N}$  in the plants.

It was further observed that N storage in different plant parts varied among plant species. Ryegrass had most of their nitrogen stored in the below ground plant parts while chenopodium and phacelia had most of their nitrogen in the above ground plant parts. Allocation of  $\delta^{15}\text{N}$  was higher in the plant parts where N accumulation is higher proving that there is no discrimination of distribution of  $^{15}\text{N}$  inside the plant. In ryegrass where much of the nitrogen is in roots had significantly higher  $^{15}\text{N}$  contents in roots.

### CONCLUSION

Nitrogen transformations are resulted in the enrichment of natural abundance of  $^{15}\text{N}$  in the mineral nitrogen of the soil. Since mineralisation is a continuous process,  $^{15}\text{N}$  content in the soil at the latter stage is comparatively higher than that of the early stage of the crop growth. Therefore, different concentration of  $^{15}\text{N}$  can be observed in different plant species and is dependent on the rate of absorption of nitrate from the soil. Higher concentrations of natural abundance of  $^{15}\text{N}$  are found in the plants, which have the slower, but longer uptake rate of nitrate from the soil solution. And as such it was revealed in the experiment that amount of  $^{15}\text{N}$  content in the plant can be used as a parameter to decide the rate of uptake of nitrate by the plants. Allocation of natural abundance of  $^{15}\text{N}$  in the above and below ground plant parts differs among plant species following a similar pattern to the allocation of nitrogen among the plant parts.

Therefore, sampling of plant for  $^{15}\text{N}$  determination should be done by considering the nitrogen storage of the plant parts.

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