

## **Effect of Increasing Rates of Sunflower Residues on the Evolution of Soil Mineral N In A Laboratory Incubation Study.**

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**Abstract:** *An aerobic soil incubation test was carried out in the laboratory to study the N immobilization-mineralization process when increasing rates of sunflower residues were incorporated into the soil at 0, 0.15, 0.5 and 1g of residues per 50g of soil. The soil obtained from a plot where sunflower has been cultivated was incubated at 32°C for 3 months. For all residues rates, nitrogen was rapidly immobilized in the first four days of incubation, then it was rapidly liberated into the soil. However, the amount of nitrogen remineralized decreased with increasing rate of residues incorporated. In terms of incubation period, the rate of N immobilized by residues relative to the control was 22, 18, 47 and 70% for R1, R2, R3 and R4, respectively.*

**Keywords:** *Aerobic incubation, immobilization, mineralization, mineral nitrogen, residues rate, sunflower residues.*

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### **I. Introduction**

Crop residue is one of the most important sustainable and ecologically sound alternative for meeting the nutrient requirement of crops (Babu et al., 2014). In addition to their role as the primary source of soil organic matter (Havlin et al., 1990; Larson et al., 1972; Uhlen, 1991; Zibilske, 1987), they have a significant positive impact on improving soil physical, chemical and biological soil properties (Gupta et al., 1987; Parr & Papendick, 1978; Sarkar et al., 2020). Moreover, incorporation of crop residues can also nourish rhizosphere biota as well as reduce farm inputs and greenhouse gases emissions (Malhi & Lemke, 2007; P. Sharma et al., 2011) responsible of climate changes.

In last years, sunflower crop has covered large areas in Meknes region (North of Morocco). It was introduced principally in biennial rotation with wheat. Sunflower is an oilseed crop which produce huge amount of crop residue. This amount of residues is neither used as feed for livestock nor suitable for fuel due to low energy value per unit mass (Babu et al., 2014). However, soil incorporation of its residues may be pertinent for replenishing the depleted soil fertility and improving production since they are nutrients rich residues (K. L. Sharma et al., 2007).

Nevertheless, the addition to the soil of an organic compound with a high C/N results in a biological immobilization of mineral nitrogen in the soil (Christensen, 1986; Jansson & Persson, 1982; S. J. Smith & Sharpley, 1990). As decomposition progresses, the initially immobilized mineral nitrogen remineralizes. This process is called Nitrogen Immobilization-Remineralization (Jansson & Persson, 1982; Kumar & Goh, 1999). It is depending on rate of residues applied to soil (Reinertsen et al., 1984; Sorensen, 1981; Zibilske, 1987). The aim of the present work is to study the immobilization and remineralization of nitrogen in the soil in the presence of increasing rates of sunflower residues known as low N content.

## II. Material and Methods:

A soil laboratory aerobic incubation test was carried out to study the evolution of soil mineral N in the presence of increasing rates of sunflower residues. Soil was sampled in autumn from a field where sunflower was cultivated. Soil initial mineral N was 8.1 mg/kg, Nitrate-N was 6.3 mg/kg.

Soil sieved at 2 mm was uniformly mixed with sunflower residues. Residues contained different parts of the plant: stems, leaves, flower heads and roots. They were ground at 1.5 mm, their %N was 0.54. The doses of residues added to the soil were  $R_0 = 0$ ;  $R_1 = 0.15$ ;  $R_2 = 0.25$ ;  $R_3 = 0.5$  and  $R_4 = 1$  g per flask containing 50 g of soil. The 0.15g dose per 50 g of soil corresponds approximately to 500g/m<sup>2</sup> (5 t/ha), which is the average rate of residues returned to the soil by sunflower under field conditions.

Before incubation, flasks were added with fertilizer in the form of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, P<sub>2</sub>O<sub>5</sub> and KCl at the respective doses 5 mg of N (100 N mg/g of soil), 1.5 mg of Pet 2 mg of K per vial. The soil was incubated in a culture chamber at 32°C and 90% humidity. The soil water was 60% of the field capacity, it was adjusted by weighing every four days. In total, the number of vials was 135. The experiment was completely randomized design with 3 repetitions. The incubation period was 3 months. During this period, samples were taken on the 2<sup>nd</sup>; 4; 7; 15; 30; 45; 60; 75 and the 90<sup>th</sup> day of incubation. In each date, 3 flasks of each treatment were sacrificed for analysis. The soil was oven dried at 40°C, nitrate and ammoniacal nitrogen were analyzed by the method of Deverda (Keeney & Nelson, 1982).

An analysis of variance (ANOVA) was performed according to the completely randomized design with 3 repetitions, at significance level of 5%.

## III. Results

### Evolution of total mineral Nitrogen

Evolution of total soil mineral nitrogen during the 90 days of incubation was characterized by a fast disappearance of mineral nitrogen in the start of incubation (figure 1). This phase was immediately followed by a rapid nitrogen remineralization. The later phase showed irregular fluctuations, but the general appearance tended towards a stable evolution of mineral nitrogen in the soil. At each sampling date, the analysis of variance showed a highly significant effect of the dose factor of sunflower residues incorporated into the soil. In general, as the amount of residue increased the mineral nitrogen decreased. For all residue treatments, the drop in the level of mineral nitrogen was observed from the 2<sup>nd</sup> day of incubation, and was maximal on the 4<sup>th</sup> day. Indeed, the disappearance of mineral nitrogen from the initial level was 74.68%, 86%, 93%, 99%, and 98%. Between the 15<sup>th</sup> and the 45<sup>th</sup> day of incubation, the level of total mineral nitrogen remained almost stable. In the control treatment, remineralization continued until day 30. The period between the 45<sup>th</sup> and the 60<sup>th</sup> day saw further nitrogen remineralization in the  $R_1$  and  $R_2$  treatments, which remained less than that of the first week of incubation. This remineralization was observed until the 60<sup>th</sup> day in the  $R_3$  and  $R_4$  and  $R_0$  control treatments. From the 75<sup>th</sup> day, the evolution of mineral nitrogen was almost stable.

After three months of incubation, the mineral nitrogen was higher in the control treatment, at the same level in  $R_1$  and  $R_2$ , and notably lower in  $R_3$  and  $R_4$  comparing to the initial soil mineral N.

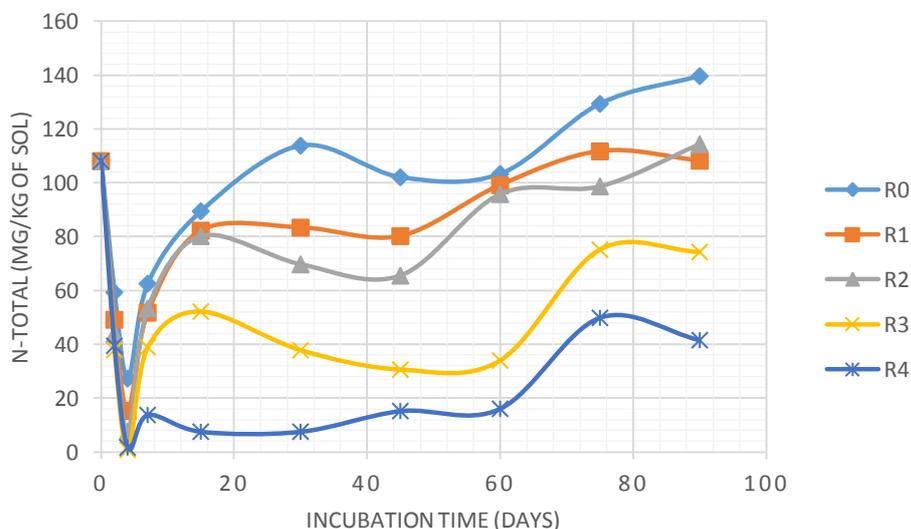


Figure 1: Evolution of total Nitrogen during incubation period

**Evolution of ammoniacal Nitrogen**

During the entire incubation period, the variation of ammoniacal nitrogen was not affected by the doses of residues brought to the soil, except on the 7<sup>th</sup> day when NH<sub>4</sub>-N was reduced by the addition of increasing doses of residues (figure 2). In fact, on the 2<sup>nd</sup> day, 93% and 95% of initial NH<sub>4</sub>-N disappeared in the control and R<sub>1</sub> treatments respectively, and of the order of 99% in the R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> treatments. On the 7<sup>th</sup> day of incubation, remineralization peaks of NH<sub>4</sub>-N appeared and their importance decreased with increasing rates of residues. These peaks of NH<sub>4</sub>-N reappearance coincided with the remineralization phase of total soil mineral nitrogen. On the 15<sup>th</sup> day, the NH<sub>4</sub>-N disappeared again, and remained at the same level until the end of incubation, with no difference in residue doses.

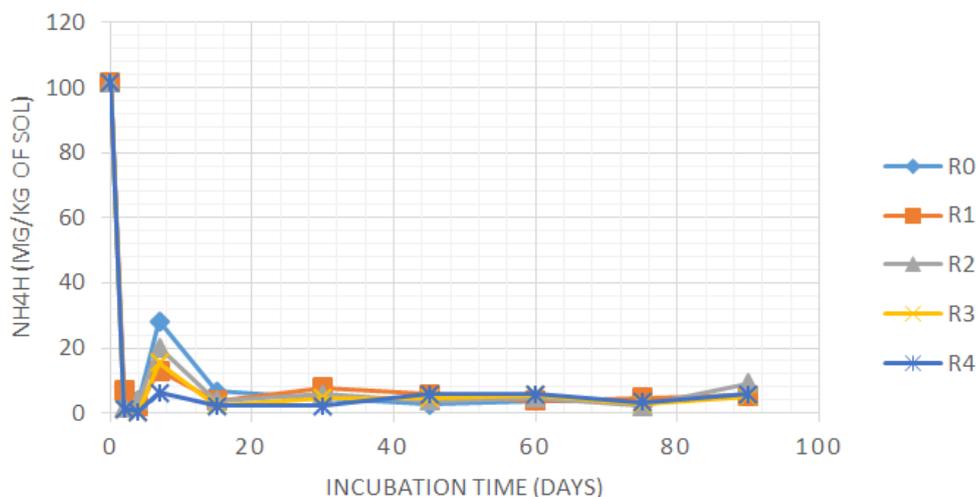


Figure 2: Evolution of ammoniacal Nitrogen during incubation period

**Evolution of nitric nitrogen in the soil**

Analysis of variance revealed a highly significant effect of increasing dose levels of sunflower residue on soil NO<sub>3</sub>-N at all sampling dates; the increase of residues rate resulted in a decrease in nitrate nitrogen in the soil (figure 3). This drop was not significant between R<sub>1</sub> to R<sub>2</sub>.

Due to the very low NH<sub>4</sub>-N content of the soil recorded throughout the incubation period, NO<sub>3</sub>-N showed variations similar to those of total mineral nitrogen. The only difference between NO<sub>3</sub>-N and total mineral nitrogen was observed in the first two days of incubation. This is because total mineral nitrogen has decreased, while NO<sub>3</sub>-N has increased. On the 4<sup>th</sup> day, an appreciable amount of the nitrified nitrogen had disappeared again. With the exception of the R<sub>4</sub> treatment, the period between the 4<sup>th</sup> and 15<sup>th</sup> day was characterized by a significant reappearance of NO<sub>3</sub>-N. During the remainder of the incubation period, the evolution of NO<sub>3</sub>-N was identical to that of total mineral nitrogen.

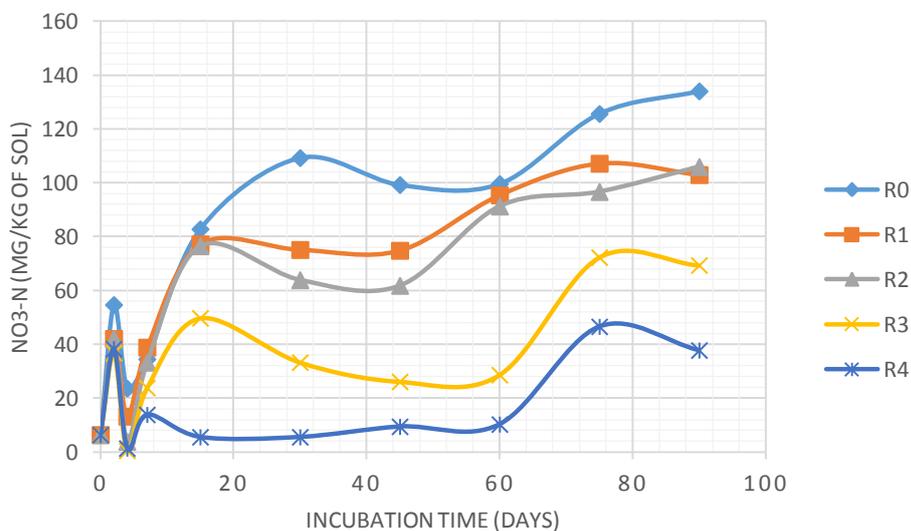


Figure 3: Evolution of nitric nitrogen during incubation period

#### **IV. Discussion**

The initial nitrogen was mostly in the form of  $\text{NH}_4\text{-N}$ . It was added to soil as fertilizer in the form of  $(\text{NH}_4)_2\text{SO}_4$  at a dose of 100 mg/kg of soil. In all residues treatments, the rapid disappearance of  $\text{NH}_4\text{-N}$  at the start of incubation indicates an intense microbial activity. Nitrification and immobilization are the most important biological processes that must have caused the consumption of  $\text{NH}_4\text{-N}$ . Concerning  $\text{NO}_3\text{-N}$ , its content increased. This increase would be due to a nitrification of  $\text{NH}_4\text{-N}$  brought to the soil. From the second day of incubation, the  $\text{NO}_3\text{-N}$  then dropped. Thus, the nitrogen nitrified in the first two days was immediately immobilized. Immobilization was therefore the predominant process during the first four days of incubation, where both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were used by microorganisms.

Several studies have reported that nitrogen is rapidly immobilized during the first days of incubation. Hdoudouch (1994) detected  $\text{NH}_4\text{-N}$  immobilization from the first 7 days of incubation in the presence of sunflower residues. In the presence of wheat straw, Jawson & Elliott (1986) found that mineral nitrogen was immobilized within the first 12 days of incubation. Following the addition of wheat straw into soils, rapid decrease of nitrate content in soil and increase of microbial biomass C and N occurred within the first week from onset of the experiment was noted by Shindo & Nishio (2005). In our experiment, the rapid consumption of nitrogen at the start of incubation can be explained by an intense proliferation of microbial biomass in the presence of sunflower residues. The significant immobilization of nitrogen in the control treatment would be explained by an activation of the microorganisms following the rewetting of the soil (De Bruin et al., 1989; Van Veen et al., 1985), and also by the presence in the soil of remaining sunflower residues from the previous season.

Once immobilized, nitrogen mineralization started since the fourth day of incubation. This phase of intense mineralization would correspond to the decomposition of the microbial biomass which immobilized nitrogen at the start of incubation. In all treatments, mineralized nitrogen was predominantly in the form of  $\text{NO}_3\text{-N}$ . Nevertheless, a brief peak of  $\text{NH}_4\text{-N}$  was recorded; it coincided with the phase of net mineralization. A similar result was found by (Ladd & Paul, 1973) in a soil supplemented with glucose and by Hdoudouch (1994) in the presence of sunflower residues. This appearance of  $\text{NH}_4\text{-N}$  would be due to the ammonification of certain decomposition products from the first generation of microorganisms, which proliferated in the first days of incubation. The period succeeding the rapid immobilization and mineralization phase was characterized by slower evolution of mineral nitrogen. This phase would correspond to the proliferation of microbial populations which use the relatively less available carbon from sunflower residues. In the same hand, Cochran et al. (1988) suggested the existence of two pools of microbial biomass which degrade wheat residues; an initial population that uses the readily available carbon from residues, and another pool of microbial biomass that simultaneously uses the less available carbon from residues and the nitrogen remineralized from the first population.

According to Reichel et al. (2018), high organic carbon amendments (HCA) consist of very different quantities and qualities of fast, moderately, and slowly degradable organic sub-fractions. In fast fraction, which is usually decomposed within a few days, immobilization can be expected to increase with increasing size and C:N ratio (Plante & Parton, 2007). Sugars, amino acids, and nucleic acids are the main constituents of this fraction (Mueller et al., 1998). It represents a substrate of a rapid growth of fermentative microorganisms (Kuz'yakov & Blagodatskaya, 2015). After death of this first microbial biomass generation, C and N released serve as additional substrates for microbes that grow more slowly on more complex organic compounds (Fontaine et al., 2004).

During the entire incubation period of our experiment, the amount of mineralized nitrogen decreased with the rate of sunflower residues added. Nitrogen immobilization in the presence of low residues N has been reported in most studies (Aulakh et al., 1991; J. H. Smith & Douglas, 1970; S. J. Smith & Sharpley, 1990), this is explained by consumption of soil nitrogen by microorganisms to degrade residues (Alexander, 1978; Bartholomew, 1965). Therefore, increasing residues rates in the soil would increase the nitrogen demand of microorganisms which would cause more immobilization. Similar results were reported by Sorensen (1981), Zibilske (1987) and Reinersten et al. (1984).

On the other hand, the high quantity of residues in the soil seems to slow down the processes of nitrogen transformation. Indeed, we noticed that the mineralization of  $\text{NO}_3\text{-N}$  observed on the 45<sup>th</sup> day for the  $\text{R}_1$  and  $\text{R}_2$  doses was only observed on the 60<sup>th</sup> day for the  $\text{R}_3$  and  $\text{R}_4$  treatments.

During the last days of incubation, mineralization was the prevalent process. However, referring to the control, an appreciable amount of nitrogen was immobilized in the treatments supplemented with sunflower residues. At the end of the incubation period (90<sup>th</sup> day), the amounts of immobilized nitrogen were 31.4, 24.58, 65.32, 98.07 mg/kg of soil for the treatments  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$  and  $\text{R}_4$  respectively. The decrease of remineralized N, with the increase of residues doses, could result from a high rate of N immobilization and/or an incorporation of the nitrogen immobilized in stable organic forms. Indeed, several authors have shown that in the presence of crop residues, an appreciable quantity of the nitrogen of the immobilized fertilizer is stabilized in the soil (Azam, Mulvaney, et al., 1989; Azam, Stevenson, et al., 1989; He et al., 1988; Sorensen, 1981).

In the term of the incubation period mineral N of the control treatment was clearly higher than the initial mineral N by a difference of 30 mg N/kg soil suggesting a priming effect due to the activation of soil microorganisms further the addition of inorganic N (Jenkinson et al., 1985; Kuzyakov, 2010).

## V. Conclusion

The incorporation of sunflower residues in the soil inducted a fast immobilization of mineral nitrogen. The nitrogen immobilized was rapidly remineralized. The quantities of remineralized N decreased with the dose of residues. In the R<sub>1</sub> treatment (the equivalent of 5t of residues/ha) and R<sub>2</sub> (the equivalent of 8t of residues/ha) the amounts of nitrogen mineralized were little different. Their impact on mineral soil nitrogen was low compared to treatments R<sub>3</sub> (equivalent to 16t of residues/ha) and R<sub>4</sub> (equivalent to 32t of residues/ha), which could cause nitrogen deficiency in soil, especially if the period of immobilization coincides with the plant's high nitrogen demands. However, for practical recommendations, this work would be experienced in the greenhouse and in the field conditions in the presence of the plant to study the synchronism between remineralization and plant needs.

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