OPEN ACCESS

Current Trends in Forest Research

Aliyat FZ, et al. Curr Trends Forest Res 6: 1032.

Research Article

DOI: 10.29011/2638-0013.101032

The Effect of Different Tree Leaves Incorporation On the Growth and Nitrogen Availability to Wheat in an ^{15}N Labelled Soil

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Citation: Aliyat FZ, Ei Mderssa M, Nassiri L, Ibijbijen J (2019) The Effect of Different Tree Leaves Incorporation On the Growth and Nitrogen Availability to Wheat in an ^{15}N Labelled Soil. Curr Trends Forest Res 6: 1032. DOI: 10.29011/2638-0013.101032

Received Date: 06 May, 2019; **Accepted Date:** 17 May, 2019; **Published Date:** 27 May, 2019

Abstract

Forest plant residues are an important source of nutrients for trees, and the decomposition of these materials has a very important role in improving soil fertility especially on the availability of Nitrogen (N). The objectives of this study were to evaluate the effect of the incorporation of different tree leaves into the soil on the soil nitrogen availability. The experiment was conducted in the greenhouse in pots, involving leaves of three types of trees: *Eucalyptus camaldulensis* (C/N: 26), *Acacia cyanophylla* (C/N: 15) and *Acacia cyclops* (C/N: 21*)*. The wheat (*Tritiucum durum* var. Karim) was grown on a soil mixed with leaves of each tree separately at rates 15, 30, 30+N, 45 and 60 g pot^{-1}. The results showed that there was a trend towards a depression of dry matter and N accumulation in the presence of *Eucalyptus* leaves, however, the reverse trend occurred with both Acacia leaves. Generally, the ^{15}N recovered decreased with the increase of the quantity of added material to the soil. The decline was more important in the *Eucalyptus* treatment than with Acacia, showing the important N immobilization in the presence of such poor N plant material. The beneficial effect of the combination of fertilizer N with leaves incorporation was important in the case of *Eucalyptus* treatment.

Keywords: *Acacia cyclops; Acacia cyanophylla; Eucalyptus camaldulensis;* Nitrogen availability; ^{15}N-recovery

Introduction

Eucalyptus (Eucalyptus sp.) is frequently planted, due to its high productivity on short-term rotations [1]. In Morocco, the first plantation was established in the Kenitra region in the 1950's [2]. Indeed, intensive and continuous tree culture of *Eucalyptus* in this region has created nutrient poor soils. Therefore, the high nutrient demand of *Eucalyptus* plantations raises questions about their sustainability, and a special focus on nutrient cycling in such tree plantations is required. Two solutions were proposed; cultivation of nitrogen fixing tree species (*Acacia sp.)* in rotation with *Eucalyptus* [3-5], or favouring litter decomposition by the burying of decayed leaves in soil.

The decomposition of plant materials is an important process in the cycle of nutrients and in the improvement of soil structure. Many studies have been conducted on this topic [6-8]. This process is influenced by the quality parameters of these materials such as Nitrogen (N). Moreover, the N is the primary limiting nutrient for forest production [9]. The availability of mineral N depends on several factors; C/N ration of plant residues is among these factors. The N concentration or the C/N ratio of incorporated plant material is often the best predictor of the plant N mineralization [10-12]. Generally, the N concentration has to be greater than a critical level of 1.5 to 1.7 %N before N mineralization will occur [13,14]. However, the high concentrations of lignin or polyphenols in plant material may slow down the mineralization of plant nitrogen despite the high concentration of N [15,9].

The objectives of this essay were to study the contribution of three tree leaves incorporated to soil on N availability in the soil and its effect on wheat crops production. Also to assess the combined effect of leaves and mineral nitrogen application on N availability in the soil.

Materials and Methods

Experimental Design

The experiment was conducted in pots under greenhouse conditions at the Faculty of Sciences, Meknes, Morocco. A complete randomized block design was established with three replications. The experimentation consisted of incorporating different tree leaves into the soil at different rates. The soil used was a mixture of sand and agricultural soil at rates of 2:1 (w / w). Each pot was filled with 5 kg of the mixture. The physicochemical properties of the agricultural soil are presented in Table 1.

Clay	Loam	Sand	pH(HO_2)	Al	Ca	Mg	P ppm	K ppm	%N	%OM
				(mEq/100 ml)						
30%	25%	35%	8	0	4	1	24.5	24	0.12	1.68

Table 1: Physical and chemical soil properties.

Dried mature leaves of three different trees: *Eucalyptus camaldulensis*, *Acacia cyanophylla* and *Acacia cyclops* were collected from trees in the forest (Kenitra region, Morocco). The characteristics of these materials are presented in Table 2.

	%N	%C	C/N	%Lignin	Lignin/N	%Polyphenol	%Cellulose
E. camaldulensis	1.74	46	26	11.5	6.6	1.87	27
A. cyanophylla	3.16	47.5	15	10.92	3.45	1.78	29.72
A.cyclops	2.03	44	21	8.61	4.24	2.04	15.1

Table 2: Chemical characteristics of the different tree leaves in % of dry weight.

The leaves of each tree were chopped (0.5 to 1 cm) and well mixed with the soil at five different rates; 15, 30, 45 and 60 g per pot. An additional treatment consisting in an addition of 40 kg N ha^{-1} for the rate 30 g plant material per pot, was included for each type of leaves. For the fertilizer nitrogen treatment, four rates were selected equivalent to 20, 40, 80, 120 kg N ha^{-1} without plant material addition. The control treatment did not receive neither plant material nor N fertilizer. Thereby the pot experiment had five treatments: (1)- Soil + *Eucalyptus camaldulensis* leaves (15, 30, 30+N, 45 and 60 g pot^{-1}). (2)-Soil + *A. cyanophylla* leaves (15, 30, 30+N, 45 and 60 g pot^{-1}). (3)-Soil + *A. cyclops* leaves (15, 30, 30+N, 45 and 60 g pot^{-1}). (4)-Soil + Ammonium sulphate (20, 40, 80 and 120 kg N ha^{-1}). (5)-Soil only (control).

^{15}N labelled fertilizer was added to the soil as a solution of ammonium sulphate 10.16 atom %^{15}N excess at the rate of 10 mg N Kg^{-1}, just after the leaves incorporation into the soil. The pots were watered regularly to field capacity for 4 weeks' incubation before planting the wheat. At the end of the pre-treatment incubation period, the wheat (*Triticum durum* cv Karim) was seeded to obtain 5 plants per pot. One week after sowing the fertilizer N (Ammonium sulphate) was applied in treatments selected for N application.

Sample Preparation and Analysis

All the shoots were harvested eight weeks after emergence. The pots were subsequently inverted and roots separated from the soil. Both roots and shoots were dried to a constant weight for 72h at 70 ˚C, then ground to less than 2 mm and preceded for different chemical analysis. Sub-samples (200 mg) were analysed for total N content using the Kjeldahl digestion followed by steam distillation [16]. After titration against sulphuric acid the resulting solutions were acidified and evaporated to dryness in small vials for the ^{15}N enrichment analysis according to [17].

Statistical Analyses

Statistical analyses were performed using the software package MSTAT-C. The data were analysed using tow way (plant material, doses) ANOVA and the means were compared using

Student's test at P=0.05. Least Significant Differences (LSD) were used to indicate significant variations within the values of different treatment.

Results and Discussion

Dry Weight and N-Uptake of Wheat

A significant difference in the dry weight of the shoots was observed for the different treatments (Table 3). *Eucalyptus* leaves addition produced significantly lower yields than any other leaves excepted in the case of rate 30+N (30 g leaves pot^{-1} supplied with 40 kgNha^{-1}) in which the dry weight yield was significantly greater than the control, whereas for the two treatments of Acacias, *A. cyanophylla* and *A. cyclops*, the dry weight of the shoots was significantly higher. While the treatment with inorganic fertilizer nitrogen showed a positive response of wheat by increasing rate of mineral N. The results show that the maximum shoot dry weight in the experiment was obtained in soil treated with the highest rate of inorganic fertilizer (120 kg N ha^{-1}).

Treatments	**Dry weight g/pot**						
Organic Materials (g/pot)	0	15	30	30+N	45	60	$LSD_{0.05}$
E. camaldulensis	5.84^{a}_{A}	5.72^{a}_{A}	5.83^{a}_{A}	6.21^{a}_{A}	5.68^{a}_{A}	5.70^{a}_{A}	0.72
A. cyanophylla	5.84^{a}_{A}	$6.52^{b}_{A,B}$	$7.12^{b}_{B,C}$	7.39^{b}_{BC}	7.67^{b}_{C}	7.74^{b}_{C}	0.95
A. cyclops	5.84^{a}_{A}	$7.01^{b}_{B,D}$	$7.15^{b}_{B,C}$	7.99^{b}_{C}	$6.15^{c}_{A,D}$	5.92^{a}_{A}	0.98
$LSD_{0.05}$	-	0.72	0.99	0.94	0.9	0.66	
Nitrogen (Rate kgN/ha)	0		20	40	80	120	$LSD_{0.05}$
	5.84_{A}		$6.84_{B,C}$	$6.46_{A,B}$	7.46_{C}	8.39_{D}	0.75

- Means followed by the same lowercase letter in same column are not significantly different by least significant difference LSD test at 5% of probability level;
- Means followed by the same capital letter in same line are not significantly different by least significant difference LSD test at 5%.

Table 3: Dry weight yield of wheat shoots after addition of different organic materials and fertilizer nitrogen to soil.

A significant difference in the wheat total N was observed for the different treatments (Table 4). Comparing to the control, the wheat total N in the *Eucalyptus* treated soil was generally lower. However, treatments with leaves of both of Acacia species had significantly higher yields of wheat total N than the control, with higher values, for soils treated with *A. cyanophylla* leaves. These results suggest that the incorporation of rich N leaves improved N availability in the soil, whereas the poor N leaves of *Eucalyptus* reduced N availability. The analysis of the results of dry weight and N yield of wheat indicate that there was significant immobilization of mineral N for the leaves of *Eucalyptus* with a low N content (1.74%N, C/N: 26) indicating that this concentration is a reasonable critical level for initial net mineralization of incorporated organic materials as indicated in literature [18-20,9].

Treatments	**Total N (mg pot^{-1})**						
Organic Materials (g pot-1)	0	15	30	30+N	45	60	$LSD_{0.05}$
E. camaldulensis	125.18^{a}_{A}	122.27^{a}_{AC}	125.04^{a}_{A}	142.03^{a}_{B}	118.67^{a}_{C}	117.29^{a}_{C}	6.24
A. cyanophylla	125.18^{a}_{A}	136.22^{b}_{A}	156.74^{b}_{B}	156.48^{a}_{B}	153.44^{b}_{B}	156.24^{b}_{B}	15.22
A. Cyclops	125.18^{a}_{A}	138.16^{b}_{B}	145.73^{b}_{B}	157.18^{a}_{C}	125.62^{c}_{A}	126.69^{c}_{A}	10.32
$LSD_{0.05}$	-	11.24	15.55	16.45	13.25	9.33	

Nitrogen (Rate kg N ha-1)	0	20	40	80	120	$LSD_{0.05}$
	125.18_A	137.20_B	140.93_B	166.77_C	178.06_D	11.22

- Means followed by the same lowercase letter in same column are not significantly different by least significant difference LSD test at 5% of probability level;
- Means followed by the same capital letter in same line are not significantly different by least significant difference LSD test at 5%.

Table 4: N uptake of wheat after addition of different organic materials and fertilizer nitrogen to soil.

Comparing between the two legume leaves, *A. cyanophylla* produced higher yields of dry weight and total N than *A. cyclops* (Table 3 and Table 4). This is due to the difference in their nitrogen content; 3.16%N (C/N: 15) for *A. cyanophylla* vs 2.03%N (C/N: 21) for *A. cyclops*. Therefore, the analysis of the results shows the significant positive correlation between C/N ratio of the leaves and availability of soil Nitrogen reflected by wheat growth and its N-uptake. Amongst these three species, *A. cyanophylla* had the lowest C/N ratio, produced highest yields of wheat dry weight and total N. In contrast, *Eucalyptus* had the highest C/N ratio, produced lowest yields of wheat dry weight and total N. The amount of N in the litter affects the mineralization of N, Similar results were reported by [21,22], which have shown that the litter with a low C/N ratio decomposes more rapidly. Thus, a rapid mineralization of N, and high mineral N contents in the soil was observed.

Some studies have demonstrated that the liberation of N is related to lignin/N ratio contents of the plant material [23-27]. As well, Talbot and Treseder, in 2012 [28] suggested that the high lignin rate prevents decomposition of the litter, this suggestion may explain the low dry weight and N-uptake of wheat of the treatment with *Eucalyptus* leaves which have a high lignin rate compared to the Acacia species (*A. cyanophylla* and *A. cyclops)*.

The application of high rate of leaves of *Eucalyptus* and *A. cyclops* (45 and 60 g per pot) decrease the top yield and N uptake of wheat (Table 3 and Table 4), this is presumably due to the excessive rate applied which created an important immobilization of soil N, it can be also due to liberation of toxins [29,30]. In fact, chemical analysis of leaves shows a relatively high content in polyphenols (Table 2) which can cause toxicity on wheat plants during decomposition in the soil. Numerous studies have assessed the influence of N availability by high content in polyphenols [31,27]. In this context Batish et al., in 2008 [32] reported that essential oil extracted from *Eucalyptus* species has an herbicidal effect against weeds. As well the studies of Jelassi, et al. in 2016 [33] showed that the phytochemical content and allelopathic activity of Acacia species, especially *A. cyclops*, showed a strong herbicidal activity on *Lactuca sativa* germination.

The addition of mineral nitrogen to decomposing leaves of *Eucalyptus* show an improvement of N uptake and dry weight of wheat (Tables 3 and Table 4). This suggest that additional fertilizer N was required to prevent loss of yield where low N organic material was incorporated into the soil. Similar findings were reported previously [34,35].

Applying of increasing rates of Ammonium sulphate to soil shows an improvement of N uptake of wheat (Table 4).

^{15}N Recovery in Wheat

Data of percent contribution of applied 15N into the total N of wheat is shown in Table 5. The results indicate that N uptake by wheat was affected by the rate of leaves incorporated. Plants in the control treatment utilized 52.21% of applied 15N Ammonium sulphate. Generally, the 15N-recovry declined with the increase rate of leaves added to soil or inorganic fertilizer Nitrogen especially for higher doses. The decrease rate was from 52.21% to 27.52% for *Eucalyptus* leaves, from 52.21% to 36.50% for *A. cyanophylla*, from 52.21% to 40.33% for *A. cyclops* and from 52.21% to 22.44% for N fertilizer. The decrease extent was more important with *Eucalyptus* than Acacias Leaves. This shows that the presence of *Eucalyptus* leaves reduced the plant uptake of 15N-Ammonium sulphate. Such results confirm increased immobilization of N in the presence of *Eucalyptus* leaves.

In a similar study, Azam et al., in 1985 [36] observed that contribution of 15N-Ammonium sulphate to different shoot parts of corn was about 40% in the presence of Sesbania aculeata residues and 50% in its absence. Results of 15N-recovry (Table 5) display that while the C/N ratio of the legume trees leaves was lower than *Eucalyptus* leaves, all the materials caused a net immobilization of soil mineral N when applied rate increase. Similar findings have been reported by Urquiaga, et al. in 1998 [15] and Sarkodie-Addo, et al. in 2006 [37] for roots of tropical forage species. Addition of increasing rates of fertilizer ammonium sulfate to soil reduced the 15N recovery of wheat. This can be explained by a preference use of easily available nitrogen of fertilizer by wheat plants rather than 15N tagged soil organic nitrogen.

Treatments	^{15}N recovery (%)						
Organic Materials (g pot^{-1})	0	15	30	30+N	45	60	$LSD_{0.05}$
Eucalyptus	52.21^{a}_{A}	49.22^{a}_{A}	38.45^{a}_{B}	33.23^{a}_{C}	$30.17^{a}_{C,D}$	27.52^{a}_{D}	3.52
A. cyanophylla	52.21^{a}_{A}	51.88^{b}_{A}	49.08^{b}_{B}	35.96^{a}_{C}	41.55^{b}_{D}	36.68^{b}_{C}	2.22
A. cyclops	52.21^{a}_{A}	$49.76^{a,b}_{A}$	49.37^{b}_{A}	44.84^{b}_{B}	45.08^{b}_{B}	40.33^{b}_{C}	2.99
$LSD_{0.05}$	-	2.45	4.55	3.54	5.52	4.55	
Nitrogen (Rate kgN ha^{-1})	0		20	40	80	120	$LSD_{0.05}$
	52.21_{A}		47.27_{B}	40.08_{C}	30.15_{D}	22.44_{E}	5.52

- Means followed by the same lowercase letter in same column are not significantly different by least significant difference LSD test at 5% of probability level;
- Means followed by the same capital letter in same line are not significantly different by least significant difference LSD test at 5%.

Table 5: ^{15}N recovery in wheat shoots after addition of different organic materials and fertilizer nitrogen to soil.

Conclusion

From the above discussion, we conclude that the incorporation of tree leaves with a high N content improve N availability in the soil. In the case of low nitrogen leaves such as *Eucalyptus* an additional mineral N is required to prevent N immobilization. The comparison between leaves organic matter and inorganic fertilizer N show that even if the quantity of leaves incorporated in soil is high, their effect did not reach the level of that of 80 kg N ha^{-1} as Ammonium sulphate. So Further studies under field conditions are needed to assess the effect of the incorporation of the leaves of the three trees studied on nitrogen availability and soil fertility.

Acknowledgements

Financial support from the FO/IAEA joint division is acknowledged. Our special thanks to Dr Robert Boddey and Dr Segundo Urquiaga from the EMBRAPA/CNPAB for their advices and the ^{15}N analysis.

Conflict of Interests: The authors declare no conflict of interest.

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