POTENTIALLY AVAILABLE SOIL NITROGEN IN FOREST ECOSYSTEMS OF THE NORTHWESTERN RIF REGION OF MOROCCO (CHEFCHAOUEN AREA)

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Abstract
Two experiments were conducted to assess potentially available soil nitrogen (N) in the forest stands around Chefchaouen (Rif mountains, Morocco) within three soil depths (root zone, A-horizon and B-horizon). Potentially available N from both anaerobic soil incubation (Nmin), and aerobic soil incubation (No)) were examined. The principal forest stands were *Pinus radiata, Pinus pinaster* and *Quercus suber* (cork oak). The values of Nmin ranged from 11 to 46 mg-N kg^{-1} and No from 29 to 54 mg-N kg^{-}1 at the surface soil with a significant decrease with depth for both. The rate constant (K) of N mineralization extended from 0.06 to 0.19 and the fraction of total kjeldahl N (TKN) that is potentially available is relatively very small (1 to 5) and varied significantly with depth.

Additional Key words: anaerobic incubation, aerobic incubation.

Introduction
The importance of nitrogen (N) for agriculture and forest productivity is well documented. Although a small part of plant needs may come from uptake of organic N, most plant N comes from the uptake in the soil of NH_4^+-N and NO_3^--N, which are made available by microbial mineralization of organic N and subsequent nitrification (Bremner and Keeney, 1965; Stevenson, 1987; Williams, 1989). In Morocco, studies related to N mineralization and the evaluation of its availability to plants have emphasized agricultural soils (Stitou *et al*., 1979; Chiang *et al*., 1983; Soudi *et al*., 1990a, b). Yet, in forest ecosystems N is most often limiting. Many different techniques for determining the rate of N mineralization have been developed (Miller and Keeney, 1982). In this study, both aerobic and anaerobic incubation techniques of soil samples under laboratory conditions were conducted to assess N mineralization. The main objective was to determine the availability of N mineralized under different forest stands and for different soil depths;

Materials and Methods
Three representative forest sites around Aïn Rami forest nursery (Chefchaouen region, Morocco) were used. The principal forest species were *Pinus radiata* and *Pinus pinaster* plantations, and *Quercus suber* (natural). The parent material is sandstone. Site elevation ranged from 600 to 900 meters. Mean annual temperature and rainfall at Chefchaouen C.T. were 18°C and 1005 mm respectively. The climate is Mediterranean and humid and the soils are mainly inceptisols and ultisols (soil taxonomy, USDA).

Two laboratory experiments were conducted to assess potentially available soil N: (i) anaerobic soil incubation during a one week time period, (ii) aerobic soil incubation during 1, 2, 3, 6, 9, 12, 15, 18, 21, 24 and 27 week time periods. In both experiments, triplicate sampling plots were selected from three forest stands: *Pinus pinaster, Pinus radiata* and *Quercus suber*. Sampling plots were selected for relative freedom of rocks to facilitate core sampling. At each plot, composite soils from three core samples were taken from root zone (a top layer with high root concentration), principal A-horizon and principal B-horizon. Because the forest floor was considered a site for N immobilization (Keeney, 1980), it was excluded from sampling. In the laboratory, soils were sieved through 2 mm-mesh to remove roots and large fragments from the samples.

Anaerobic incubation
The anaerobic incubation test proposed by Waring and Bremner (1964) was used. It involved the determination of NH_4^+-N produced when soil was incubated under waterlogged conditions at 40°C for 7 days. Soil samples were analyzed at time zero (to) before incubation and after one week. Soil samples weighing 5g were added to 16 by 150 mm test tubes containing 12.5 ml of distilled water. The soil was added gradually to ensure thorough wetting so that air pockets would not develop in the soil column. Preparation and soil incubation were done according to Keeney and Bremner (1966) using 4M KCl solution.

Static aerobic incubation
This technique was described by Harmsen (1955) and Vitousek et al. (1982). It involved the determination of NH_4^+-N and NO_3^--N produced when 25g of soil is incubated aerobically in small glass containers (5 cm diameter) at 30°C for 21 weeks. Soil moisture was kept at field capacity by monitoring water loss from the cups

gravimetrically and adding distilled water weekly as necessary. For aeration purpose, the cups were covered with plastic film containing small holes. In this experiment, soil samples weighing 10g were transferred into 250 ml wide mouth bottles to which 100 ml of 2M KCl were added. The potentially available N (No) and the rate constant of N mineralization (K) were estimated from the first order kinetic model (Smith *et al*., 1980).

Chemical analysis

Aliquot of soil KCl extracts were distilled with steam until 25 ml of distillate was trapped in 5 ml of boric acid mixed indicator solution (Bremner and Keeney, 1965). Magnesium oxide (MgO) and Devarda alloy were used to separate different forms of exchangeable inorganic N (NH_4^+-N and NO_3^--N). Titration was performed using 0.005N H_2SO_4. Results were corrected for distilled water blanks and adjusted for oven dry weights. Total Kjeldahl N (TKN) was determined according to a modified Bremner (1965) technique (Sefrioui *et al*., 1971).

Statistical analysis

For both experiments, the means and standard deviations were determined and compared for all measured N mineralization parameters. Site and depth effects on potentially available N from anaerobic and aerobic incubations were tested by one way ANOVA for root zone samples and by two-way factorial ANOVA for A and B-horizon samples (Statgraphics program).

Results and Discussion

Soil characteristics

Soil is an essential link in the ecological system. In forests, mineral nutrition and water supply to plants are dependent on soil. Also, N mineralization and microbial activity are related to its physicochemical qualities (Allison, 1973). Needed soil features of the studied sites are given on table1.

Anaerobic incubation

In this experiment, available N in the root zone (Nmin), does not show much variation among the three sites (Table 2). As expected, there were significant differences of Nmin between A and B-horizon soil material at all sites (Table 2). The much lower Nmin of B-horizons relative to A-horizons is consistent with the generally lower TKN of the B-horizons (Table 1); however, the ratio of Nmin to TKN, also, decreased significantly with depth (Table 2). This suggests that not only is there less N in the B horizon, but also that it is less available to microbial mineralization. The decrease of TKN with depth (Table 1) is similar to the trend reported elsewhere (Vitousek, 1982, Ben Jelloun, 1993). However, both TKN Table 1) and Nmin values (Table 2) are lower than those reported in other forest sites at higher elevations in the area (Ben Jelloun, 1993), but are somewhat similar to the ones reported by Powers (1980). One possible explanation is that, at lower altitudinal sites there is less N input into the system through litterfall. This litterfall N may be quickly mineralized, leached or exported by runoff resulting in low soil TKN which is also relatively unavailable for N mineralization.

Table 1. Selected soil characteristics at three soil depths (root zone, A-horizon and B-horizon) of the studied sites.

	Soil* depths	PH (H_2O)	Org.C.* (%)	TKN* (%)	C/TKN	BD* (g/cm3)	Mechanical analysis (%)		
							Sand	Silt	Clay
P. radiata	RZ	5.2	2.1	0.1	21	1.0	62.4	21.8	15.8
	A	5.2	1.8	0.1	16	1.0	62.7	21.1	16.2
	B	5.1	1.4	0.1	14	1.1	62.6	19.6	17.8
P. pinaster	RZ	5.9	3.9	0.2	26	0.9	31.6	30.4	38.0
	A	5.9	3.9	0.1	35	0.9	31.6	30.4	38.0
	B	5.0	1.9	0.1	19	1.0	30.8	20.9	48.3
Q. suber	RZ	5.5	4.0	0.1	31	1.0	28.0	41.9	30.1
	A	5.6	5.2	0.2	35	1.0	29.3	42.7	28.0
	B	5.1	2.5	0.1	26	0.9	12.3	22.8	64.9

*** RZ** : root zone ; **A** : A-horizon ; **B** : B-horizon. **Org. C**. : organic carbon. **TKN** : total Kjeldahl nitrogen.
BD : bulk density. All values are rounded to one decimal.

Table 2.Variation of anaerobic potentially available soil nitrogen (Nmin) and of the ratio Nmin/TKN (mean values and their CV,s).

	Variables	Test statistique P≤0,05*	Radiata pine	Pinaster pine	Cork oak
Root zone	Nmin (mg kg^{-1})	NSS	17.8 (42)**	22.8 (23)	18.2 (10)
	Nmin/TKN (%)	NSS	1.6 (35)	1.7 (37)	1.5 (20)
A horizon	Nmin (mg kg^{-1})	SS	11.5 (44)a	30.9 (17)ab	45.6 (22)b
	Nmin/TKN (%)	SS	1.0 (33)a	3.0 (23)b	3.0 (01)b
B horizon	Nmin (mg kg^{-1})	NSS	7.2 (90)	3.7 (08)	3.4 (0.1)
	Nmin/TKN (%)	NSS	0.6 (83)	0.4 (10)	0.4 (17)

* NSS : No statistically significant difference among sites at 5 % P value. SS : there is a statistically significant difference among sites at 5 % P value.
** Values in brackets are coefficients of variation (CV,s) Values with same alphabet letters in horizontal cells constitute homogenous groups (Fisher's least significant difference : LSD)

Aerobic incubation
Net N mineralization as measured by static aerobic incubation occurred continually throughout the incubation period. During this time, the N produced in the root zone samples ranged from about 50 mg-N kg^{-1} in *Quercus suber* (ork oak) and *Pinus pinaster* sites to over 75 mg-N kg^{-1} in *Pinus radiata* sites. For these three sites, net N mineralization in the A-horizon was quite similar to that in the root zone and both were about two folds or more, greater than in B-horizon soils.

In this study, attempts were made to fit a first order kinetic model (Stanford *et al.*, 1972) to aerobic N mineralization data. N mineralization potential N (No), as measured from the model, has been defined as the fraction of organic N pool that is susceptible to mineralization. The goodness of fit of the model to the data as expressed by r^2 (Table 3) showed higher values for surface horizons (root zone and A-horizon) relative to the subsurface B-horizon.

Table 3. Estimated mean values and their coefficients of variation (CV's) of potentially available Nitrogen (No), rate constant (K), maximized r^2 and ratio No/TKN for study sites. The model used is : Nm* = No.[1-Exp(-K.t)].

	Root zone				A horizon				B horizon			
	No	K	r^2 **	No/TKN	No	K	r^2	No/TKN	No	K	r^2	No/TKN
Radiata pine	45 (21)	0.19 (5)	87 (5)	4.79 (24)	54 (24)	0.18 (11)	86 (3)	4.99 (14)	27 (46)	0.13 (14)	64 (15)	2.51 (33)
Pinaster pine	45 (51)	0.14 (50)	90 (25)	2.68 (35)	31 (5)	0.16 (32)	81 (13)	3.03 (38)	11 (5)	0.12 (5)	64 (17)	1.14 (8)
Cork oak	29 (36)	0.11 (31)	78 (14)	2.67 (53)	32 (23)	0.16 (14)	82 (9)	2.15 (17)	17 (39)	0.06 (20)	42 (44)	1.78 (21)

* Nm : quantity of mineralized N at time t (after 1, 2, 3, 6, 9, 12, 15, 18, 21, 24 and 27 weeks) of aerobic incubation, No : estimated potentially available N, K : rate constant of N mineralization, t : time of N mineralization and r^2 : coefficient of determination.
** Each value in table is the mean of three observations (triplicates) and the values in brackets are coefficients of variation (in %) between triplicates.

Nitrogen mineralization potentials (No) and the rate constant (K) as estimated from the first-order model are variable among sites and within sites (CV values). However, the among site variation is not statistically significant (P≤0.05) for either No, K and No/TKN may be, because of high within site variability (Table 3). As expected, No and K decreased significantly with depth. This decrease with depth is consistent with the lower values of TKN at