Nitrogen Mineralization Experiment under Fir Stand with Different Measurement Methods

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ABSTRACT— ***Within the current study nitrogen mineralization under East Black Sea Fir (Abies nordmanniana* (Steven) Spach*) stand at Atatürk Arboretum has been investigated. Closed top solid cylinder, buried bag, laboratory incubation and ground samples were taken from a 30 x 30 m^2 sampling area during a year. Soil samplings were carried out from April 2013 to 2014 for field and laboratory incubation. We tested (i) to what extend is the method efficient on the amount of mineralization, (ii) to what extend mineralization amount differs seasonally and (iii) is there any correlation between nitrogen pool of soil and mineralization rates under in situ and in vitro conditions. Our results revealed that both seasonality and incubation methods were efficient on mineral N amounts. Ammonium-N accumulation values with tube incubation samples were significantly higher at summer incubation periods while opposite at samples incubated at laboratory conditions. We found significant difference in terms of incubation method (Closed top cylinder and buried bags) at August and December periods for Nitrate-N (mg-N/kg/day) values. The lack of correlation between total nitrogen and/or initial total mineral nitrogen and ammonium mineralization or nitrification implies unimportance of mineral N pool instead, furthermore possible addition or removal of organic and mineral nitrogen fluxes takes accounts to a more determinant factor.***

Keywords— Ammonium, nitrate, fir, nitrogen.

1. INTRODUCTION

Nitrogen plays a key element role at plant nutrition as a fertilizer (Kim et al. 2008), its transformation and fluxes create mutual influences with global climate change (Vitousek et al. 1997, Rustad et al. 2001) and is a determinant factor of site productivity (Reich et al. 1997). At the temperate zone like Turkey the nitrogen mineralization is under the intensive manipulation of climatic conditions by its efficient parameters such as temperature and precipitation. Nitrogen is generally a predominant element on plant growth (Stump and Binkley 1993) and mineralization of nitrogen highly dependent upon plant species (Klopatek 1987; Finzi et al. 1998), soil organic carbon amount, microbial population functions and microbial biodiversity (Merillä et al., 2002; Smithwick et al., 2005) and the climate reigns at the region (Rovira and Vallejo 1997). Understanding the accumulation or loss of N, characteristics of nutrient cycles and the effects on the amount of N in the compartments of the ecosystem requests comprehensive studies on N mineralization (Ross et al. 2004). The study site Atatürk Arboretum has been established in 1949 on 296 ha area by Kayacık at Belgrad forest in Istanbul with educational purposes for public and scientists (Yaltırık 1988). In context of arboretum approximately 2000 plant taxa including Turkey's main plant species were planted and sample stands from representative species of surrounding climatic regions were structured in the area.

Black sea fir (*Abies nordmanniana* (Steven) Spach) makes approximately 406 989 ha at its natural distribution areas (OGM 2014) in Turkey. Its surficial and highly dense root stem supports fir species advantageously competitor against the accompanying tree species and very limited ground vegetation is permitted. Fir with its shade tolerant physiology thus providing limited light penetration, suppressed evapotranspiration, and higher water potential causing lower temperature makes significant restraint on soil nitrogen mineralization (Sierra 1997). Studies on nitrogen mineralization modelling concludes indirect effect of water potential (Paul et al. 2003, Pastor and Post 1986).

On the other hand, the N mineralization rate is highly dependent upon the chemistry of the tree species by being determinant on the quality of leaf litter sourced organic matter. McNulty et al. (1996) found a strong positive correlation between forest floor and net nitrogen mineralization.

Nitrogen budget and nutrition status of a given site can be determined by many methods that some of which provide indirect estimation about the nutrition status such as microbial nitrogen biomass, nitrogen nutrition of plants; and some of which provide precisely status of nitrogen forms but they are expensive methods such as isotopic dilution methods, isotopic enrichment methods (Davidson et al. 1991). In this study two practical *in situ* methods and the *in vitro* laboratory incubation method results were compared. Within the target of study we intended to clarify micro differences sourced from the incubation methods in the field and made laboratory incubation to compare the field sampling results. We questioned that (I) to what extend does the method efficient on the amount of mineralization, (ii) to what extend mineralization amount differs seasonally and (iii) is there any correlation between nitrogen pool of soil and mineralization rates under *in situ* and *in vitro* conditions.

2. MATERIAL AND METHODS

The study area (Atatürk Arboretum) is located in Istanbul (Turkey) near Belgrade forest. Geographical position of the area is 41°10'70'' northern and 28°59'00'' eastern latitudes and longitudes respectively. The climate of the area is Mediterranean climate with dry summers according to Köppen-Geiger with the code Csa. Elevation is 131 m above sea level with peneplain topography. The study site is at the ridge position, receiving 888mm annual precipitation and annual average temperature is 13.4°C (Anon.). Soil is formed on plyosen accumulation material. Soils are sandy clay.

Soil samplings were carried out from April 2013 to 2014 for field and laboratory incubation. The sampling points in the sample area were chosen to be excluding the rhizosphere effect of trees. At the beginning of the study 210 pvc tubes (50 mm inner diameter and 250 mm length) were installed to the sampling points providing to harvest 10 tripled sub samples. At first sampling date (10th of April 2013) the tops of the first three pvc tubes were covered with the stretch film that allows the gas transfer but prevents the entrance of water inside. Additionally sealed soil bags were prepared and buried due to the method procedure to be incubated for 60 days. Immediately after initiation of the in situ incubations another 10 tripled sub samples were taken from near the sub sampling points. Subsamples of soils were collected with an excavating dug and stored in polyethylene bags until reaching the laboratory in a cooler (2-5 °C) to impede biological activity.

Soils from cores are composited in the laboratory for extraction and analysis (10 composite samples for the sampled area per incubation type). Coarse fraction from each soil sample was removed and the fine fraction was saved for analysis. These soil samples provided the initial (t_o) concentrations of NH_4^+-N and NO_3^--N for the incubation. A well-mixed and sieved from 4mm mesh size 10-g dry-soil equivalent subsample is extracted with 50 ml of 2 M KCl. The suspensions are shaken on a mechanical shaker for 1 h and centrifuged for 5 min. at 10 000 rpm. The filtered extracts are then analyzed for NH_4^+-N and NO_3^--N with a Foss Fiastar 5000 Auto Analyzer system. Then, 10 g equivalent soils were weighed to the plastic seals and covered with thin plastic wraps to allow gas exchange but minimize water loss, and incubated for 30 days at room temperature (21°C). The plastic seals had 5 cm inner radius. Separate soil samples are taken from each soil to determine the gravimetric soil water content to calculate net soil weights subjected to mineral N content analyzes. Net N mineralization is calculated by subtracting the initial quantity of soil inorganic-N from the post-incubation quantity of soil inorganic N. Similarly, net nitrification is calculated as the change in NO_3^--N pool size over the incubation period.

Soil samples were air-dried and then the skeleton and coarse woody debris, containing the roots, were removed, ground and sieved from a 2 mm-sized mesh for physical soil analysis. Four soil samples were collected for physical soil analysis per plot. Separate soil sub-samples were oven-dried at 105°C for 24 h (at hygroscopic moisture). The soil particle distribution was determined according to Bouyoucous hydrometer method and the soil texture composition was found with the International Particle Size Distribution triangle. Actual soil pH was determined in a suspension of 1:2.5 of sample in distilled water. Soil organic carbon (SOC) was determined by Walkley-Black method, the total N was detected with a Buchi Auto Kjeldahl Unit K-370.

The data collected from laboratory incubations and samplings were subjected to proper statistical evaluation. As the assumptions of normality and homogeneity of variances were met, then the ammonium-N and nitrate-N budgets were analyzed with ANOVA Tukey HSD. The significance levels were applied at $p<0.05$ and 0.01 in convenience. Analysis of variance and descriptive statistics were executed with anova and summary functions under Stats package version 3.2.2. in free software R statistical program version 3.2.2.

3. RESULTS

Total nitrogen and organic carbon amounts of buried bag incubation samples were almost double and two of third of the other incubation types respectively. pH and electrical conductivity values were very close to each other (Table 1).

Table 1. Descriptive statistical data for soil properties per incubation type.

Incubation Type*	Measured Parameter	N	Min.	Max.	Mean	Std. Dev.
Tube	Total Nitrogen (%)	35	0	0.29	0.069	0.068
	Organic Carbon (%)	50	0.01	4.31	2.32	0.936
	pH	42	4.82	6.02	5.327	0.260
	Electrical Cond. µS/cm	50	64	348	151.06	64.270
Bag	Total Nitrogen (%)	35	0.02	0.38	0.125	0.109
	Organic Carbon (%)	57	0.79	5.87	3.048	0.903
	pH	42	4.57	6	5.319	0.324
	Electrical Cond. µS/cm	50	33.3	338	149.434	62.06
Ground	Total Nitrogen (%)	42	0	0.17	0.067	0.049
	Organic Carbon (%)	66	1.07	4.88	2.427	0.864
	pH	50	4.55	6.04	5.224	0.320
	Electrical Cond. µS/cm	58	55.9	291.1	121.833	45.321

*Laboratory incubation samples were subsamples of ground samples so they are omitted here.

Laboratory incubations revealed the highest mineralization amounts when we examined the data (mg-N/kg/day) in terms of incubation method except the incubation period in June (Table 2). Ammonium-N amounts (mg-N/kg/day) of closed top cylinder and buried bags did not show significant differences at incubation method base (Table 2, Figure 1).

We found significant difference in terms of incubation method (Closed top cylinder and buried bags) at August and December periods for Nitrate-N (mg-N/kg/day) values. Closed top cylinder method revealed the highest nitrification during June, August and October periods. However laboratory incubation periods revealed the highest nitrification amounts at December, February and April periods (Table 3, Figure 2).

Table 2. Daily ammonium-N accumulation at sampling periods per incubation method.

	Sample Type	Jun^{ns}	Aug*	Oct ^{ns}	Dec*	Feb ^{ns}	Apr*
NH_4^+-N mg kg^{-1} d^{-1}	Tube	2.022 ^{a}	0.2332 ^{a}	1.5173 ^{a}	0.1573 ^{a}	0.3249 ^{a}	0.0732 ^{a}
	Bag	0.076 ^{a}	0.1849 ^{a}	0.1280 ^{a}	0.1903 ^{a}	0.1210 ^{a}	0.1106 ^{a}
	Ground	-0.066 ^{a}	0.0148 ^{a}	-0.0056 ^{a}	0.184 ^{a}	-4.2764 ^{a}	-0.0991 ^{a}
	Lab	0.6127 ^{a}	0.6256 ^{b}	1.6270 ^{a}	0.835 ^{b}	0.7261 ^{a}	1.2743 ^{b}

Note: Column values with different superscript letters are significantly different from one another. Significance levels are: NS not significant, and *P < 0.05.

Table 3. Daily nitrate-N accumulation at sampling periods per incubation method.

	Sample Type	Jun^{ns}	Aug*	Oct^{ns}	Dec*	Feb*	Apr*
NO_3^--N mg kg^{-1} d^{-1}	Tube	0.6092 a	0.3117 b	1.9578 a	0.6989 bc	0.3593 ab	0.2446 a
	Bag	0.1518 a	0.164 ab	0.0785 a	0.3186 ab	0.418 ab	0.1767 a
	Ground	0.0133 a	0.0046 a	-0.0123 a	0.1336 a	-0.5408 a	-0.1451 b
	Lab	0.119 a	0.2235 b	0.0895 a	1.0407 c	1.326 b	0.3361 a

Note: Column values with different superscript letters are significantly different from one another. Significance levels are: NS not significant, *P < 0.05.

We determined ammonium mineralization of tube incubation samples at spring, summer and autumn periods (from April to June, June to August, August to October and October to December). The tube incubation samples ranged between -6.908 – 9.482 mg NH_4^+-N kg^{-1} ammonium mineralization for April to June and December to February periods respectively. At bag incubation samples we determined consistently minus values revealing microbial immobilization except June to August and October to December incubation periods. Highest ammonium mineralization at bag incubation samples was detected at June to August incubation period as 4.137 mg NH_4^+-N kg^{-1} and the lowest value was at December to February incubation period as -13.204 mg NH_4^+-N kg^{-1}. Laboratory incubation samples resulted significantly higher ammonium mineralization at all incubation periods with a range 9.741 and 41.309 mg NH_4^+-N kg^{-1} at December to February and August to October incubation periods respectively (Table 4).

We determined the highest nitrification at tube incubation samples at April to June, June to August, August to October and October to December incubation periods. The tube incubation samples ranged between 1.193 – 39.276 mg NO_3^--N kg^{-1} nitrification for February to April and October to December periods respectively. At bag incubation samples we determined minus nitrification value only at the February to April incubation period whilst illustrating a wavy line

during the year. The lowest nitrification at laboratory incubation samples were at April to June, June to August, August to October and October to December incubation periods with a range 1.588 and 25.763 mg NO_3^--N kg^{-1} at August to October and December to February incubation periods respectively (Table 5).

Table 4. Year round ammonium-N mineralization at sampling periods per incubation method.

	Sample Type	Apr to Jun*	Jun to Aug ns	Aug to Oct*	Oct to Dec ns	Dec to Feb*	Feb to Apr*
NH_4^+-N mg kg^{-1}	Tube	9.482 a	7.046	9.295 a	1.305	-0.964 a	-6.908 a
	Bag	-6.345 b	4.137	-0.160 a	2.347	-13.204 a	-4.049 a
	Lab	11.426 a	13.393	41.309 b	10.420	9.7411 b	30.233 b

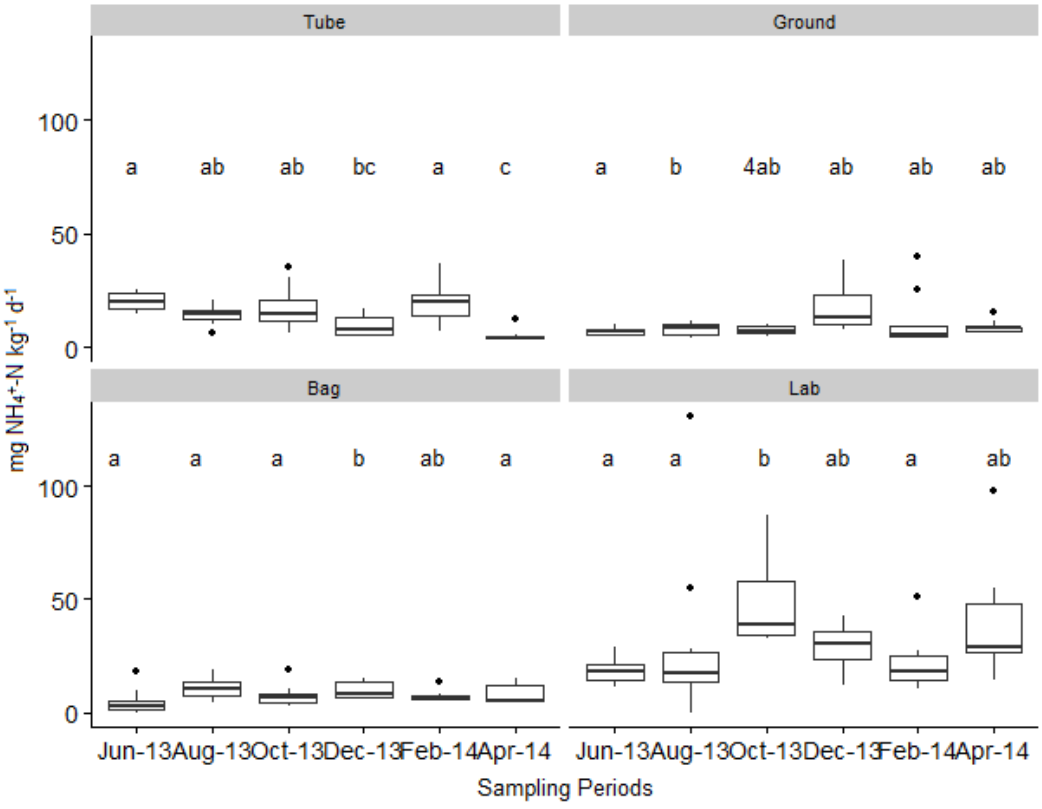
Note: Column values with different superscript letters are significantly different from one another. Significance levels are: NS not significant, *P < 0.05.

Table 5. Year round nitrification at sampling periods per incubation method.

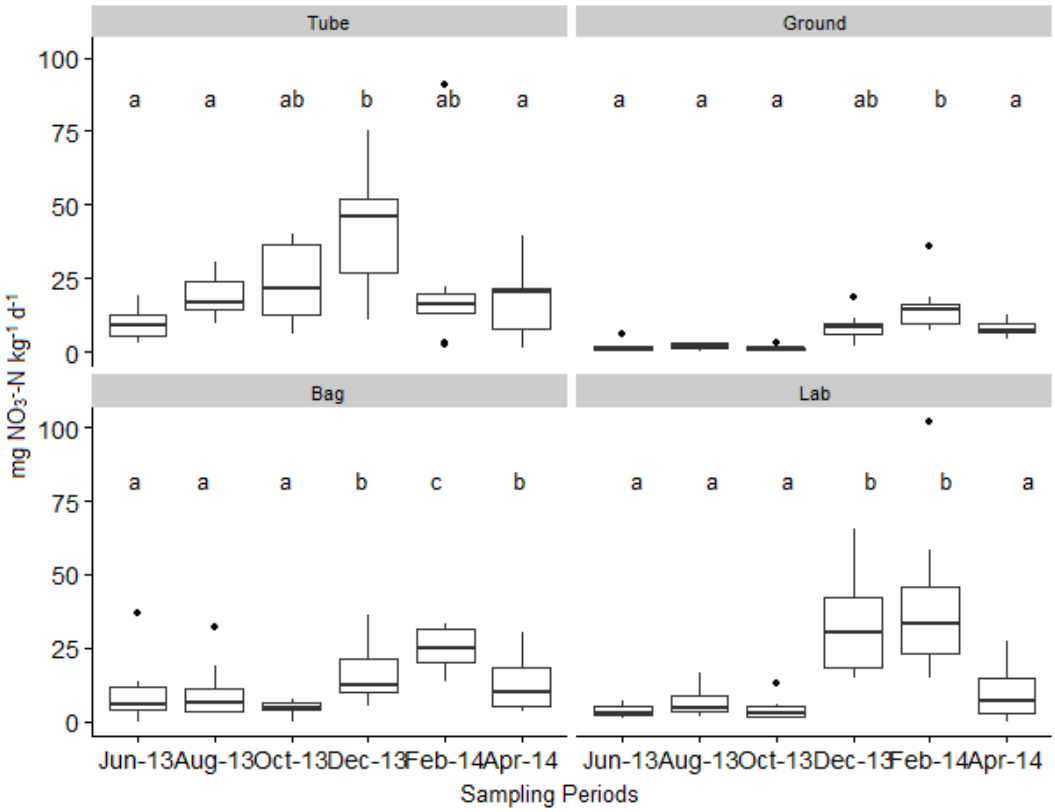
	Sample Type	Apr to Jun ns	Jun to Aug	Aug to Oct	Oct to Dec	Dec to Feb ns	Feb to Apr ns
NO_3^--N mg kg^{-1}	Tube	8.985	17.150 a	22.136 a	39.276 a	11.508	1.193
	Bag	8.333	8.281 b	2.863 b	15.095 b	15.023	-3.224
	Lab	2.004	5.027 b	1.588 b	23.924 ab	25.763	3.071

Note: Column values with different superscript letters are significantly different from one another. Significance levels are: NS not significant, *P < 0.05.

Ammonium-N amount did not show significant difference according to sampling period while nitrate-N amounts increased disregarding the incubation method (Figure 1 a, b). Nitrification amounts for laboratory incubation were the highest amongst the incubation types while tube, bag and ground samplings did not show significant difference among each other.



(a)



(b)

Figure 1. Method based comparison of (a) ammonium-N mineralization and (b) nitrification amounts during the year.

Significant correlations could be detected between nitrate-N amounts and organic carbon (%) and electrical conductivity with r^2 values 0.372 and 0.433 respectively ($p<0.01$). Total nitrogen had significant correlation with organic carbon (%) and electrical conductivity too with r^2 values 0.334 and 0.367 respectively ($p<0.01$).

4. DISCUSSION AND CONCLUSION

Main issues executed in this study were seasonal change of nitrogen mineralization and comparison of different incubation methods under an introduced species of fir stand. The tree species, ecosystem type, soil properties and related parameters that are accounted to be influential on net nitrogen mineralization are crucial for both basic and the most complicated ecosystem studies (Aber et al. 1998, Allison 1965, Binkley 1992, Côté et al. 2000). Indeed Adams (2003) concluded higher attention on nitrogen mineralization as to bring a better understanding to quantification of N cycle in soil. We determined mineral nitrogen leaching plus microbial immobilization phenomenon only at ground measurements. We also determined significant differences between *in situ* and *in vitro* incubation methods mainly to the advantageous to laboratory incubation method. The main difference at status of soils exposed during incubation between closed top cylinder and buried bag method are the slight effect on soil compaction and disturbance of intact soil (Raison et al. 1987) respectively. While ground soil samples are subjected to higher evaporation, radiation, leaching, plant uptake and microbial sequestration, the *in situ* methods are exceptional from leaching, plant uptake.

Ground measurements represent the mineral nitrogen pool per period prior to incubation and year round mineral nitrogen change. According to ground measurements during June, August and October we detected almost zero daily nitrate and ammonium-N accumulation. Since those days received the lowest precipitation, this result could be referred to microbial immobilization (Figure 1). However we assume that the microbial population is not significantly different per incubation method since the sampled soils are from adjacent points, ground soils subject to be infectious microbial migration. Possible microbial population caused differences could be inferred from the results of less mineralization or immobilized mineral nitrogen at ground samples (Figure 2). Besides, higher ammonium-N, nitrate-N accumulation, ammonium mineralization and nitrification for the year round samplings at tube, bag and laboratory incubations give more evidence to differences at microbial populations.

We detected correlation between nitrification and organic carbon amount revealing the organic carbon induced nitrification (Taylor and Townsend 2010).-Studies on saline soils expressed less ammonification at higher amounts of

salt, stimulated at lower concentrations while salinity is notably makes sensitive the nitrification (Westerman and Tucker, 1974; McCormick and Wolf, 1980). Our findings on correlation between soil electrical conductivity and nitrification (r^2 0.433 at $p<0.01$) shows the nitrate production of soils are under the management of microbial enzyme's activity that are functional at this environment.

From the results of current study, we can conclude that while applying any in situ incubation method for net nitrogen mineralization measurements, the data for the surrounding conditions and soil disturbance at which extend has been done should clearly be stated. Although Khanna and Raison (2013) tested changes in soil temperature and moisture conditions, effect of disturbance of soil structure, effect of the tube material used, effect of root severance on N dynamics, effect of mineral N accumulation and N losses, changes in microbial and faunal composition and activity, and the consequences of uptake of organic N by roots, they still are unsure about using this method for routinely managing the N cycles. To be valid limited to current study, the lack of correlation between total nitrogen and/or initial total mineral nitrogen and ammonium mineralization or nitrification implies unimportance of mineral N pool instead, furthermore possible addition or removal of organic and mineral nitrogen fluxes takes accounts to a more determinant factor.

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