E3 Journal of Environmental Research and Management Vol. 4(4). pp. 0234-0241, May, 2013
Available online http://www.e3journals.org
ISSN 2141-7466 © E3 Journals 2013

Full Length Research Paper

Nitrogen cycling in seasonally dry coffee (*Coffea arabica* L.) agroecosystem of southern Ethiopia

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Accepted 18 April, 2013

Nitrogen (N) cycling is presumed to be closed in most low input agroecosystems. This is because N cycles in a short circuit between vegetation, soil and microorganisms. However, information is lacking with this regard in low input coffee soils of Ethiopia. To this end, two experiments were conducted; the first experiment assessed carbon (C) and N mineralization in laboratory incubation under different moisture regimes (25%FC, 50%FC, 75%FC and 100%FC), whereas the second experiment investigated N mineralization via unconfined in-situ soil sampling. The soil revealed low bulk density, good aggregate fraction and higher aggregate stability (82%). Drought stress significantly reduced the rates of C (89%) and N (75%) mineralization, suggesting low N cycling in dry season. The net N mineralization and nitrification rates tended to be greater under in-situ than laboratory incubation. Average in-situ nitrification rate was about 30-40% of mineral N, despite the fact that the soil is relatively acidic. Hence, leaching and denitrification loss of N might be possible as the soil moisture in the wet season was found to be higher. This finding suggests that the anticipated climate change that results in reduction of rainfall may substantially reduce the rates of SOM decomposition and N cycling.

Keywords: Carbon mineralization, climate change, carbon sequestration, denitrification, drought stress, nitrate leaching

INTRODUCTION

In low external input farming systems of the tropics, the main sources of nutrients are decomposition of soil organic matter (SOM) and crop residues (Abera, 2012). These organic resources provide a significant proportion of nitrogen (N) necessary for plant growth, which is most limiting after availability of water in dry tropical climate conditions (Burke et al., 1997; Schlesinger, 1997). However, N mineralization is substantially affected by SOM quality, soil moisture, temperature, management practices and soil types (Goncalves and Carlyle, 1994; Abera, 2012).

Southern and southwestern parts of Ethiopia are the largest coffee production zones in the country. More than 80% of the coffee production of Ethiopia has been characterized to come from low input smallholder farming systems under overstory (shade trees). This overstory includes some leguminous shade trees such as *Millettia ferruginea*, *Albizia gummifera* and *Albzia schimperiana* (Hundera et al., 2013) that fix atmospheric N_2 in association with symbiotic rhizobia. This legume trees contribute biologically fixed N fertilizer for the low input coffee production systems, besides sequestering substantial amount of C in its biomass and soil.

The coffee production system of Ethiopia predominantly relies on low input that concentrates on use of organic materials such as compost and farmyard manures (Abera et al., 2012a). In fact, the litter and root exudates of coffee and its shade trees are the largest sources of input in this system. The litter serves as sources of fertilizer through decomposition over a short term, while building soil organic matter over long term. This biologically mediated process is regulated by the amount and chemistry of organic matter returned to the soil from above and below ground plant parts (Babbar and Zak, 1994). Nevertheless, studies are very much limited with regards to rates of litter production from both coffee and shade trees and its rates of mineralization to provide essential nutrients. Soil organic matter provides a

slow but important release of inorganic nitrogen to plants via decomposition, thus crucial for low input systems such as smallholder coffee farms of Ethiopia. Generally our understanding of the coffee agroecosystem N budget is incomplete; however, this information is very crucial from agronomic and environmental perspective.

Optimum N fertilization depends on the proper knowledge of the rates of SOM mineralization, which is important to improve N use efficiency and minimize leaching and denitrification loss of N. Mineral N in soil may exist either in ammonium or nitrate form. This N species depends on soil climate, substrate availability and microbial activity involved in the formation process of each specie. According to Babbar and Zak (1994), if nitrification is an important process within coffee agroecosystems, any N in excess of plant demands has the potential to be lost through leaching and denitrification.

Efficient N recycling in this dry agroecosystem depends on a synchrony between the production of mineral N and plant N uptake. For example, accumulated NO_3 during dry season, could easily leach and/or denitrified if large rain events follow, complicating the ability to meet goals of agricultural management. Exploring the effect of drought stress on soil N mineralization helps to understand the rate of N release in relation with crop N requirement. Furthermore, understanding the rate of N mineralization potential of soil could help to identify and develop appropriate management interventions necessary to maximize the N use efficiency, thereby reduces the N fertilizer cost and loss to the environment (Abera et al., 2012a). Thus, the primary objective of this study is to examine the effect of drought stress on N mineralization and cycling in coffee agroecosystem soils under laboratory and in-situ conditions.

MATERIALS AND METHODS

Soil Sampling And Site Description

Fifteen soil samples were randomly collected from 0-15 cm depth of standing coffee plots of Yirgalem area, southern Ethiopia for laboratory incubation. Each of five samples was bulked into one composite, thus representing a total of three replications. The sampling site was situated 6°45′ N and 38°38′ E, at about 1835 m a.s.l. Texture of the soil was clay, while its pH was slightly acidic (5.5). The soil had 2.44% total carbon, 2.26% organic carbon, 0.15% total nitrogen and 2.5 ppm phosphorous (Abera et al., 2012b). It is well aerated at field capacity (23.2 vol %), low in bulk density (0.96 g cm^{-3}) and holds higher (40-64%) water across field capacity to saturation water potentials. The long year average temperature of the study site was 18.7 °C yr^{-1}, while its total annual rainfall was 1230 mm yr^{-1}. The site was characterized by sub-humid agroclimatic condition (Abera, 2012). From the same site, unconfined in-situ soil samples were collected every 30 days during December 2009 to August 2010 from a fixed plot area (5 x 5 m). Soil moisture (vol%) was periodically measured by Time Domain Reflectrometry (TDR), concurrently with soil sampling for N mineralization. The relationship between soil moisture measured during December 2009 to August 2010 and long term rainfall (r^2=0.89) of the study site is given in Figure 1.

Soil Physical Analyses

Soil samples were air dried at Hawassa University and subsequently freighted to Norwegian University of Life Sciences, Norway for undertaking most of the measurements. First the whole samples were mechanically shaken for three minutes using nested dry sieves of 0.6, 2, 6 and 20 mm and aggregates were collected under each sieve. The aggregate sizes collected were: <0.6, 0.6-2, 2-6, 6-20 and >20 mm (Børresen, 2003).

The aggregate stability (AS) was measured with 20 g air dry soil for the two macro aggregate fractions (0.6-2 and 2-6 mm) using an artificial rainfall simulator (Børresen, 2003). The dry aggregate samples were placed on a wet 0.5 mm sieves. The sieves were then placed in a rotating disk at a distance of 31.5 cm from the nozzles. Water drops were applied by 4 nozzles in a form of rain simulation. The water pressure of 1.5 kg cm^{-2} is applied for 3 minutes. The AS of soils was estimated as weight of dry sample after rain simulation divided by weight of dry sample before rain simulation.

The soil bulk density was determined based on core sampled soils by an oven drying at 105 °C for 24 h. Soil samples were gently ground and sieved through a 2 mm size sieve before the particle size analysis was performed according to the pipette method (Day, 1965). Total porosity (P_t), the percentage of bulk volume of soil not occupied by solid particles was determined by saturation. Water-filled pore space (WFPS) was calculated as the ratio of the volumetric soil water content to the total pore space (Saggar et al., 2004).

Laboratory Incubation Under Different Moisture Regimes

First, soil moisture was set at pF2 by a pressure plate apparatus and afterwards desired soil moisture levels (treatments, 25%FC, 50%FC, 75%FC and 100%FC) were obtained by proportionally mixing air dry and wet soils. Accordingly, the moisture content of wet and air dry soils were determined by oven drying for 24 h at 105 °C. Soils (each 10 g dry soil) were incubated separately for carbon (120 mL serum flasks) and nitrogen (50 mL tubes) mineralization determinations. After applying soils, the

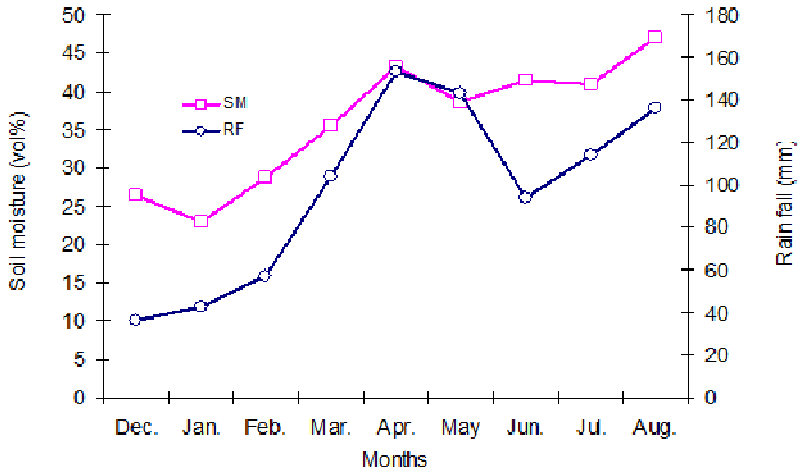

Figure 1: Relationship between soil moisture, SM (vol%) measured during in-situ soil sampling (December 2009 to August 2010) and long term rainfall, RF (mm) of the study site.

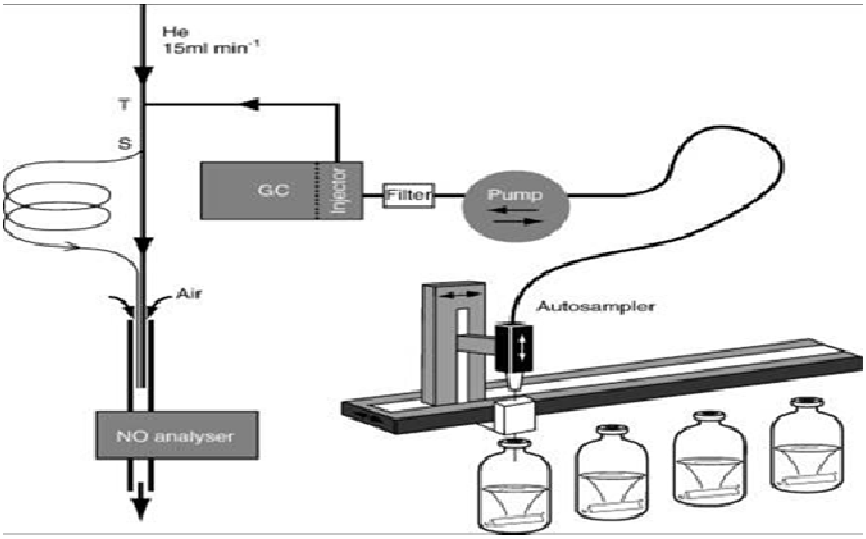

Figure 2: The robotized gas sampling and analysis system (Molstad et al., 2007).

flasks were immediately sealed with butyl rubber septa, while tubes were capped to avoid moisture loss. Three gas standards (low, air and high standard) were employed for calibration and assessment of the sampling dilution (Molstad et al., 2007). The system monitors CO_2 flux and O_2 consumption, as well as CH_4, N_2, NO and N_2O (Figure 2). Thus, CO_2-flux was used as a measure of respiration.

Mineral Nitrogen Determination

Mineral N (NH_4^+-N and NO_3^--N) content was determined for laboratory incubation and unconfined in-situ soil sampling experiments. Three and five replicates were involved, respectively for laboratory incubation and in-situ sampling. Mineral N extraction was done by dispersing 10 g soil dry weight in 25 mL of 2 M KCl. The soil slurries were shaken at 180 strokes per minute in 50 mL flasks for 30 minutes in reciprocal shaker, and settled for 10 minutes and filtered through Whatman #42 filter paper. The filter paper was pre-washed with 0.2 M KCl to avoid mineral N contamination. Subsequently NH_4^+-N and NO_3^--N contents were measured by colorimetric method using spectrophotometer (Spectroquant test kits, www.merk-chemicals.com) at Hawassa University or by flow

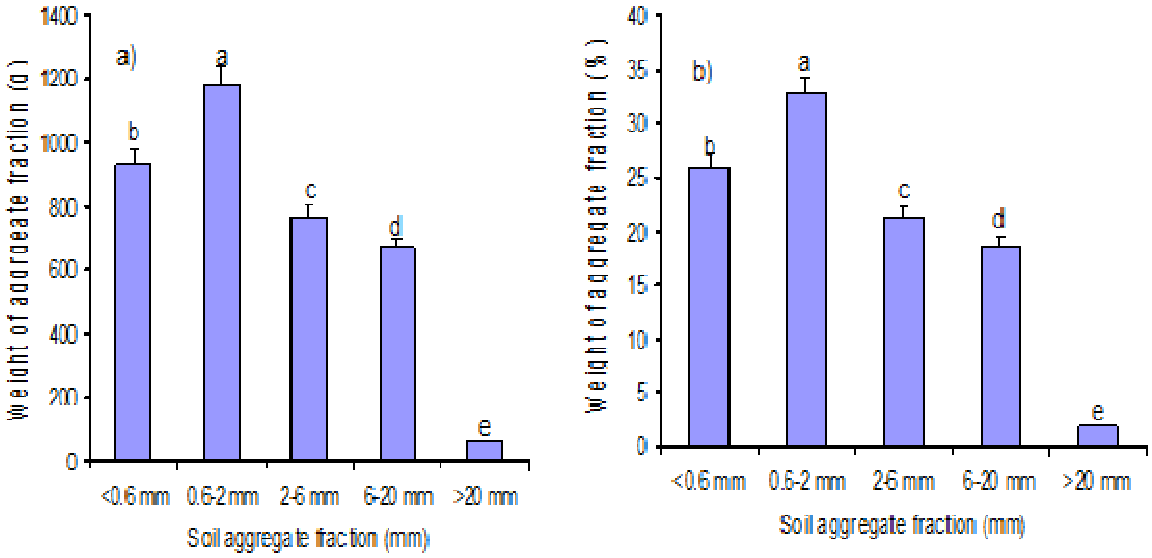

Figure 3a-b: The soil aggregate fraction of soil samples.

injection analyzer at Norwegian University of Life Sciences.

Statistical Analyses

The data were subjected to analyses of variance using GLM Model in MINITAB Statistical Software Program for Windows Release 14 (Minitab, State College, Pa.). The mean differences were declared at 5% probability. Descriptive statistics were also employed whenever necessary.

RESULTS AND DISCUSSION

Soil Aggregate Fraction And Aggregate Stability

Analyses of variance revealed significant difference among aggregate fractions (Figure 3 a-b). Soil aggregate fraction (AF) of the study samples ranged from 1.8% (>20 mm) to 32.7% (0.6-2 mm). Fairly high proportion (53.9%) of the AF ranges from 0.6-6 mm, which is supposed to be good for seed bed preparation. Normally a good AF is a distribution where most aggregates are neither very big, nor very small. It is favorable that at least 50% of the aggregates should be between 0.5 to 5 mm (Børresen, 2003). The 0.6-2 mm AF exhibited 82% aggregate stability (AS), while 2-6 mm AF displayed 81% AS. These AS found fairly high as compared to earlier reported results from crop lands (Shrestha et al., 2007; Barreto et al., 2009; Abera, 2012). The higher AS of the coffee soil in present study might be attributed to the less frequent disturbance of soils and its higher organic C content due to leaf litter fall and root biomass. The AS values in current study are comparable with most forest and pasture land soils (Shrestha et al., 2007; Barreto et al., 2009).

The aggregate stability depends on both the forces that bind particles together and the nature and magnitude of the disruptive stress (Beare and Bruce, 1993). With this view, AS is a measure of the vulnerability of soil aggregates to external destructive forces (Hillel, 1982). Therefore, the currently identified stable aggregates of coffee soil is supposed to be resistant to external destructive forces such as rainfall, thus less erosion problem would be expected. This soil retains sufficient amount of water as determined based on its water potential across pF0 to pF2, 64 to 38.9 (vol%), respectively. The soil displayed a water filled pore space (WFPS) of 68-77% during June to August based on field soil moisture determination that supposed to result in denitrification loss of mineral N. Accordingly many previous reports indicated that denitrification exponentially increase when WFPS exceeding 60% in combination with the availability of high soil mineral-N and soluble carbon substrates (Dobbie and Smith, 2001; Saggar et al., 2004).

Rate Of CO_2-C Flux

The rates of SOM mineralization in response to soil moisture was estimated at different sampling periods (Figure 4). Analyses of variance revealed that there were significant differences in the rates of CO_2-C flux among soil moisture levels. The current rate of respiration (0.3-5.2 mg kg^{-1} d^{-1}) was relatively lower than the ranges of

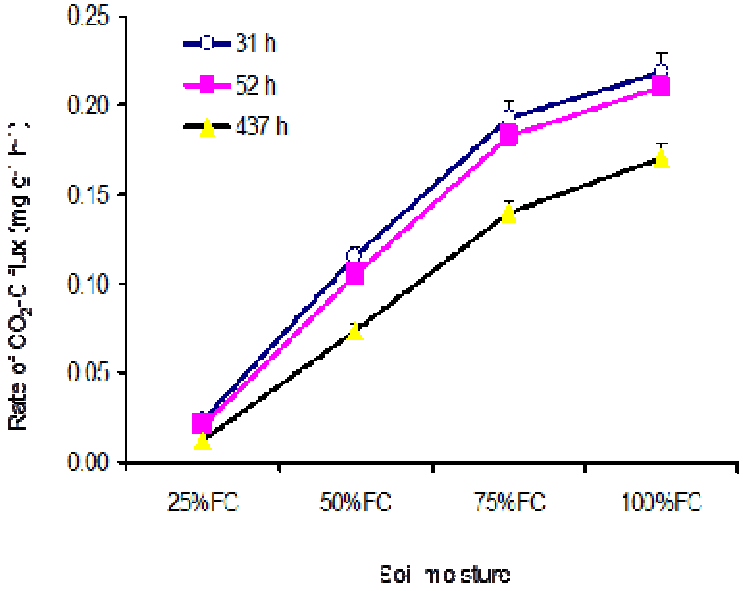

Figure 4: The rates of CO_2-C flux (mg g^{-1} h^{-1}) of coffee agroecosystem soil incubated under laboratory condition at different soil moisture regimes. The measured values are average of three replications (+SE).

Table 1: Calculated net N mineralization and nitrification rates (mg N kg $soil^{-1}$ day^{-1}) of laboratory incubated soil samples

Soil moisture	Mineralization	S.D	Nitrification	S.D
25%FC	0.27d	0.02	0.03c	0.00
50%FC	0.49c	0.04	0.14b	0.00
75%FC	0.86b	0.17	0.39a	0.04
100%FC	1.02a	0.14	0.37a	0.03

Key: S.D = standard deviation

documented values (2.9-61.4 mg kg^{-1} d^{-1}) with many soils (Conant et al., 2004). Rate of CO_2-C flux was linearly increased in response to soil moisture increase from 25 to 100%FC over the incubation period. Similar soil moisture effect on carbon mineralization has been reported elsewhere (Orchard and Cook, 1983; Andrén and Paustian, 1987; Orchard et al., 1992; Andrén et al., 1993; Abera et al., 2012b).

Indeed, the effect of drought stress on soil respiration was tremendous. For example, the rate of CO_2-C flux was declined by 89, 47 and 12%, respectively at 25, 50 and 75%FC as contrast to 100%FC, during the first 31 h of sampling. Similar trends of CO_2-C flux were exhibited during 52 h and 437 h of sampling. The rates of CO_2-C flux across all soil moisture levels were lower during 437 h than 52 h and 31 h of sampling periods (Figure 4), suggesting the decline in the availability of carbon substrate. The results displayed that under drier condition microbial activity (soil respiration) was limited, which might be attributed to limitation of substrate access, death/dormancy and restricted microbial mobility and/or low intracellular water potential (Griffin 1981; Killhma, 1993; Schjønning et al. 2003; Conant et al., 2004, Abera et al., 2012b). This finding suggest that climate change that results in reduction of rainfall with resultant soil moisture reduction, may substantially reduce the rate of SOM decomposition by negatively affecting soil microbial activity and biomass. This scenario may enhance soil carbon sequestration. Likewise, a number of soil warming experiments have concluded that decreased soil moisture associated with increased temperature is the likely explanation for limited positive responses of soil respiration to artificial warming (Rustad et al., 2001; Conant et al., 2004).

Net Nitrogen Mineralization And Nitrification Rates

Laboratory Incubation

The rate of N mineralization under laboratory incubation ranges from 0.27-1.02 (mg N kg $soil^{-1}$ day^{-1}), while nitrification rate ranges from 0.03-0.37 (mg N kg $soil^{-1}$ day^{-1}) in response to soil moisture increases from 25 to

Table 2: Calculated net N mineralization and nitrification rates (mg N kg soil^{-1} day^{-1}) of in-situ sampling

Month	Mineralization	S.D	Nitrification	S.D
Jan.	0.82b	0.04	0.55b	0.10
Feb.	1.18a	0.05	0.36bc	0.04
Mar.	1.34a	0.15	0.39b	0.04
Apr.	0.74b	0.17	0.70ab	0.02
May	1.03ab	0.13	0.15c	0.13
Jun.	1.02ab	0.12	0.68ab	0.11
Jul.	1.18a	0.14	0.16c	0.05
Aug.	0.95ab	0.04	0.59ab	0.06

Key: S.D = standard deviation.

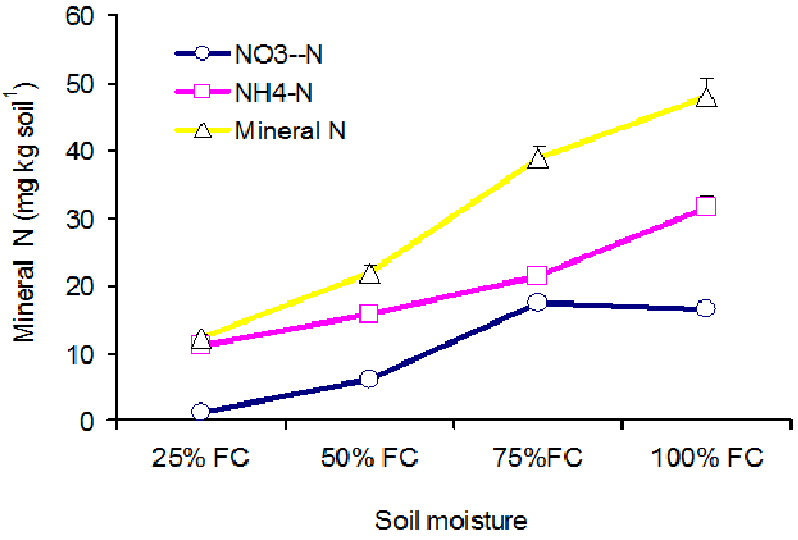

Figure 5: Extractable mineral N (mg N kg soil^{-1}) during 45 days of laboratory incubation.

100%FC. The low mineralization and nitrification rates observed at lower soil moisture than at higher soils moisture condition are consistent with earlier findings (Miller and Johnson, 1964; Franzluebbers, 1999;Unkovich et al., 1998; Abera, 2012a). Many previous works have shown that N mineralization and microbial N biomass greatly affected by soil moisture and seasons (Kushwah et al., 2000; Qi et al., 2011). In the present study, laboratory incubation exhibited lower mineralization and nitrification rates than in-situ sampling (Tables 1 and 2). This could be partly attributed to temperature effect as laboratory incubation was carried out at 15 ℃, in contrast to annual average temperature of the study site was 18.7 ℃. The other possible explanation might be due to fresh and continuous labile C supply during the in-situ sampling. The extractable mineral N concentration was linearly increased in response to soil moisture increase. Indeed, extractable mineral N was declined by 75%, while extractable NO_3^--N was declined by 93% as soil moisture declines from 100%FC to 25%FC (Figure 5). Earlier reports indicated that nitrogen mineralization increases in response to soil moisture increase, but strongly declines as the soils become wetter in different ecosystems due to O_2 limitation (Miller and Johnson, 1964; Franzluebbers, 1999). In general the proportion of nitrification contribution to mineral N was found to be low under laboratory condition.

In-Situ Soil Sampling

The net mineralization and nitrification rates were calculated by assuming the December dry month N mineralization value as initial value (Figure 1). Thus, this initial value was subtracted from other month sampling period. The mineralization rate tends to be slightly (37%) different during the sampling months, indicating that N mineralization in coffee agroecosystem was not much varied across seasons. By contrast, nitrification rate appeared to vary largely (79%) among the sampling months, suggesting that nitrification is more sensitive to

climate variables, plausibly soil moisture. Similar effect of climate variables (mainly reflected on soil moisture) on mineralization and nitrification has been documented (Haynes, 1986; Unkovich et al., 1998; Abera et al., 2012b). The rates of mineralization (0.8-1.3 mg N kg soil^{-1} day^{-1}) and nitrification (0.1-0.7 mg N kg soil^{-1} day^{-1}) values are very close to gross mineralization and nitrification values of most results reported elsewhere (Davidson et al., 1991; Unkovich et al., 1998). This net rate of N mineralization in standing coffee plot implied the production of more amount of mineral N than consumption. Another plausible explanation is that the mineral N produced was sampled from shallower depth than the root depth of coffee and shade trees. This suggests further N mineralization investigation across different soil profiles.

It is possible that net N mineralization may also vary in response to seasonal changes in labile C inputs, although this was not determined in the present study. In this regard, earlier investigations indicated that net N mineralization vary in response to changes in potentially important sources of bioavailable C such as light fraction, potentially mineralizable C, dissolved organic C and fine root turnover (Unkovich et al., 1998).

It was evident that average extractable NO_3^--N concentration of the coffee soil when incubated under laboratory as well as in-situ condition was relatively low (30-40%) of mineral N across moisture levels. This might be because the nitrifiers activity might be highly sensitive to low soil pH and/or likely low abundance of nitrifiers (Chu and Grogan, 2010). Similar study conducted by Cookson et al. (2007) suggested soil pH impacted the microbial activity and microbial composition, and thus regulating gross N fluxes. In previous study Abera (2012) has shown that nitrification tends to be low in coffee soils could be attributed to the low soil pH and competition for NH_4^+-N between the heterotrophic decomposer and nitrifiers. Another explanation could be the selective uptake of nitrate by the perennial plants (coffee and shade trees) since it is highly mobile in the soil matrix. Therefore, there would be low N loss via leaching and denitrification in coffee agroecosystems (Abrea 2012). Similar to this finding, low N leaching loss in shaded than non-shaded coffee agroecosystems was reported by Babbar and Zak (1994). Furthermore, lower net nitrification was documented in acidic soils as compared to high pH upland soils of the subtropics (Khalil et al., 2005). Generally, drought stress significantly reduced the rates of C (89%) and N (75%) mineralization, suggesting that N cycling would be low during the seasonally dry tropical climate condition, while C sequestration could be higher.

CONCLUSION

Coffee agroecosystem soil has shown better aggregation and aggregate stability consistent with earlier reports under pasture and forest soils. The results revealed that the rates of C and N mineralization under laboratory and N mineralization in-situ tended to be negatively affected by drought stress. This suggests that the anticipated climate change might have significant effect on N cycling and C sequestration in coffee agroecosystems. Furthermore, the results exhibited that nitrification was more affected by drought stress than N mineralization, confirming that nitrifiers are more sensitive to drought stress than ammonifiers. The net rates of nitrification of the present study appear to be lower across all moisture levels as compared to our pervious results, suggesting the sensitivity of nitrifiers to acidic soil. The net N mineralization and nitrification rates tended to be greater under in-situ than laboratory condition might be because optimum moisture and temperature attained in field condition. In nutshell, nitrate leaching and denitrification might not be underestimated in this agroecosystem as soil moisture and organic C of the study site appears higher, suggesting further detailed studies of such processes.

ACKNOWLEDGEMENT

The author thanks the Norwegian Program for Development, Research and Higher Education (NUFU) Rhizobium project (NUFUPR 2007/10144) for financing this research work. The author would like to thank Dr. Endalkachew Wolde-meskel for reading the draft of this article.

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