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Nitrogen mineralization, nitrification and nitrifier population in a protected grassland and rainfed agricultural soil

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Abstract: Establishing effects of land cover change on soil properties have implications for devising land management strategies for sustainable use. This study appraises the effects of land cover change on soil properties and two potentially sensitive indicators of short-term soil biochemical changes, namely N-mineralization and nitrification in a protected grassland and an adjacent agricultural land. The soil of the agricultural land showed significantly higher soil pH, moisture and mineral–N. The cultivated soil had significantly higher rate of N-mineralization, nitrification. The change in N transformation rates may be the result of differences in the nitrifier population and their activity and differences in other soil microbial community associated with the type of organic matter substrate present in the grassland and agricultural soil. Land cover change enhanced the rates of N transformation consequently the rate of N loss may also increase especially in absence of adequate plant cover leading to decrease in soil N content. Therefore, measures should be taken to reduce conversion of grasslands.

Resumen: Conocer los efectos de los cambios en las coberturas del suelo sobre las propiedades edáficas tiene implicaciones para el diseño de estrategias de manejo de la tierra que estén orientadas hacia un uso sostenible. Este estudio examina los efectos del cambio en las coberturas del suelo sobre propiedades del suelo y dos indicadores potencialmente sensibles de corto plazo de cambios bioquímicos en el suelo: la mineralización del nitrógeno y la nitrificación, en un pastizal protegido y una tierra agrícola adyacente en la región occidental de los Himalayas. El suelo de la tierra agrícola mostró valores significativamente más altos de pH, humedad y nitrógeno mineral. El suelo cultivado tuvo tasas de mineralización de N y de nitrificación significativamente mayores. El cambio en las tasas de transformación de N puede resultar de las diferencias en la población nitrificante y su actividad, y de diferencias en otras comunidades microbianas del suelo asociadas con el tipo de sustrato de materia orgánica presente en el pastizal y el suelo agrícola. El cambio en las coberturas del suelo incrementó las tasas de transformación de N; consecuentemente, la tasa de pérdida de N también puede aumentar, especialmente en ausencia de una cobertura vegetal adecuada, conduciendo a un decremento en el contenido de N en el suelo. Por lo tanto, deberían tomarse medidas para reducir la conversión de pastizales en tierras de cultivo.

Resumo: O estabelecimento dos efeitos das mudanças do revestimento da terra nas propriedades do solo tem implicações para a concepção das estratégias de gestão para o seu uso sustentado. Este estudo avalia os efeitos das mudanças no coberto vegetal nas propriedades do solo e em dois indicadores potencialmente sensíveis às mudanças bioquímicas de curto termo, nomeadamente a mineralização do N e a nitrificação num prado protegido e num campo de cultura adjacente na região ocidental da região dos Himalaias. O solo da parcela agrícola mostrou um pH, humidade e N-mineral significativamente elevado. O solo cultivado tinha mais elevadas taxas de mineralização de N e de nitrificação. A mudança nas taxas de transformação do N pode ser o resultado de diferenças na população nitrificante e na sua actividade e diferenças em outra comunidade microbiana associada com o tipo de substrato orgânico presente no prado e no solo agrícola. A mudança na cobertura do solo acentuou as taxas de transformação do N e, consequentemente, a taxa de perda de N pode também aumentar especialmente na ausência de uma adequada cobertura do solo conduzindo ao decréscimo do teor em N no solo. Por isso, devem ser tomadas medidas para reduzir a conversão dos prados em campos de cultivo.

Key words: Agricultural land, ammonium oxidizing bacteria, nitrification, nitrite oxidizing bacteria, N– mineralization, protected grassland.

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Introduction

The plant available N is produced through N mineralization, a process where more complex proteins and allied compounds of organic matter are hydrolyzed first to ammonium (NH_4^+) and then to nitrate (NO_3^-). Nitrification is an aerobic process, which is important in linking the reduced and oxidized forms of nitrogen within the nitrogen cycle. Its significance in soil is related to plant nutrition with both ammonia and nitrate acting as nitrogen sources. Nitrification is brought about by two distinct physiological groups of gram–negative chemoautotrophic bacteria belonging to Nitrobacteraceae (Schmidt 1982). The first step, conversion of ammonium to nitrite, is mediated by ammonia oxidizing bacteria (AOB) and the conversion of nitrite to nitrate is brought about by nitrite–oxidizing bacteria (NOB). Size of nitrifier population could be an important factor in controlling nitrification (Sabey *et al.* 1959). The effects of forest management on nitrification are better indicated by nitrifier counts than by soil incubations (Martikainen 1985). Rates of soil N mineralization and nitrification are indicators of the ability of soils to supply N for plant growth and to retain N following disturbances (Neill *et al.* 1995).

Human density in the central Himalayan region is about 17 to 18 person ha^{-1} of cultivated land, naturally the demand from the land is high and is constantly increasing due to low productivity of croplands (Shah 1996). Conversion of forest into open cropped area is mainly due to population growth and fragmentation of upland farm families. This has been a general trend in the entire Hindu-Kush Himalayan region (Rai *et al.* 1994). The nutrient status of soil changes quickly when land under forest or grassland vegetation is brought under cultivation. Therefore, there is a need to characterize the nutrient supplies in these land use types in order to have a better knowledge of the rate of supply of nutrients like nitrogen, for practical purposes, for example, in evaluation of sustainability of land use systems. It has been suggested that many of the problems in minimizing nitrogen losses are related to inadequate knowledge of the amounts, forms and patterns of change of mineral nitrogen in agricultural soils (Jarvis 1993). The bulk of nitrogen lost from soils via leaching, nitrate reduction and denitrification is attributable to nitrification which is the prime engine of nitrogen pollution in agroecosystems and the main cause of low nitrogen use efficiency in crops. Though there is abundant literature on the magnitude and rate of soil erosion in the central Himalayan region, changes in soil physical and biochemical properties associated with land cover change are poorly documented. Ramakrishnan (1993) and Sharma *et al.* (2004) have studied the effects of land use change on soil microbial biomass and nutrient transformations in the northeast and Sikkim Himalayan region.

In the present investigation we quantified the rates of N mineralization, nitrification, mineral–N pool, nitrifier population fluctuations and other soil properties in protected grassland and a rainfed agricultural soil with a view to evaluate the effects of land cover change. The study will be of considerable importance in enhancing our knowledge in regional soil N transformations under different land cover.

Materials and methods

Study site

The study site was situated in the Katarmal experimental station of the institute (GBPIHED). It is located $29^0\ 36'$ N latitude and $79^0\ 37'$ E longitude and 1250 meters above mean sea level. The slope of the land was less than 5%. The region has a warm temperate climate with typical monsoonal character. May and June are the warmest months of the year with a maximum temperature around 33^0C, January is the coldest month with minimum temperature dipping below the freezing point. In general, June–September receive approximately 800 mm, October–December 78 mm, January–March 237 mm and April–May 118 mm of rainfall which constitutes 65, 6, 19 and 10% of the annual precipitation respectively.

The soil is an Inceptisol, sandy loam in texture (clay 19.6%, sand 57. 9% and silt 22.5%) and has a neutral reaction. In general the soil is well drained and moderately fertile having 0.70% to 0.91% organic C, 0.06% to 0.09% total N and 0.04 to 0.06% total P. It has a bulk density of 1.55 g cm^{-3} and water holding capacity of 33.5% (Singh *et al.* 2000).

Experimental design

In the absence of prior history, changes in soil properties induced by land use dynamics need to be evaluated by establishing experimental plots under different land use treatments and monitoring them for a long time, which is costly. An alternative approach is to take soil samples from plots of land under different use and compare their physicochemical properties. This approach substitutes space for time and is called the 'spatial analogue method'. A prerequisite for this method is that the land use treatments must be located such that the differences in geologic, topographic and climatic conditions are negligible. Under this condition, any differences in soil properties can be attributed to the differences in land use types. Because of this assumption, the conclusions drawn would remain provisional. This approach has been employed in several studies (Abubakar 1996; Lumbanraja *et al.* 1998). The present study also used this inferential approach.

The treatments were set up in a completely randomized block design with three replicates. Experimental plots in the protected grassland and the agricultural field consisted of three homogenous sub–plots (5 × 3m). The protected grassland was dominated by *Dichanthium* spp. and the agricultural land was subjected to rainfed cultivation of paddy. Following ploughing of all the agricultural plots to a depth of 20 cm farmyard manure was applied at a rate of 1000 kg ha^{-1}. The farmyard manure was surface–applied and lightly incorporated (upto 10 cm) in the soil. The farmyard manure consisted of dung, animal urine, bedding leaves and feed leftovers. The percentage nutrient composition of the farm yard manure applied was (mean±SE) 33.17±2.33 C, 0.83±0.20 N, 0.26±0.09 P. Seeds of paddy were sown by dibbling method with 4–6 seeds per hill with a row to row distance of 20 cm and hill to hill distance of 15 cm. Rainfall was the only source of irrigation during the cultivation period.

Soil sampling

Soil samples were collected every 10 to 15 days from the date of land clearing. A short term time scale is important in case of annual crops, where over a period of 90–150 days there are large changes in plant demand. Three soil samples were collected randomly from each treatment plot from the upper 10 cm soil layer and mixed to form a composite sample to account for spatial variation in the field. Soil monoliths (10×10×10 cm) were removed and stored in polyethylene bags and brought to the laboratory. Each composite soil sample was divided into two parts. One part in the field moist condition was used for determination of pH, soil moisture, and mineral–N (NH_4^+–N and NO_3^- –N). The second part also in field moist condition was used for assessing the N–mineralization, nitrification and nitrifier population. Soil samples were taken approximately10, 25, 35, 50, 60 and 75 days after land clearing. The samples were brought to the laboratory, spread on paper sheets and visible roots and fragments of organic debris were removed and the soil was sieved (2 mm mesh).

Soil analyses

Particle size analysis was done by using sieves of different mesh size (Anderson & Ingram 1989). Bulk density was determined by using a soil corer and measuring the weight of dry soil of a unit volume to a depth of 10 cm. Water holding capacity was determined by using perforated circular brass boxes (Piper 1944). Organic carbon in soil was analysed by using dichromate oxidation and titration with ferrous ammonium sulphate (Walkley 1947). The percent soil organic matter (SOM) was calculated by multiplying the percent organic carbon by a factor of 1.72, following the standard practice that organic matter is composed of 58% carbon (Brady 1985). Total P was measured colorimetrically after $HClO_4$ digestion (Jackson 1958). Total N was analysed by macrokjeldahl digestion (Jackson 1958).

Soil pH (1 : 2, soil : water) was measured using a pH meter equipped with glass electrode. Gravimetric soil moisture content was measured with freshly pulled out soil according to the following equation (Buresh 1991).

$$M = \frac{WCWS - WCDS}{WCDS - WC} \times 100$$

Where, M= gravimetric soil moisture content (%); WCWS= weight of can plus wet soil (g); WCDS = weight of can plus dry soil (g); WC= weight of moisture can (g).

Extractable soil ammonium nitrogen was estimated colorimetrically by the phenate method (APHA 1985). Nitrate nitrogen was measured by phenol disulphonic acid method (Jackson 1958). *In situ* rates of N mineralization were measured at the sampling points using *in situ* incubations (Binkley & Hart 1989). A portion of the fresh, field moist soil sample (about 100 g) was incubated in the soil at 10 cm depth using a large sealed polyethylene tube. Coarse roots and large organic debris were removed. Ammonium and nitrate were determined at time zero and after 30 days of field incubation. The increase in concentration of ammonium and nitrate N during the course of field incubation is defined as net N mineralization (Hart *et al.* 1994). Net nitrification was calculated as the difference in the NO_3–N concentration in the incubated and initial sample

(Hart *et al.* 1994). Rate of N mineralization and nitrification are expressed in units of μg N per g dry soil per thirty day. Unless otherwise stated, all results were calculated on an oven-dry (105^0 C) soil weight basis.

Counts of nitrifiers

The viable population of nitrifiers i.e. ammonium oxidizers and nitrite oxidizers was estimated by the most probable number (MPN) technique (Alexander 1965). The number of tubes positive or negative to the test was noted and the most probable number of organisms present was calculated from a MPN table (Cochran 1950).

Statistical data analysis

All data were analysed statistically according to Snedecor & Cochran 1989. Plot–level soil properties were compared across the two land cover categories (protected grassland and agricultural land) with analysis of variance with sampling of the same plots across the season being treated as repeated measure. Relationships between soil properties were compared using Pearson's correlation.

Results and discussion

Continued emphasis on sustainable agriculture especially in the fragile Himalayan region and on soil and environmental quality has generated renewed interest in evaluating effect of different land management systems on N dynamics in soil in this region. In the present study we focussed on surface (0–10 cm) horizons because processes in these horizons are likely to have an important influence on system level patterns of nitrogen transformation. Plants, microbes and soils consume and compete for NH_4^+ and NO_3^--N over a broad range of temporal scales. In the present study we studied the short term cycling (period of days to weeks) as defined by Kaye & Hart (1997). The results from the study permit us to understand how nitrogen that is produced in the soil environment as NH_4^+ or NO_3^- is retained and recycled by different soil pools in the short term (days to weeks).

The average values for general soil properties determined for different land cover situations are shown in Table 1. It is evident that with a few exceptions, values of most variables are higher in the protected grassland plots. The sand fraction was lower in protected grassland, while clay content was higher in grassland, therefore, a general observation is that after grassland has been converted to agricultural land the soil texture became sandy. This was because under sparse vegetation the clay fraction is likely to be lost to processes of selective erosion and migration down the soil profile.

Table 1. General soil physico-chemical properties under two land cover. Values are means of three replicates ± 1 SE.

Soil property	Protected grassland	Agricultural soil
Soil texture		
Sand (%)	63.26 ± 2.72	65.26 ± 0.26
Silt (%)	27.5 ± 2.56	30.13 ± 1.10
Clay (%)	9.23 ± 5.18	4.6 ± 1.04
Bulk density (g cm^{-3})	1.39 ±0.08	1.00 ±0.02
Water holding capacity (%)	30.76 ± 1.46	26.86 ± 0.78
Organic carbon (%)	0.92 ± 0.13	0.83 ± 0.12
Soil organic matter (SOM)	1.58 ± 0.23	1.42 ± 0.18
Total N (%)	0.11 ± 0.04	0.07 ± 0.08
Total P (%)	0.06 ± 0.04	0.03 ± 0.01
C:N ratio	12.09 ± 1.28	7.58 ± 1.02

The pH values in the 0–10 cm surface soil of the grassland ranged from 7.3 to 7.6 while that of cropland ranged from 7.6 to 7.8 (Fig. 1). ANOVA indicated significant (F = 17.08, $p<0.0002$) difference in pH due to cultivation. Soil pH had significant correlation with mineral–N ($r^2=0.47$, $p< 0.001$), rates of N mineralization ($r^2=0.54$, $p< 0.001$), nitrification ($r^2 = 0.35$, $p<0.001$), NOB ($r^2=0.66$, $p<0.001$), and soil moisture ($r^2= 0.56$, $p<0.001$) in grassland. It had significant correlation with rate of nitrification ($r^2=0.56$, $p<0.001$), AOB ($r^2 =0.41$, $p<0.001$), NOB ($r^2=0.41$, $p<0.001$) and soil moisture ($r^2= – 0.48$, $p<0.001$) in cultivated soil. Rates of N cycling are known to be strongly affected by soil pH (Killham 1994). Agricultural practices are known to cause elevation of soil pH (Ollinger *et al.* 2002). In the present study a correlation between nitrification rates and pH suggest that nitrifying bacteria are pH sensitive (Ste–Marie & Paré 1999).

The bulk density was lower in agricultural soil (Table 1) which has implications for the moisture and air that would be available for life forms in the soil. The bulk density difference may be attributed to differences in soil organic matter content.

As expected the soil organic matter was higher in protected grassland and conversion to agricultural land led to a drop in SOM content. In cultivated fields the type of crop grown contributes to the differences in SOM. Land use practices that have detrimental effects on SOM content have far reaching implications because of the multiple roles SOM plays in governing soil quality (Wild 1996). Cleveland *et al.* (2003) also

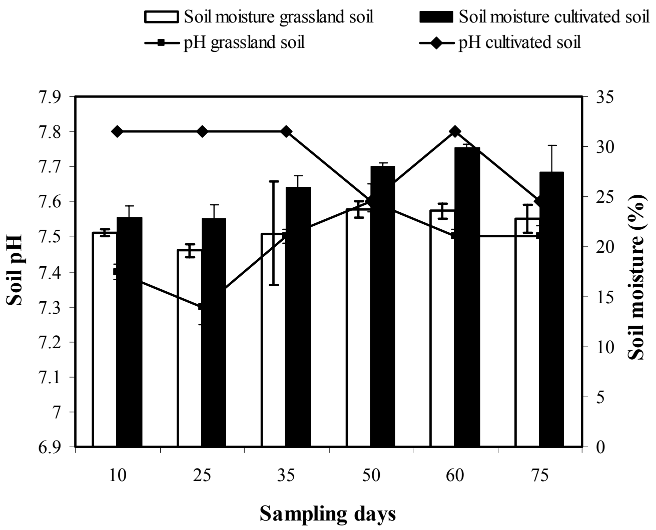

Fig. 1. Temporal variation in soil pH and soil moisture (%) in grassland and agricultural soil. Error bars represent ± 1SE.

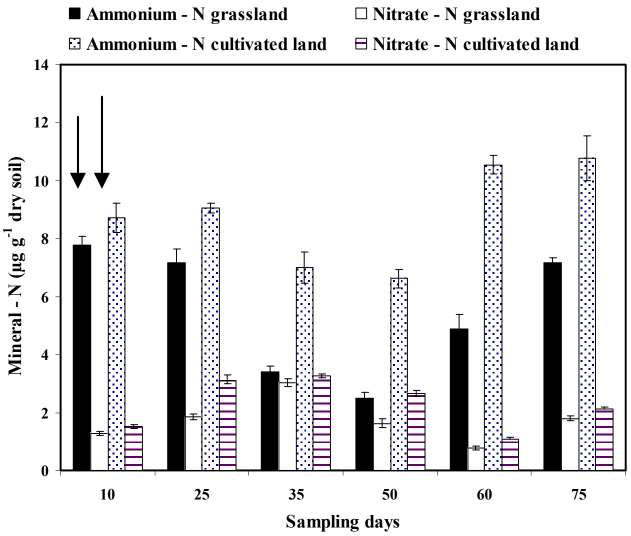

Fig. 2. Temporal variation in mineral–N (µg g^{-1} dry soil) in grassland and agricultural soil. Error bars represent ±1SE. Arrows indicate days of cultivation and application of farmyard manure respectively.

reported that land transformations caused reduction in organic C and other nutrients in soil. Total N and Total P content also showed similar trend as organic C. The decline in the general soil properties as observed in the present study is characteristics of native forest or grassland soils when they are subjected to cultivation (Haynes & Beare 1996). Under reduced tillage, organic C and N tend to accumulate in surface soils as a result of diminished oxidative microbial metabolism (Wood & Edwards 1992 and references cited therein). The conversion of a grassland ecosystem to arable farmland is tantamount to reversing the successional process and reintroducing an immature seral vegetative stage.

The soil moisture ranged from 19.65 to 23.64% in the protected grassland while it ranged from 22.82% to 29.93% in the cultivated plots (Fig. 1). The moisture content was significantly higher in the cultivated plots (F=15.61, $p<0.003$). The soil moisture had significant correlation with mineral –N ($r^2=0.24$, $p<0.01$), nitrification ($r^2=0.33$, $p<0.01$), AOB ($r^2=0.31$, $p<0.01$), and NOB ($r^2=0.44$, $p<0.001$), in the grassland soil while it had significant correlation with rate of N-mineralization ($r^2=0.37$, $p<0.01$), nitrification ($r^2=0.37$, $p<0.01$), mineral–N ($r^2=0.36$, $p<0.01$), AOB ($r^2=0.65$, $p<0.001$), and NOB ($r^2=0.75$, $p<0.001$), in cultivated soil. Neill *et al.* (1995) reported that soil moisture may be an important controller of soil inorganic–N pools and N transformation rates leading to availability of NO_3, but the relationship may be complex and mediated by the balance between net N-mineralization and N immobilization as soil microorganisms respond to soil wetting and drying.

The mineral–N content in soil increased significantly (F=42.41, $p<0.0001$) due to cultivation, later it declined during rapid plant growth phase and again registered an increase late in the season (Fig. 2). The mineral–N content in soil varied from 4.17 µg g^{-1} dry soil to 9.06 µg g^{-1} dry soil in the grassland soil while it ranged from 9.27 µg g^{-1} dry soil to 12.89 µg g^{-1} dry soil in the cultivated soil. Maximum value of mineral–N was recorded few days after FYM application as expected and at the end of the season (Fig. 2). Mineral–N showed correlation with N-mineralization in both grassland ($r^2= 0.60$, $p<0.001$) and cultivated soil ($r^2= 0.40$, $p<0.01$). The NH_4^+–N/ NO_3^-–N ratio was always greater than 1 (Fig. 2). Cultivated soils typically have greater concentrations of inorganic N than their undisturbed counterparts (Woods 1989). Physical disruption of the soil increases both aeration potential and microbial access to readily mineralizable soil organic matter (Cabrera & Kissel 1988) and provides at least a partial explanation for the flush of mineral N that is released when grassland is ploughed. In our study the decrease in inorganic N coincided with active crop growth and was evidently due to N–uptake by the crop. NH_4^+–N was the dominant form of inorganic N in the present study accounting for 53.07% to 85.76% of

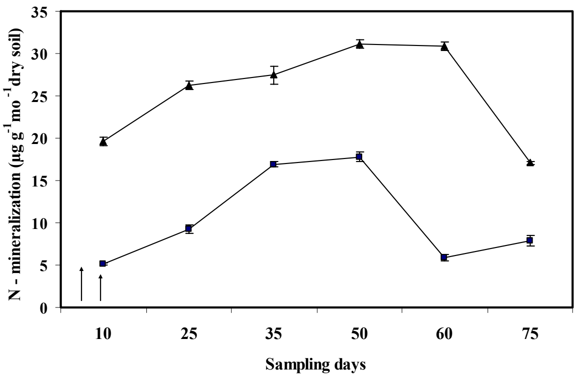

Fig. 3. Temporal variation in rate of N– mineralization (µg g^{-1} dry soil $month^{-1}$) in grassland (■) and agricultural **(▲)** soil. Error bars represent ± 1SE. Arrows indicate days of cultivation and application of farmyard manure respectively.

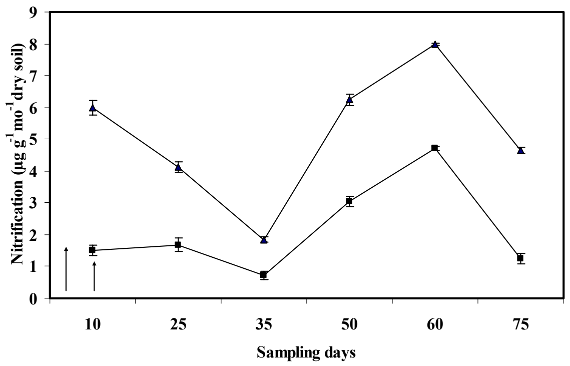

Fig. 4. Temporal variation in rate of nitrification (µg g^{-1} dry soil $month^{-1}$) in grassland (■) and agricultural(▲) soil. Error bars represent±1SE. Arrows indicate days of cultivation and application of farmyard manure, respectively.

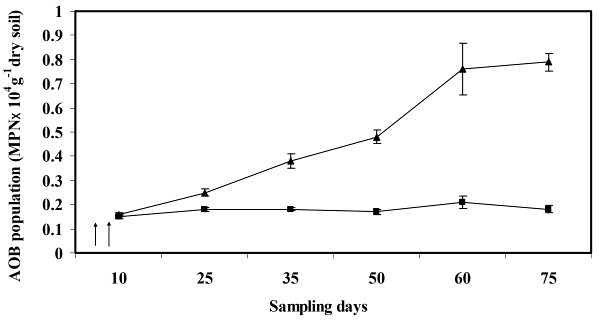

Fig. 5. Temporal variation in ammonium oxidizer (AOB) population (MPN × 10^4 g^{-1} dry soil) in grassland(■) and agricultural(▲) soil. Error bars represent ± 1SE. Arrows indicate days of cultivation and application of farmyard manure respectively.

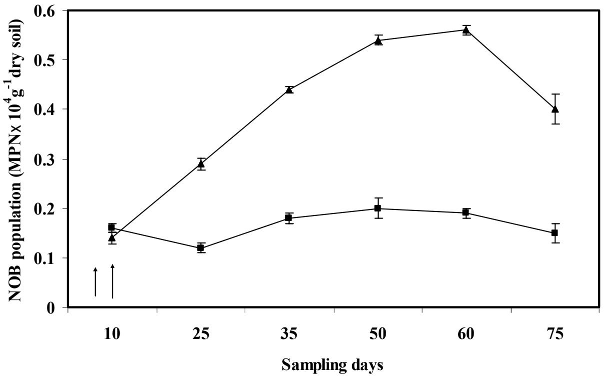

Fig. 6. Temporal variation in nitrite oxidizer (NOB) population (MPN × 10^4 g^{-1} dry soil) in grassland (■) and agricultural (▲) soil. Error bars represent ± 1SE. Arrows indicate days of cultivation and application of farmyard manure respectively.

inorganic N in the grassland and 71.41% to 83.55 % in the cultivated soil (Fig. 2).

N–mineralization rates were significantly greater in cultivated soil than grassland soil (F= 69.01, p< 0.001). In the grassland it ranged from 5.11 µg g^{-1} $month^{-1}$ to 17.78 µg g^{-1} $month^{-1}$ whereas in the cultivated plots it ranged from 17.08 µg g^{-1} $month^{-1}$ to 31.10 µg g^{-1} $month^{-1}$ (Fig. 3). It correlated with nitrite oxidizers in both grassland (r^2= 0.36, p<0.01) and cultivated soil (r^2=0.66, p<0.001) and nitrification (r^2=0.21, p<0.01) in cultivated soil. The nitrification rates ranged from 0.72 to 4.72 µg g^{-1} $month^{-1}$ in grassland to 1.84 to 7.88 µg g^{-1} $month^{-1}$ in cultivated soil (Fig. 4.). There was significant rise in nitrification rate due to cultivation (F=27.25, p<0.001). Nitrification rates correlated with AOB (r^2=0.43, p<0.001; r^2=0.32, p<0.01), NOB (r^2=0.45, p<0.001; r^2=0.22, p<0.01), soil moisture (r^2=0.33, p<0.001; r^2=0.36, p<0.001) and pH (r^2=0.37, p<0.01; r^2=0.56, p<0.001) in grassland as well as in cultivated soil. Cultivation is generally considered an oxidative process since it promotes soil aeration and exposes soil surfaces to the atmosphere (Power 1981). Consequently cultivation enhances processes such as oxidation of organic matter, mineralization of organic N and nitrification. Our study showed that N mineralization from native soil organic matter was dependent on the soil moisture content. Nitrification accounted for only 17.16% to 29.55% in the grassland and 20.1% to 27.29% of N mineralization indicating the rest to be accounted for ammonification. Mechanical disruption of the soil structure makes previously protected soil organic matter available for degradation and increased rates of mineralization have been observed in disturbed soils (Ballesdent *et al.* 1990).

The high nitrification rates observed in cultivated soil was probably caused by greater NH_4^+–N availability, higher soil pH and higher soil moisture resulting from FYM application (Vitousek & Matson 1985). Phillips *et al.* (2000) observed that increased aeration might be a reason for higher potential nitrification rates in cultivated soils than in native soils and successional grassland soils.

The number of viable cells of AOB and NOB showed a marked variation due to cultivation ($F = 23.46$, $p<0.005$; $F=40.12$, $p< 0.001$). The population of both AOB and NOB were more or less stable across the sampling dates in the grassland (Figs. 5 & 6) but it increased significantly throughout the season in the cultivated plots. The AOB population fluctuated from 0.15×10^4 to 0.21×10^4 g^{-1} dry soil in the grassland and 0.16×10^4 to 0.79×10^4 g^{-1} in cultivated soil, while that of NOB fluctuated from 0.12×10^4 g^{-1} dry soil to 0.20×10^4 g^{-1} dry soil in grassland to 0.14×10^4 g^{-1} to 0.56×10^4 g^{-1} in cultivated soil. The numbers of AOB and NOB were significantly correlated to each other in cultivated soil ($r^2=0.68$, $p<0.001$) and to soil moisture ($r^2=0.30$, $p<0.01$; $r^2=0.65$, $p<0.001$; NOB $r^2=0.44$, $p<0.001$; $r^2=0.75$, $p<0.001$) and nitrification ($r^2=0.43$, $p<0.001$; $r^2=0.32$, $p<0.001$; NOB $r^2=0.45$, $p<0.001$; $r^2=0.22$, $p<0.01$) in both grassland and cultivated soil. Nitrification proceeds most readily in well–aerated soils and nitrifier populations are greatest near the soil surface. They are present in untreated (no ammonium fertilizer) agricultural soils at levels of 1×10^3 to 1×10^4 g^{-1} (Adrakani *et al.* 1974). Doran (1980) enumerated nitrifiers in no–till and plowed soils from several locations, and observed that in the top 15–cm soil the ammonium oxidizer population was smaller in the no–till soils. The AOB population was significantly correlated to NOB population. This confirms the fact that both processes carried out by these bacteria are coupled (Woldendorp & Laanbroek 1989). Various cultural operations including ploughing also stimulate soil microorganisms (Yoshida & Sakai 1962). In the present study, a strong positive relationship of both AOB and NOB existed with soil moisture. The soil moisture affects the activity of nitrifying bacteria through dehydration as well as through substrate limitation (Stark & Firestone 1995). There are several reasons for the weak/or no correlation between nitrifying bacterial population and nitrification rates. The treatments imposed during land cover change i.e., tillage and fertilization may not drive nitrifying bacterial community structure, which may be dependent on soil properties which were initially the same for all treatments. The results of MPN enumeration are, however, influenced by several factors such as medium composition (including pH and substrate concentration), incubation conditions and the method used to extract bacteria from soil.

Conclusions

Our finding of significant higher rates of N mineralization and nitrification following land cover change from protected grassland to cultivated land in the fragile Himalayan ecosystem has important implications for both land–water and land–atmosphere interactions and its consequences thereof. High mineral–N pools and nitrification rates in the cultivated soil suggest that the potential for N loss from agricultural land may be considerable. However, the occurrence of NH_4^+ in greater proportions in the cultivated soil is expected to reduce N losses by denitrification or leaching. Informations from other sites in the central Himalayan region, on rates of N transformations, need adequate investigation to determine to what extent these vary. Immediate action should be taken to stop conversion of grasslands to agricultural land in this region.

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