Biogeochemical cycling of nitrogen in terrestrial ecosystems of India

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By

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CERTIFICATE

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Dedicated to Papa

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<u>Abstract</u>

Nitrogen is an essential element required to sustain life on this planet. In many ecosystems, nitrogen is a growth limiting nutrient; whereas in other ecosystems, excess amount of nitrogen is causing severe environmental problems. Therefore, in order to ascertain the movement and availability of nitrogen at global scale, studies focussed on understanding nitrogen transformation processes at ecosystem level are desirable. Use of stable isotopes can provide detailed insight into mechanisms of processes involved in nitrogen cycle along with quantification of their rates. To the best of our knowledge, studies focussed on understanding nitrogen transformation processes, particularly their gross rates, using stable isotope approach in soils of the Indian subcontinent are non-existent. In view of this, the aim of present thesis was to quantitatively understand biogeochemical cycling of nitrogen in terrestrial ecosystems of India by measuring gross rates of nitrogen transformation processes in soils of different climatic zones. The ¹⁵N isotope dilution technique was employed to estimate rates of nitrogen transformation processes in soils to gain better insights into nitrogen and carbon dynamics.

The experiments conducted in the montane soils of the Himalayas indicated significant increase in gross rates of nitrogen mineralization with increase in temperature. Change in temperature did not show significant change in gross nitrification rates, indicating low sensitivity of nitrifiers towards temperature change.

Study of nitrogen cycling in soils from different land-types present in semi-arid region (Kutch, Gujarat) indicated cumulative effect of vegetation, moisture, and salinity on the soil microbial activity and nitrogen transformations in this region. However, analyses of different factors revealed salinity to be the principal regulator of nitrogen biogeochemistry in the region.

Rates of nitrogen transformation processes were measured in soils of the Western Ghats to understand nitrogen dynamics in tropical humid forest soils. Results indicated higher potential for nutrient consumption than their

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production in these forest soils. The potential for consumption of ammonium was higher compared to nitrate indicating active nutrient conservation mechanism in these soils. Despite the higher consumption rates, accumulation of nitrogen was observed in soils of the Western Ghats suggesting significant contribution of external sources of nitrogen, such as atmospheric nitrogen deposition and biological nitrogen fixation, to the soil nitrogen pool of the Western Ghats.

Effect of mono-plantation (rubber plantation) by replacing natural forested ecosystems on soil nitrogen dynamics was studied in soils of Kerala, India. Gross nitrogen mineralization and ammonium consumption were considerably lower in soils of the rubber plantation compared to the managed and unmanaged forests. This was attributed to change in tree species composition from mixed to mono-culture of rubber plants. Longer residence time of ammonium under rubber canopy also supported slow-down in N turnover due to conversion of mixed forest to mono-plantation.

Assessment of stable isotopic compositions of soils collected from different climatic zones of India showed soils from semi-arid climate to be enriched in the heavier isotopes of carbon and nitrogen compared to soils from the humid climate, possibly due to low organic matter content and relatively disproportionate mineralization. Results indicated that change in climatic conditions experienced by soils under different climate has the potential to significantly control the organic matter dynamics.

Overall, soils collected from different locations showed decrease in nitrogen transformation rates with depth indicating lowering in microbial activity in the deeper soils. Mineralization of organic matter was susceptible to change in surrounding conditions, whereas nitrification was relatively unaffected by climatic factors such as temperature and moisture. Ammonium immobilization was the major pathway for ammonium consumption compared to nitrification.

Keywords: Mineralization, Nitrification, Tropical soils, ¹⁵N isotope dilution, Terrestrial nitrogen cycle.

Abbreviations

‰	Per mil
$\delta^{13}C$	Isotopic composition of carbon with respect to V-PDB
$\delta^{15}N$	Isotopic composition of nitrogen with respect to Air-N $_2$
BNF	Biological Nitrogen Fixation
С	Carbon
GWC	Gravimetric Water Content
GWB	Ground Water Board
IAEA	International Atomic Energy Agency
IPCC	Intergovernmental Panel on Climate Change
IRMS	Isotope Ratio Mass Spectrometer
Ν	Nitrogen
Р	Phosphorous
SOC	Soil Organic Carbon
TN	Total Nitrogen
ТОС	Total Organic Carbon
VOCs	Volatile Organic Compounds
V-PDB	Vienna-Pee Dee Belemnite
WFPS	Water Filled Pore Spaces

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Chapter 1 Introduction

For millions of years, plants have been taking carbon dioxide (CO₂) out of the air and storing it into their biomass, keeping a crucial check on the global climate. However, like all other life forms, the normal functioning of plants is also dependent on the availability of various elements. Nitrogen (N) is among the fundamental elements that are required to sustain life as it acts as a nutrient and is necessary to form basic blocks of life, such as amino acids and nucleic acids. For plants and other primary producers, N is essential for conducting photosynthesis, i.e., the process of converting atmospheric CO_2 into sugar, which is stored in the biomass. The availability of N in terrestrial ecosystems is governed by a series of microbially mediated processes, which constitute the 'nitrogen cycle'. Due to continuous soil-vegetation interactions and importance of N in regulating primary productivity, cycling of N is closely coupled with carbon (C) cycle in the pedosphere (Soussana and Lemaire, 2014; Figure 1.1). The efficient coupling of C and N in soil not only contributes to increase in CO₂ sink capacity of soil, but also modulates movement of reactive forms of N, such as nitrate (NO_3) to the hydrosphere and nitrous oxide (N_2O) to the atmosphere (Thornton et al., 2007; Singh, 2011).

Terrestrial ecosystems mediate a large portion of land-atmosphere CO_2 flux with the removal of approximately 120 Pg C annually from the atmosphere through

photosynthesis and release a significant amount of CO₂ back to the atmosphere through microbial respiration during decomposition of soil organic matter (Figure 1.1; Chen and Coops, 2009). During the degradation, decomposers use plant residue C for respiration to meet their energy requirements and release CO_2 as a by-product (Chen et al., 2014). With the energy derived from decomposition, microbes are capable of absorbing nutrients (such as N) required for their growth. If the amount of N, also derived from decaying organic matter, does not fulfill the needs for microbial growth, it is immobilized into the microbial biomass (Nieder and Benbi, 2008; Chen et al., 2014). However, if the microbial demand for N is satisfied, the excess N remains in mineralized form for uptake by plants. Therefore, the quality of available organic substrate governs C and N use efficiency of the system, such that high quality plant residue (high N and low C:N) leads to high N mineralization rates, whereas low quality plant residue (low N and high C:N) results in high N immobilization rates (Nieder and Benbi, 2008; Chen et al., 2014; Hagemann et al., 2016). This helps in maintaining the balance between N mineralization and immobilization, eventually deciding the fate of C and N in the soil pool.



Figure 1.1: Coupled carbon and nitrogen cycles in soils. Black and blue arrows represent the movement of carbon and nitrogen, respectively.

In summary, as illustrated in Figure 1.1, organic matter decomposition in soils acts as a bridge between C and N cycles, where soils with limited N tend to accumulate it in microbial biomass while the soils with elevated N reduce belowground allocation of C and increase C uptake by plants (Shibata et al., 2015).

However, as there are other pathways besides organic matter decomposition in the N cycle to make inorganic N available in soils, they can also affect the availability of N, and in turn C dynamics, in the soils. Therefore, a detailed understanding of the cycling of N in terrestrial ecosystems is desirable.

1.1 Nitrogen cycle in terrestrial ecosystems

Despite the fact that more than 70% of the atmosphere is composed of di-nitrogen gas (N₂), N is a limiting nutrient in most of the ecosystems worldwide (Vitousek and Howarth, 1991). This is because most of the plants and other primary producers cannot use atmospheric N₂ due to its inert nature and higher energy requirement to break the strong triple bond of the N₂ molecule (N \equiv N). In general, only free living or symbiotic N-fixers are capable of converting atmospheric N₂ to reactive N and creating a pathway via N cycle for N to enter into living biomass and its transportation to higher species through the food chain. N cycle is a complex biogeochemical cycle of N within the biosphere, geosphere, and atmosphere, where inert atmospheric N₂ is converted microbially to reactive forms of N such as, ammonium (NH₄⁺), nitrite (NO₂⁻), and NO₃⁻ and released back into the atmosphere as N₂ through enzymatic redox reactions. Figure 1.2 depicts a simplified flow diagram of the terrestrial N cycle.



Figure 1.2: Simplified nitrogen cycle in terrestrial ecosystems.

The important steps involved in the N cycle are described below:

- N-fixation: Atmospheric N₂ is fixed into organic form by the process of Nfixation. This process is carried out by free living or symbiotic bacteria known as diazotrophs. The N fixed by these bacteria is converted into organic form and is incorporated in the biomass.
- Nitrogen Mineralization (or Ammonification): The death and decomposition of biota result in degradation of organic N and facilitates the release of inorganic N as NH₄⁺.

$\textit{Organic N} \xrightarrow{\textit{Mineralization}} \textit{Inorganic N}$

This process of breakdown of complex organic matter to produce inorganic N from the organic matter is called N mineralization. The NH₄⁺ produced as a result of ammonification either participate further in N cycle or is taken up by plants and/or microorganisms.

 Immobilization (or Assimilation): Immobilization is the opposite of mineralization, i.e., uptake of mobile inorganic N (NH₄⁺ or NO₃⁻) by plants or organisms and its conversion into organic form.

Inorganic N $\xrightarrow{Immobilization}$ Organic N

In general, mineralization of organic N is favored under C-limited conditions, whereas immobilization of inorganic N is favored under N-limited conditions.

Nitrification: It is the microbial conversion of NH₄⁺ to NO₃⁻. Nitrification is a two-step process, where first the NH₄⁺ is converted into NO₂⁻ by *Nitrosomonas*, which is followed by conversion of NO₂⁻ to NO₃⁻ by *Nitrobacter*.

$$2NH_{4}^{+} + 3O_{2} \xrightarrow{Nitrosomonas} 2NO_{2}^{-} + 2H_{2}O$$
$$2NO_{2}^{-} + O_{2} \xrightarrow{Nitrobacter} 2NO_{3}^{-}$$

The NO_3^- produced as a result of nitrification either undergoes denitrification or is taken up by plants and/or microorganisms.

Denitrification: It is the reduction of NO₃⁻ to gaseous forms of N under low oxygen supply with N₂ as the end product. The intermediate gases such as nitric oxide (NO) and N₂O emitted to the atmosphere during denitrification are potent climate threats.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO_{(g)} \longrightarrow N_2O_{(g)} \longrightarrow N_{2(g)}$$

Other than the above discussed processes, biological processes like anammox, nitrifier nitrification, nitrifier denitrification, dissimilatory nitrate reduction to ammonia, nitrite oxidation, and abiotic processes like fixation of NH4⁺ on clay, ammonia (NH₃) volatilization, and leaching of NO₃⁻ also form important components of the N cycle (Vitousek et al., 1997; Pajares and Bohannan, 2016). Out of various steps in the N cycle, mineralization of organic matter to NH_4^+ and its subsequent nitrification to NO_3^- are the two major steps which provide nutrients, i.e., NH_4^+ and NO_3^- , to the soils for uptake by both plants and microorganisms. However, availability of these nutrients in the terrestrial ecosystem is subject to change as the processes regulating their production and consumption are governed by microbes, which are very prone to change in their surrounding conditions. Changes in both biotic and abiotic factors such as temperature, moisture, and vegetation cover can greatly alter the N production and consumption rates (Classen et al., 2015). It is therefore important to understand the factors controlling the N cycle and the availability of N in the ecosystem. Quantification of rates of these processes would be helpful to understand N and in turn C biogeochemistry of any ecosystem.

1.2 Perturbations to natural nitrogen cycling

In the first half of 19^{th} century, the increase in agricultural production raised the demand for inorganic N as it was becoming difficult to feed the world's growing population with limited food resources. To rely on natural N-fixation was not an option as it provided only a small amount of bioavailable N compared to the rising demands (Erisman et al., 2008). However, since the discovery of artificial fixation of atmospheric N₂ through Haber-Bosch process (20th century) there has been an immense increase in agricultural production; which, along with the industrial revolution, resulted in over 50% rise in anthropogenically fixed N (Galloway et al.,

2004). In this regard, the conversion of woodland to agricultural land, with intensified use of fertilizers, has greatly perturbed natural N cycling (Cameron et al., 2013). Nitrogen addition to the terrestrial biosphere through atmospheric deposition of industrial and vehicular exhausts, along with other sources such as fossil fuel burning, biomass burning, drainage of wetlands, and clearing of forests, has also contributed significantly towards alteration of natural biogeochemical cycling of N (Asner et al., 1997; Vitousek et al., 1997; Galloway et al., 2004; Zaehle, 2013; Reşitoğlu et al., 2015). These additional N inputs have greatly affected the coupling of C and N in various ecosystems. Both positive and negative effects of N addition to terrestrial ecosystems have been observed which primarily depends upon the N status of the system and the rate and duration of N deposition (Chen and Coops, 2009). Nitrogen addition increases net primary productivity of an N-limited ecosystem, whereas N-saturation is observed in ecosystems where N addition exceeds the ecosystem's capacity for N uptake eventually leading to forest decline (Nihlgård, 1985; Ågren and Bosatta, 1988; Bauer et al., 2004).

Increased N addition leading to N enrichment can have other environmental consequences as well. Large quantities of fertilizers can cause soil acidification or leach from the agricultural fields and contribute to N toxicity and eutrophication in the water bodies (Wallace, 1994; Barak et al., 1997; Fowler et al., 2013; Withers et al., 2014). Since the industrial revolution, annual inputs of reactive N have more than doubled in terrestrial and aquatic bodies, which are primarily driven by anthropogenic sources (Galloway et al., 2004). Accumulated N in the soils can also be lost as NO and N_2O under favourable conditions. Large scale emission of these gases cause negative impact on the environment as one of these gases (N_2O) contributes to ozone layer depletion and acts as a potent greenhouse gas, whereas the other (NO) behaves as a precursor for acid rain and photochemical smog (IPCC et al., 2007; Ravishankara et al., 2009). It is very clear that anthropogenic perturbations caused to natural N cycling have a large impact on the environment, biodiversity, ecosystem health, and mankind at both regional and global scale (Sutton et al., 2011). Apparently, biogeochemical cycling of N is one of the most disturbed among the basic elemental cycles. Given the issues associated with disturbance of the natural N cycle and its coupling with the C cycle, it has become
a necessity to resolve the complexity associated with N cycle and to understand its shift due to anthropogenic, and as a feedback, climatic disturbances (Field et al., 2007; Bernal et al., 2012; Shibata et al., 2015). Stable isotope applications are among the state-of-the-art techniques which can be employed to understand the processes involved in the biogeochemical cycling of the elements in great detail.

1.3 Use of stable isotopes

Isotopes are atoms of the same element with slightly different masses as their nuclei contain the same number of protons but different numbers of neutrons. Unlike radioactive isotopes, which decay over time, stable isotopes have a stable nucleus which makes their abundance constant over time. For this reason, stable isotope study of lighter elements offers an excellent tool for solving biogeochemical problems. Owing to their similar electronic structure, isotopes of an element undergo similar chemical, physical, and biological processes. However, due to minor difference in their masses, these processes happen with a slightly and consistently different rate, with faster reaction rate for the lighter isotope compared to the heavier isotope. This leads to isotopic fractionation, a phenomenon which causes change in the relative abundance of isotopes due to their mass differences.

In soil system science, stable isotopes of N and C are widely studied as they provide detailed information regarding sources, fates, and sinks of these elements and a better insight into the biogeochemical processes involved in their transformation. The application of stable isotope techniques in soil and environmental research has long been identified, and the use of ¹⁵N enriched fertilizers in agro-ecosystems is continuing since 1940s (Norman and Werkman, 1943). However, due to cost limitations of expensive ¹⁵N enriched fertilizers, the stable isotope studies based on measurement of natural abundances of rare isotope (¹⁵N) relative to abundant isotope (¹⁴N) have gained significant popularity in the recent decades (Robinson, 2001). The natural abundance level stable isotope approach provides meaningful information regarding natural ecosystems with minimal disturbance of the system. Given the importance of soil organic matter in storing CO₂ and regulating fluxes of major greenhouse gases, the stable isotope signatures of C (δ^{13} C) and N (δ^{15} N) in soils at natural abundance level are mostly

used to study organic matter turnover and related processes (Balesdent et al., 1988; Tiunov, 2007). Along with organic matter turnover, information regarding soil age, topography, soil parent material, vegetation shift, and impact of human activities can also be identified using stable isotopes at natural abundance level (Natelhoffer and Fry, 1988; Billings and Richter, 2006).

Despite recent advances in the application of stable isotopes at natural abundance level, the addition of artificially enriched ¹⁵N remains a useful technique to study biogeochemical cycling of elements. One of the most widely used applications of ¹⁵N tracer technique is in agricultural ecosystems, commonly used to estimate plant N yield and fertilizer use efficiency (Hauck and Bremner, 1976; Follett, 2001). In this technique, soils are artificially enriched with ¹⁵N fertilizer and the recovery and fate of fertilizer N is traced in different reservoirs. A very recent example of the use of ¹⁵N tracer technique is a study by Lenka et al. (2019), where they demonstrated the effect of elevated CO₂ and/or temperature on plant N uptake of a soybean-wheat cropping sequence. The ¹⁵N tracer technique, with the goal to assess elemental transfer from one pool to another (e.g., fertilizer to plant), can not only be used to study the impacts of environmental and anthropogenic perturbations on N allocation, but also can be used to identify mineralization-immobilization turnover and denitrification (Ellert and Rock, 2008). Isotope dilution is another technique using ¹⁵N fertilizer salt, which is used to decipher gross rates of N transformation processes. Unlike ¹⁵N tracer technique, ¹⁵N isotope pool dilution technique is based on labelling a pool with a known quantity of ¹⁵N salt which is allowed to equilibrate for a defined period after which pool size and isotopic abundance are measured. The amount of dilution in the initially added enriched salt indicates the size of the pool accessible to the tracer during the equilibration period (Ellert and Rock, 2008).

1.4 Gross nitrogen transformation rates

Prior to the introduction of the isotope dilution technique, the N transformation processes were determined by more popular non-isotopic net rate assay (Schimel and Bennett, 2004). Measurement of net rates of N transformations, which is still a popular technique due to its ease and low assessment cost, is conducted by

incubating the soils for certain time period, and the rates are calculated based on the change in nutrient's concentrations at defined time intervals (Drury et al., 2008). Results obtained from net rate assessments helped to develop three major concepts regarding N cycling: (i) plants can take only inorganic forms of N, (ii) N mineralization is the limiting step of N cycling, and (iii) plants are poor competitors to soil microorganisms for the available N and utilize excess N left after microbial demand (Schimel and Bennett, 2004; Hood-Nowotny, 2018). However, in past few decades, ¹⁵N isotope dilution based measurements of gross N transformation rates (total production; i.e., net production + immobilization) have resolved great complexities within N cycle which were not possible to be identified using net rate measurements. For instance, research based on ¹⁵N isotope dilution methods have provided a major paradigm shift in the understanding of N cycle (Figure 1.3) suggesting that plants are not only effective in competing with microorganisms for inorganic N, but in many cases they are also capable of absorbing N in the form of much simpler 'monomers' of the complex organic matter to fulfil their nutritional requirements (Schimel and Bennett, 2004).

The principal of ¹⁵N isotope dilution technique lies in isotopically spiking the product pool of the process involved with heavy N isotope (¹⁵N) and monitoring the isotopic dilution and change in pool size of the product with time (Kirkham and Bartholomew, 1954). For example, to measure gross N mineralization rates, the soil NH₄⁺ pool (i.e., product of N mineralization) is spiked artificially with ¹⁵N and the isotopic dilution (in atom %) of the spiked ¹⁵N together with the change in NH₄⁺ pool size is monitored with time (Figure 1.4). The isotopic dilution and change in soil NH₄⁺ pool occur due to the production of ¹⁴NH₄⁺ as a result of microbial preference for lighter isotope during N mineralization. Therefore, quantification of gross N mineralization rates is conducted by measuring the change in the initial (immediately after ¹⁵N spiking) and final (after incubation period) ¹⁵N enrichment and NH₄⁺ pool size. Consumptive processes can change the size of the nutrient pool but do not affect % atom enrichment (isotopic dilution); thus the quantification of gross consumption rates can also be performed using this method (Davidson et al., 1991; Drury et al., 2008).



Figure 1.3: Shift in the understanding of the N cycle from (a) classical paradigm to (b) new paradigm. Green and blue arrows indicate N uptake by plants and microorganisms, respectively. The conceptual advances during the two paradigms are represented by red text. Model adapted from Schimel and Bennett (2004).



Figure 1.4: Principle of gross N transformation rates measurement. MBN stands for microbial biomass N. Figure adapted from- Handbook of Soil Sciences: Properties and Processes, Second Edition.

Despite the potential of measurement of gross N transformation rates to provide detailed knowledge about the capacity of a system to produce nutrients along with mechanism of its production and uptake by both plants and microbes (Myrold and Tiedje, 1986; Davidson et al., 1991; Hart et al., 1994), there remains various challenges in using ¹⁵N isotope dilution techniques. For example, the ¹⁵N isotope dilution technique is based on several assumptions (Davidson et al., 1991; Kirkham and Bartholomew, 1954) such as:

- (i) The rates of N transformation processes remain constant during the incubation period.
- Microorganisms cannot discriminate between ¹⁵N and ¹⁴N during the incubation period.
- (iii) The amount of ¹⁵N assimilated during the incubation does not get remineralized.

These assumptions have been found to be violated in several studies and are reported to be valid for a small incubation period of not greater than one week (Davidson et al., 1991). Apart from these assumptions, one of the major reasons behind the limited number of studies using ¹⁵N isotope dilution technique is its high measurement cost and use of expensive tracer salts (Robinson, 2001). Another issue is the cumbersome experimental methodology which allows independent measurement of only one process (such as, gross N mineralization or gross nitrification) at a time. Nevertheless, measurement of gross N transformation processes by using ¹⁵N isotope dilution technique remains widely accepted and much preferred and reliable method to study gross N transformations and understand biogeochemical cycling of N in various ecosystems.

1.5 Soil nitrogen cycling: a global perspective

Soil N research has undergone various phases since its beginning. Where the initial phase of research focused on the availability of reactive N to fulfil demands of food security, the present day research is centred on mitigating N management after identifying harmful impacts of excess reactive N on the environment and ecosystems of the world (Galloway et al., 2013). Due to increasing identification of

negative effects of excess reactive N on biodiversity and ecosystem health (Compton et al., 2011; Galloway et al., 2013), the focus of soil N research also shifted to identify N loss pathways from ecosystems. This becomes of immense importance as the soil N cycle is closely linked to the terrestrial C cycle and a smallest shift in N cycle holds the potential to significantly release large proportions of stored CO₂ from the soils. However, there remains uncertainty in N budget, as the N inputs in soils of agricultural ecosystems are significantly larger than N outputs observed in hydrological systems (Howarth et al., 1996; Boyer et al., 2002). On the other hand, ecosystems, such as forests, experienced accumulation of N under elevated CO₂ levels, indicating improper characterization of N input processes (Zak et al., 2003; Yanai et al., 2013). Large uncertainties still remain in quantifying the budget of gaseous emissions (of NO, N_2O , and N_2) through the soil N cycle. These uncertainties regarding the fate of N entering into a system, and its feedback to the climate, will remain unresolved unless a detailed understanding of mechanisms behind N transformation processes and their rates in the soils is not acquired. This information regarding the quantification of N transformations and impact of their ecological interactions is lacking on a global scale (van Groenigen et al., 2015).

During 1990s, after increasing reports of gross N transformation processes, scientific understanding of N transformations was widened and mechanism behind N transformations in different ecosystems was identified (Schimel and Bennett, 2004). This was especially useful in low N ecosystems, where the measurements of gross N mineralization rates allowed to calculate net mineralization and immobilization rates and helped to estimate the rate at which NH₄⁺ becomes available for plant-microbe competition (Schimel and Bennett, 2004). Not just the mechanism, but also correlation of different N transformation processes and their regulators in different ecosystems have been comprehended using ¹⁵N isotope dilution assay. For example, soil C and N showed strong relationships with gross N mineralization rates in the United States and Australia, respectively, implying these to be effective predictors of mineralization in soils (Barrett and Burke, 2000; Wang et al., 2001). In a meta-analysis, microbial activity, substrate concentrations, and soil organic matter composition were shown to increase the production of inorganic

N in soils (Booth et al., 2005). Relationships between various N transformation processes such as N mineralization, NH₄⁺ consumption, NH₄⁺ immobilization, NH₄⁺ concentrations, and nitrification in different climates and ecosystem types, such as conifer and deciduous woodland, grassland, and shrub land were also explored (Booth et al., 2005). This meta-analysis also highlighted lack of sufficient studies on gross N transformation rates from tropical regions of the world, which restricted these regions to be included in the meta-analysis, limiting their conclusions to temperate, arctic/montane, and semi-arid ecosystems. Even among the limited studies on gross N transformations from the tropical ecosystems, data were completely lacking from the Indian subcontinent.

1.6 Soil nitrogen cycling: an Indian perspective

Being a developing nation with the world's second largest population, India is predicted to contribute significantly towards the increasing reactive N loads experienced worldwide (Galloway et al., 2008). Due to its emphasis on agriculture, most estimates of N cycling in India are related to agro-ecosystems; where the principal source of N in agricultural soils is industrial or biological N fixation and main pathway for N removal is crop harvest (Velmurugan et al., 2008). Organic matter mineralization, another natural source for inorganic N to plants, is not quantified with certainty (Velmurugan et al., 2008). Other research in the field of soil science have focused on soil properties, nutrient availability, fertilizer application, carbon inputs to soils, NO₃⁻ leaching and net rates of N transformations (Verma et al., 2011; Kujur and Patel, 2012; Bhattacharyya et al., 2013; Lal et al., 2013). Net rates of N mineralization, ammonification, and nitrification have also been identified in different agro-ecosystems to analyse their relative importance in different cropping sequences (Bhuyan et al., 2014).

Apart from agriculture, there are also studies focussed on understanding nutrient dynamics under different settings. For example, Sharma et al. (2009) investigated the impact of C, N, and phosphorous (P) dynamics in a humid tropical forest chronosequence in the Himalayas. In the field of agroforestry, Arunachalam and Arunachalam (2006) characterized N availability and net N mineralization rates in a paddy field, shifting agricultural fallow and forested soils to show the relative contribution of ecosystem characteristics in N transformations. On the same line, Pandey et al. (2007) investigated dynamics of mineral N pools and N mineralization rates in different seasons in the humid climate of the Andaman to identify their response in a home garden as compared to natural forest ecosystems. To identify controls on N transformations, Ganguli et al. (2016) conducted experiments in two differently managed dry deciduous forests in West Bengal in different seasons and identified soil moisture, nutrient, and organic matter content as the regulators of net N transformation processes. Ecosystem quality based research, such as the impact of savannization and influence of the land-use change of dry forests on soil N transformations, have also been studied in tropical forests of India (Tripathi and Singh, 2009, 2012). Effect of species invasion, such as Lantana camara L., on N mineralization and its feedback to nutrient cycle, was studied in dry deciduous Vindhyan forests of India (Sharma and Raghubanshi, 2009). N mineralization and its relation to microbial biomass, effects of cultivation, soil properties, burning and grazing in savannah, fertilizer and organic matter inputs, and topography are among few other topics on which research has been carried out in the Indian soils (Singh et al., 1991; Raghubanshi, 1992; Jha et al., 1996; Das et al., 1997; Tripathi and Singh, 2007; Pandey et al., 2010). Although there exists a spectrum of N transformation processes based research in the Indian sub-continent, all these rate estimates are based on measurements of net N transformations and do not provide details on gross N transformations in the Indian soils.

1.7 Scope and objectives of the present work

Given the increasing agricultural productivity, fertilizer use, biomass and fossil fuel burning, thermal power production, population, deforestation, etc., sources of reactive N are immense in the Indian subcontinent (Galloway et al., 2008). The subcontinent is also experiencing indications of climate change through the delayed monsoon, increasing mean annual temperature, and increased number of extreme events (Goswami et al., 2006), all of which hold the potential to significantly affect soil microbial functioning and N transformations, and in turn, C storage capacity of the soils. Under such a scenario, the absence of data related to gross N transformations from soils of the subcontinent creates a big black box in soil N based research of the world.

Therefore, the aim of this thesis is to quantify rates of gross N transformation processes (i.e., gross N mineralization, gross nitrification, NH₄⁺ consumption, NH₄⁺ immobilization, and NO₃⁻ consumption) and comprehensively understand some important aspects of N cycling in soils of different climatic zones of India. To fulfil the objectives of the thesis, along with ¹⁵N isotope dilution experiments, physico-chemical properties of soils, nutrients concentration, total organic C (TOC) and total N (TN) contents, and natural C and N isotopic compositions in soils collected from different ecozones of India were measured. Specifically, focus of the present study was to:

- Understand the impacts of temperature and elevation change on rates of gross N transformations in tropical montane soils. A decrease in gross N transformation rates was hypothesized with increasing elevation and decreasing temperature.
- (ii) Investigate N dynamics in soils under different land-use practices in semiarid climate. With the hypothesis of decrease in gross N transformation rates with increasing salinity, moisture, and decreasing vegetation, this study also aimed to identify the controls of salinity, moisture, vegetation, and their interactions on soil N cycling.
- (iii) Understand gross N transformation processes in soils of a tropical humid forest and compare rates with temperate counterparts. It was hypothesized that the N transformation rates in tropical forest soils would exceed the rates in temperate ecosystems.
- (iv) Identify the effects of agro-forestry on gross N transformation processes and nutrient dynamics in soils. It was hypothesized that monoculture practices acquired in agro-forestry would diminish N cycling as compared to the mixed forests.
- (v) Identify major controls on N and C dynamics of soils from different ecozones of India through their isotopic signatures. Climatic conditions of the ecosystems were hypothesized to exert a major hold on N and C dynamics and soil properties.

1.8 Outline of the thesis

This thesis consists of eight chapters, the details of which are as follows:

Chapter 1: Introduction

This chapter provides a brief overview of N cycling in terrestrial ecosystems and its importance in relation to C dynamics. The chapter discusses the use of stable isotopes in identifying and understanding processes associated with N in an ecosystem. This is followed by global and Indian perspective *vis-à-vis* soil N research. Last part of the chapter provides scope and major scientific objectives of the present study.

Chapter 2: Methodology

This chapter presents a brief description of the sampling regions. This is followed by discussion on methodologies for sample preparation, soil physico-chemical properties, nutrient analysis, and TN-TOC contents along with natural isotopic measurements of C and N in soils. Lastly, the protocol for the ¹⁵N isotope dilution experiment is described in detail.

Chapter 3: Nitrogen dynamics in montane soils of the Himalayas

This chapter discusses gross N transformations in tropical montane soils of the Himalayas. Based on the results of ¹⁵N isotope dilution experiments, this chapter addresses the potential changes in rates of N transformations in soil with depth as affected by the change in temperature conditions. Next part of the chapter identifies the role of elevation (altitude) in controlling N transformation processes in soils of a tropical montane ecosystem.

Chapter 4: Nitrogen cycling in semi-arid soils

This chapter presents the N dynamics in soils of different land-types of a semi-arid climate. Relationships of different N transformation processes with various characteristics of semi-arid ecosystems such as salinity, availability of moisture, and soil organic matter are explained in this chapter.

Chapter 5: Nitrogen transformations in tropical humid forest soils

This chapter focuses on the N dynamics in soils of a tropical humid mixed broadleaf forest. This chapter discusses external sources of N in a natural forested ecosystem and the microbial adaptations in response to it. The rates of gross N transformations of this study are also compared with the rates obtained in other temperate and tropical forests around the world.

Chapter 6: Nitrogen dynamics in differently managed forest soils

This chapter deals with N transformations in soils of differently managed forests to study the effects of forest type alteration and agro-forestry on soil microbial N dynamics.

Chapter 7: Organic matter content and its stable isotopic composition in soils of India

This chapter discusses C and N dynamics using stable isotope signatures of soils from different climatic zones of India.

Chapter 8: Summary and scope for future works

This chapter summarizes the results obtained from this thesis and provides the scope for the future works.

Chapter 2

Methodology

To fulfil the objectives of this thesis, samples were collected from the following study areas.

- (i) Tropical montane ecosystem: Soils were sampled from the Garhwal Himalayas in the Uttarakhand state of India. The soils were collected from five elevations, i.e., 3000 m, 2500 m, 2000 m, 1500 m, and 1000 m above mean sea level.
- Semi-arid ecosystem: Soils were collected from the Kutch district of the state of Gujarat, India. The sampling was performed based on geomorphological differences in land-types.
- (iii) Tropical humid forests: Soil sampling was carried out in a mixed broadleaf tropical forest at the Bhagwan Mahaveer Wildlife Sanctuary, Goa, situated in the northern part of the Western Ghats, India.
- (iv) Tropical forests under different land use: Soil were collected from differently organized forested ecosystems, i.e., managed forest, unmanaged forest, and rubber plantation, in Nilambur region of Kerala, India.

These study areas differed from each other with respect to climatic conditions as described by the Köppen climate classification system (Figure 2.1). Further details of the study areas are described in the respective chapters.



Figure 2.1: Sampling regions based on the Köppen climate classification system. Figure modified after Mishra (2014).

The soil sampling was performed at five locations in the first three sampling regions, whereas samples were collected from only three locations in the tropical forests with different land use in Kerala. Considering the heterogeneous nature of soils, samples were collected from three sites at each location of each sampling region, which were approximately 50 m apart. The sampling at each site was performed at two depth intervals, i.e., 0 - 20 cm (Top layer-T) and 20 - 40 cm (Bottom layer-B) leading to 30 samples from first three sampling regions (5 locations \times 3 sites \times 2 depths (n) = 30). Samples from only top layers were collected for bulk

density analysis. Samples were sieved through 4 mm mesh to remove stones and roots, and stored at 4 °C to be transported to the laboratory for immediate processing.

2.1 Soil physical properties

To determine the gravimetric water content (GWC), a measure of soil moisture, a subsample of soils was oven dried at 105 °C for 48 hours, until a constant dry weight was obtained. The weight difference of field moist and dry soil was used to calculate GWC (Drury et al., 2008). Bulk density of soils was determined by using the dry weight of soils collected from core and volume of the core. Water filled pore space (WFPS), indicative of aerobic/anaerobic condition of the soil, was calculated using GWC, bulk density, and total porosity of soils using the following equation (Equation 2.1).

WFPS (%) =
$$\frac{\text{Gravimetric water content } \times \text{bulk density}}{\text{total porosity}} \times 100$$
 (Eq. 2.1)

where, total porosity was calculated as: 1 - (bulk density/particle density). The average value of particle density was taken as 2.6 g cm⁻³ (Hao et al., 2008).

For measurement of soil pH, a mixture of oven dried soil and ultrapure water was prepared in 1:5 ratio (Rayment and Higginson, 1992), where the mixture was shaken well and subsequently allowed the sediments to settle down. A pre-calibrated pH electrode was immersed in soil suspension to record the pH value (PC 2700 - Eutech Instruments, Oakton). Where desired, the salinity of the soils was also measured in 1:5 soil-water suspension using bench-top PC 2700 (Eutech Instruments, Oakton) (Hardie and Doyle, 2012).

2.2 Mineral nitrogen concentration in soils

Nutrient concentrations for mineral N, i.e., NH_4^+ and NO_3^- , were determined in triplicate for each sample using soil KCl extracts (Drury et al., 2008; Maynard et al., 2008). For this purpose, 20 g oven-dry equivalent field moist soils were mixed with 150 mL of 2 M KCl solution in the Erlenmeyer flasks (Borosil[®]). The mixture was

shaken for 1 hour, followed by filtration with Whatman no. 1 filter paper. The soil KCl extracts containing NH_4^+ and NO_3^- were analyzed for NH_4^+ and NO_3^- concentrations by colorimetric technique. The analysis was performed using a continuous flow injection San⁺⁺ autoanalyzer (Skalar) with an analytical precision of ± 0.01 mg L⁻¹.

2.2.1 Autoanalyzer

Autoanalyzer works on the principle of segmented flow analysis and is a continuous flow method of wet chemistry in which a stream of samples and reagents, segmented with bubbles, is pumped through a manifold for reactions to take place before entering a flow cell to be detected. The main parts of the autoanalyzer system are autosampler, chemistry unit with pump, rinsing valves and chemistry module, detectors, and data handling unit (Figure 2.2).



Figure 2.2: San++ autoanalyzer system at the Physical Research Laboratory.

A modified Berthelot reaction method was used for determination of NH_{4^+} concentrations in the soils (Krom, 1980). In this method, NH_{4^+} is chlorinated to monochloramine, which reacts with salicylate to form 5-aminosalicylate. After oxidation and oxidative coupling, a green-colored complex is formed which is measured at 660 nm. The automated determination for NO_3^- and NO_2^- in soils was based on the cadmium reduction method (Dorich and Nelson, 1984; Maynard et al.,

2008) modified with imidazole buffer. In this method, the sample is buffered at pH 8.2 and is passed through a column containing granulated copper-cadmium to reduce NO_3^- to NO_2^- . The NO_2^- , already present and produced through the reduction of NO_3^- , is determined by diazotizing with sulfanilamide and coupling with N-1-naphthyl-ethylenediamine dihydrochloride to form a reddish purple azo dye which is measured at 540 nm. To determine the concentration of total NO_3^- in the samples, amount of NO_2^- (measured without using cadmium reduction column) is subtracted from total NO_2^- determined as $NO_3^- + NO_2^-$.

2.3 Total nitrogen and total organic carbon contents and their isotopic compositions

The soil samples were analyzed for TN and TOC contents in bulk soils along with their isotopic compositions using thermal oxidation (dry combustion) method modified after Giovannini et al. (1975). For this purpose, a subsample of collected soils was dried in an oven at 105 °C for 48 h. After grinding, dried soils were packed in ultraclean tin capsules (~ 15 - 20 mg) for measurement of TN and δ^{15} N. For TOC and δ^{13} C, the soils were first decarbonated using 1 M HCl to remove inorganic C fraction present in soils. For decarbonation, 40 mL of 1 M HCl solution was added to 2 g of soils in centrifuge tubes and kept overnight at 80 °C on a hot plate. Subsequently, the mixture was centrifuged and the supernatant was decanted. The soils were washed several times with ultrapure water until a neutral pH was obtained. The soils were dried again, ground, and packed in tin capsules (~ 5 mg) for TOC and δ^{13} C analysis. The analysis for TN and TOC along with their isotopic compositions was performed using an Elemental Analyzer (Flash 2000) connected to an isotope ratio mass spectrometer (IRMS-Delta V plus - Thermo Scientific) via ConFlo (Figure 2.3). TOC and TN contents in soil samples were calculated using a straight line calibration equation generated using standards of known %C (IAEA-CH-3 (cellulose): 44.40%) and %N (IAEA-N-2 (ammonium sulphate): 21.21%) against area under the curve. The analytical precisions for TOC and TN analyses were less than 10% for duplicate measurements. The analytical precision for δ^{13} C and δ^{15} N measurements were less than 0.1‰ and 0.3‰, respectively. IAEA-CH-3 (– 24.7‰) and IAEA-N-2 (20.3‰) were used as standards during the measurements of δ^{13} C and δ^{15} N, respectively. Isotopic compositions are denoted by δ notation (Equation 2.2) and expressed in ‰ unit:

$$\delta(\%_0) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$
 (Eq. 2.2)

where, R is the ratio of the heavier isotope to the lighter isotope. Final values are reported with respect to the international standards, i.e., Vienna- Pee Dee Belemnite (V-PDB) for δ^{13} C and Air-N₂ for δ^{15} N.



Figure 2.3: Delta V plus isotope ratio mass spectrometer and Flash 2000 Elemental Analyzer at the Physical Research Laboratory.

2.4 Soil organic carbon stock

Soil organic C stock, one of the most important reservoirs of global C, is represented largely by C held within soil microbial biomass, dead plants, and decomposed animals. Soil organic C stock was measured in the top 10 cm of soils, which were sieved through < 2 mm mesh before analysis. After obtaining soil TOC content, organic C stock in soils was calculated using Equation 2.3 (Evrendilek et al., 2004).

SOC $(kg ha^{-1}) = (\%TOC/100) \times soil mass (kg ha^{-1})$ (Eq. 2.3)

where, soil mass (kg ha⁻¹) = depth (m) × bulk density (Mg m⁻³) × 10000 m² ha⁻¹ × 1000 kg Mg⁻¹.

2.5 Isotope dilution experiment

For the measurement of gross N transformation rates, the ¹⁵N isotope dilution technique was employed during the present study (Davidson et al., 1991; Drury et al., 2008). In this technique, the soil nutrient pool is labeled with enriched salt (¹⁵NH₄⁺ for mineralization and ¹⁵NO₃⁻ for nitrification) and a dilution in the enrichment as atom % excess is observed with time which indicates the progress of the process (Davidson et al., 1991). As the consumptive processes do not affect atom % excess and only change the nutrient pool size, rates of gross consumption of nutrients can be calculated along with their production rates.

For the isotope dilution experiments, soils from the top and bottom layers at each site of each location of the respective study areas were divided into three parts for experiments/measurements to be performed in triplicates. To begin the experiments, 80 g sieved soils, from each site, were transferred in 30×30 cm ziplock polyethylene bags in triplicate for both gross N mineralization and gross nitrification experiments. After homogenizing the soils, bags were kept on a flat surface and the samples were spread in a thin layer for the addition of enriched salts. Using a fine gauge needle and a syringe, 4 mL of 98 atom % (¹⁵NH₄)₂SO₄ and K¹⁵NO₃ tracer solution (Sigma Aldrich) containing 40 mg N L⁻¹ was added drop-wise to the soil samples for gross N mineralization and gross nitrification experiments, respectively (Davidson et al., 1991; Drury et al., 2008). For even mixing, 1 mL of solution was added evenly across the soils and the treated soils were remixed and were spread again into a thin layer to repeat the procedure another three times. Immediately after tracer addition, a soil subsample (20 g oven-dry equivalent) was extracted in 2 M KCl solution to determine pre-incubation (time-0) concentration and ¹⁵N enrichment of soils. The amended soils were incubated for 2 days (48 to 49 hours) in the dark at room temperature (Davidson et al., 1991; Drury et al., 2008). After completion of the incubation period, soils were mixed well and a final soil extract in 2 M KCl was taken for post-incubation (time - t) NH_4^+ concentration and ^{15}N enrichment.

All the soil KCl extracts obtained during the experiments were divided into two parts. One aliquot was used to determine the concentration of the nutrients while the other was used for the measurement of isotopic enrichment (atom %). To determine pre- and post-incubation nutrient pool size, nutrients (NH₄⁺ and NO₃⁻) concentrations in pre- and post-incubation soil KCl extracts were measured using colorimetric techniques as described in Section 2.2. Diffusion method (Stark and Hart, 1996) was followed for the measurement of ¹⁵N enrichment in pre- and postincubation nutrient pool.

2.5.1 Diffusion experiment

Diffusion method, described by Stark and Hart (1996), was used to extract dissolved nutrients into the solid phase for isotope analysis. An aliquot of soil KCl extracts was used for diffusion experiment for which 70 mL aliquots of the pre-incubation and post-incubation KCl extract were placed separately in 150 mL specimen cups (Figure 2.4).



Figure 2.4: Diffusion experiment conducted in specimen cups for extraction of dissolved nutrients (in soil KCl extracts) to the solid phase (on GF/D filters).

In the samples treated for mineralization experiments, acidified GF/D filter paper disks were placed on the soil KCl extracts followed by addition of 200 mg MgO. To prepare the acidified GF/D filter paper disks, 25μ L of 2 M H₂SO₄ was used to acidify GF/D filter paper which was followed by pressing the filter paper between two hydrophobic Teflon membranes (Merck, Millipore). The cups were sealed immediately after the addition of MgO to avoid loss of NH₃ which generates from dissolved NH₄⁺ in the alkaline medium created by MgO (Brooks et al., 1989). The generated NH₃ was collected on acidified discs as ammonium sulphate ((NH₄)₂SO₄) complex. The cups were kept sealed for six days and were shaken in between for proper reaction required for complete conversion of dissolved NH₄⁺ to NH₃ gas. After 6 days, the filter disks were taken out and the GF/D filter papers were dried in H₂SO₄ fumes.

For the KCl extracts of gross nitrification experiments, first 200 mg MgO was added to the solution and the cups were kept open for six days with occasional stirring for removal of all dissolved NH_4^+ from the solution. Afterward, acidified GF/D filter disks and 400 mg Devarda's alloy were added to the aliquots (Brooks et al., 1989; Sørensen and Jensen, 1991) and the cups were sealed immediately for another six days with periodical gentle shaking. Devarda's alloy, being a reducing agent, reduces NO_3^- to NH_4^+ which gets collected as $(NH_4)_2SO_4$ on the filter disk in the basic medium. The filter disks were taken out after six days. A schematic diagram representing the isotope dilution experiments is shown in Figure 2.5.

After drying with concentrated H₂SO₄ fumes, all the GF/D filter papers were packed in tin capsules for isotope analysis. The ¹⁵N enrichment was determined on an isotope ratio mass spectrometer (Delta V plus-Thermo Fisher Scientific) connected to an Elemental Analyser (Flash 2000).



Figure 2.5: Schematic presentation of ¹⁵N isotope dilution experiment for the measurement of gross mineralization and gross nitrification rates.

The $\delta^{15}N$ values for the nutrients obtained from mass spectrometer were converted into atom % using following Equation 2.4:

Atom % =
$$\left[\frac{R_{sample}}{1 + R_{sample}}\right] \times 100$$
 (Eq. 2.4)

where, R_{sample} can be calculated from the $\delta^{15}N$ values obtained from the mass spectrometer.

2.6. Calculations and data analyses

The calculations for the gross rates of N mineralization and nitrification (Equation 2.5) along with the consumption rates of NH_4^+ and NO_3^- (Equation 2.6) were carried out using the equations given by Kirkham and Bartholomew (1954) and Hart et al. (1994).

$$GPR = \frac{N_0 - N_t}{t} \times \frac{\log(A_0/A_t)}{\log(N_0/N_t)}$$
(Eq. 2.5)

$$CR = GPR - \frac{N_t - N_0}{t}$$
(Eq. 2.6)

where, GPR = gross production rate (N mineralization or nitrification) of nutrient (NH₄⁺ or NO₃⁻) in soils (mg N kg⁻¹ day⁻¹), CR = consumption rate of NH₄⁺ or NO₃⁻ in soils (mg N kg⁻¹ day⁻¹), t = 2 days (time of incubation), A₀ = atom % ¹⁵N excess of NH₄⁺ or NO₃⁻ pool at time 0, A_t = atom % ¹⁵N excess of NH₄⁺ or NO₃⁻ pool at time 0, A_t = atom % ¹⁵N excess of NH₄⁺ or NO₃⁻ pool at time 0, A_t = atom % ¹⁵N excess of NH₄⁺ or NO₃⁻ pool at time 1, N₀ = total nutrient (NH₄⁺ or NO₃⁻) concentration (mg N kg⁻¹) at time 0, N_t = total (NH₄⁺ or NO₃⁻) concentration (mg N kg⁻¹) at time t.

The NH_4^+ immobilization (Equation 2.7) was calculated as a difference in gross NH_4^+ consumption and gross nitrification (Hooker and Stark, 2008).

 NH_{4^+} immobilization = Gross NH_{4^+} consumption – Gross nitrification (Eq. 2.7)

Mean residence time of nutrients in the soil pool (Equation 2.8) was calculated by dividing nutrient pool size $(NH_4^+ \text{ or } NO_3^-)$ by its production rates.

Mean residence time =
$$\frac{\text{nutrient pool size}}{\text{nutrient production rate}}$$
 (Eq. 2.8)

After triplicate analysis of the samples, results in this thesis are expressed on a dry weight basis. The data obtained for N transformation rates, nutrient pools, and other soil parameters were subjected to statistical analyses. The data were tested for normality of distribution using Shapiro-Wilk and homogeneity of variation using Levene's test. Student's t-test was used to compare N transformation rates and nutrient pools in normally distributed data, whereas the Mann-Whitney Rank Sum Test was used for randomly distributed data. With a significance of p < 0.05, linear regression approach (Pearson correlation) was used to establish the relationships among gross N transformations and associated variables. With Tukey's honestly significant different (HSD) test for post-hoc analysis, analysis of variance (ANOVA) was used for multiple comparison of soil properties and N transformation rates at different locations. The tests were conducted with 95% confidence and were considered significant at p < 0.05. The statistical analyses of results were performed using Statistica Version 6.1 (Stat Soft. Inc.) and SigmaPlot Version 11.0 (Systat Soft. Inc.).

Chapter 3

Nitrogen dynamics in montane soils of the Himalayas

Soils store ~ 1500 Pg of total C and 92 - 140 Pg of total N in the top one meter of global soil layer representing the largest terrestrial pool for C and N (Oertel et al., 2016). Forest soils form one of the important components of this global soil C and N pool. However, C in forested ecosystems is stored not only in soils but in biomass as well, the relative proportion of which is regulated by the climatic condition of the region. For example, the dominant fraction of total C in boreal forests is stored in soils, whereas in tropical forests biomass stores larger fraction (Pan et al., 2011). In tropical montane regions, forest soils are the dominant reservoir of C due to low temperature and slow rate of organic matter decomposition (Moser et al., 2011; Nottingham et al., 2016). Because of the same reasons, montane soils are also limited by availability of nutrients, such as N, as low temperature and slow decomposition rates reduce the production of mineral N (Tanner et al., 1998; Stewart, 2000), which in turn can affect both above- and below-ground productivity in these ecosystems.

High sensitivity of microorganisms towards changing environmental conditions results in the production of variable soil nutrients pool and thus can improve or diminish ecosystem productivity. Change in temperature and elevation are the two closely linked factors which govern microbial N dynamics in the montane ecosystems. Many studies have focused on the effect of temperature and elevation on soil C dynamics to identify their relationship with soil respiration and primary productivity in tropical montane ecosystems (Nottingham et al., 2015, 2016, Selmants et al., 2016). However, most studies addressing effects of temperature and elevation change on N transformation rates remain confined to high-latitudes (Knoepp and Swank, 1998; Shaw and Harte, 2001; Knoepp and Vose, 2007; Zhang et al., 2012; Schütt et al., 2014) with limited focus on tropical high-altitude regions (Kitayama et al., 1998; Marrs et al., 1988; Wilcke et al., 2013; Bauters et al., 2019).

For the low temperature ecosystems present in high-latitudes and highaltitudes, even slight increase in the temperature can significantly contribute to increase in rates of organic matter mineralization, soil respiration, and apparent nutrient availability (Nadelhoffer et al., 1997; Knorr et al., 2005; Davidson and Janssens, 2006). Based on the temperature sensitivity of microbially mediated reactions, often measured as Q10 coefficient, several studies have observed significant change in the microbial activity related to N transformations (Dalias et al., 2002; Wang et al., 2006; Auyeung et al., 2012; Fraser et al., 2013). It has also been suggested that this temperature sensitivity, i.e., increase in rates of N transformations at higher temperature relative to ambient, is highly dependent on organic C supply (Dessureault-Rompré et al., 2010; Gutiérrez-Girón et al., 2015). Other than the Q10 coefficient, many studies investigated the direct effect of temperature change on N dynamics to address the response of different N transformation processes to increase in temperature. Largely agreeing with Q10 coefficient studies, most of these studies indicated increase in N transformation rates and related microbial activity with increase in temperature. This implies that climate warming in future has the potential to shift the pattern and emission flux of greenhouse gases, respiration rates, and nutrient turnover in soils of colder regions due to high temperature sensitivity of the below-ground biota. However, such studies addressing the effect of temperature change on gross N transformations in tropical montane ecosystems remain limited (Wang et al., 2016; Yuan et al., 2016).

In the montane ecosystems, temperature becomes a function of elevation with a general trend of decrease in temperature with increasing elevation. However, similar to temperature change, variation in N transformation rates due to change in elevation also remain unexplored in tropical montane regions as most of such studies were conducted in high-latitude regions (Powers, 1990; Knoepp and Swank, 1998; Hart and Perry, 2001; Zhang et al., 2012) with focus on net rates (Kitayama et al., 1998; Marrs et al., 1988). In general, decrease in available nutrients in the litter, particularly N, with increasing elevation owing to low nutrient supply; and enhancement in the litter production due to the addition of N and P was noticed in tropical montane ecosystems (Tanner et al., 1998). This indicates the importance of elevation in regulating productivity in the montane ecosystems. However, both increase and decrease in rates of N transformations with increase in elevation have been reported (Kitayama et al., 1998; Knoepp and Swank, 1998; Marrs et al., 1988; Zhang et al., 2012), further complicating the understanding towards the relationship of N transformations with elevation.

Despite the importance of temperature and elevation in shaping nutrient dynamics and primary productivity in montane ecosystems, a large part of tropical montane regions remains understudied with no reports so far from the Himalayas, one of the youngest and ecologically sensitive high-altitude regions of the world. Therefore, the present study aimed to decipher potential changes in N transformation rates under different temperature and elevation conditions in the Himalayas by quantifying the gross rates of N transformations using the ¹⁵N isotope dilution technique. For this purpose, experiments to measure gross N mineralization and nitrification along with NH₄⁺ consumption and immobilization rates were performed on soils from five elevations incubated under two temperature conditions. We hypothesized an increase in rates of N transformations with increasing soil temperature and lowering in rates with increase in elevation.

3.1. Sampling and experiment

The sampling region is located in the Garhwal Himalayas in the Uttarakhand state of India (Figure 3.1 & 3.2). The climate in this region is controlled by monsoon and varies from cold temperate to tropical (Bagchi and Singh, 2011; Rawat and Chandra, 2014). The mean annual precipitation in the region is approximately 1395 mm (Bagchi and Singh, 2011). Winter precipitation is associated with the Western

Disturbances in the form of snowfall at higher elevations (Bagchi and Singh, 2011). January is the coldest month with mean maximum temperature of 19.6 °C and the mean minimum temperature of 4.6 °C, whereas June is the warmest month with mean minimum and mean maximum temperatures of 32.6 °C and 36.5 °C, respectively (Bagchi and Singh, 2011). The major part of the region is covered by soils of the Lesser Himalaya, which may be broadly subdivided into three types, i.e., Dystric Eutrudepts, Lithic Udorthents, and Typic Udorthents (Bagchi and Singh, 2011). The vegetation diversity of the Himalayas is influenced by topography, soil, climate, and geographical location of the region (Chandra et al., 2010).



Figure 3.1: Sampling locations at different elevations above mean sea level at the Garhwal Himalayas.



Figure 3.2: Field photographs showing different types of vegetation and topography of the sampling region.

For the experiments during this study, soils were collected at two depth intervals from three sites of five elevations (3000 m, 2500 m, 2000 m, 1500 m, and

1000 m above sea level (asl)) in the Garhwal Himalayas (Figure 3.2) during November 2017 (n = 30). With a mean of 12 °C, temperature variation along the elevation gradient was from 8 °C to 17 °C during the time of sample collection (Table 3.1). Vegetation pattern differed among the sampling locations and showed change in vegetation cover with elevation (Table 3.1). Samples were bought to the laboratory and processed for measurements of the parameters discussed in Chapter 2. For discussion, 3000 m and 2500 m asl elevations are termed as upper elevations; whereas 2000 m, 1500 m, and 1000 m asl are combinedly referred as lower elevations, unless mentioned specifically.

Table 3.1: Soil physical properties, vegetation, and climate type at different elevations.

	3000 m	2500 m	2000 m	1500 m	1000 m
Location	30° 31' N, 79° 33' E	30° 32' N, 79° 33' E	30° 29' N, 79° 36' E	30° 31' N, 79° 31' E	30° 24' N, 79° 22' E
Temperature	8 °C	9 °C	11 °C	17 °C	16 °C
pН	4.5 ± 0.3	5.0 ± 0.6	6.1 ± 0.7	6.5 ± 0.2	6.3 ± 0.0
Bulk density (g cm ⁻³)	1.0 ± 0.2	1.0 ± 0.1	1.1 ± 0.2	1.3 ± 0.1	1.0 ± 0.2
GWC (%)	21.7 ± 10.8	33.3 ± 5.9	11.4 ± 5.2	8.8 ± 6.2	6.4 ± 2.6
Major vegetation	Rhododendron arboreum, Quercus floribunda, Cedrus deodara, Betula utilis, Pinus wallichiana	Rosaceae, Fragaria sp, Rubus foliolosum, Rhododendron arboreum, Acer oblongum, Quercus incana	Thuja orientalis, Berberis sp, Cedrus deodara, Arenaria neilgherrensis, Pinus roxburghii	Rubus macilentus, Cersium sp, Lantana camara	Aegle marmelos, Pinus roxburghii, Shorea robusta
Climate zone	Alpine-cold temperate	Cold temperate	Warm temperate	Warm temperate	Sub- tropical
Forest type	Himadri forest	Moist semi temperate	Moist semi temperate- tropical pine	Tropical pine	Tropical deciduous

The soils collected from different elevations were subjected to ¹⁵N isotope dilution experiments for quantification of gross N transformation rates as described in Chapter 2. However, to determine the effect of temperature on microbial N transformations, incubation of amended soils was conducted at two different temperature conditions during the experiment. Incubation at low temperature condition was carried out at 10 ± 2 °C, whereas the high temperature incubation was conducted at the room temperature. Daily minimum and maximum room temperatures during the incubation period varied between 17 - 30 °C with an average of 23 °C. During the ¹⁵N isotope dilution experiments, immediately after the addition of tracer to the soils, a subsample of amended soils was extracted in 2 M KCl solution for measurement of initial (pre-incubation) nutrients concentrations and their isotopic (¹⁵N atom %) enrichments. The remaining soils were divided into two parts to be incubated in the dark for two days at two different temperature conditions, as mentioned earlier. Therefore, the initial (pre-incubation) nutrient pool size and isotopic enrichment were same for the two incubation conditions, while the final (post-incubation) nutrient pool size and isotopic enrichment depended on the incubation temperature conditions.

3.2 Physico-chemical properties of soils

Despite the change in elevation, bulk density of soils was low (~ 1.0 g cm⁻³) and remained nearly constant with low soil moisture throughout (< 40%; Table 3.1) indicating soils at all elevations to be sufficiently porous to support respiration and root growth (Kakaire et al., 2015). Porosity of soils appeared to be the highest at the upper elevations, which were more susceptible to weathering as these monsoon-influenced forested regions experienced low temperatures and high wind speed compared to the lower elevation soils (Carroll, 1970). Soil pH was significantly lower at upper elevations (F_{4,25} = 25.73, p < 0.001) and showed decrease in acidity at lower elevations (Table 3.1). High weathering of the bedrock probably resulted in the acidic pH of soils present at the upper elevations compared to the lower.

Overall, NH_4^+ was the predominant form of mineral N in the study area with significantly higher NH_4^+ in the top layers than the bottom (p < 0.01, Figure 3.3a). The NO_3^- concentrations showed no significant change with depth (Figure 3.3b). The average nutrients (NH_4^+ and NO_3^-) concentrations during the present study were higher in the top soils from upper elevations as compared to the lower (Figure 3.3) with variability in the middle. One of the potential factors affecting nutrient dynamics at different elevations is the change in organic matter quantity and quality due to the presence of varied species diversity across elevations. The vegetation in this region also varies with elevation, showing increasing species richness at lower altitudes (Gairola et al., 2011).



Figure 3.3: Layer wise variation in (a) NH_4^+ and (b) NO_3^- concentrations in the Himalayan soils at different elevations (mean $\pm 1\sigma$).

Showing large variability at upper elevations, average TN and TOC contents of soils were relatively higher at the upper compared to the lower elevations (Figure 3.4). Despite the variation in soil physico-chemical properties and vegetation, there was no significant difference in TN ($F_{4,25} = 0.91$, p > 0.05) and TOC ($F_{4,25} = 0.82$, p > 0.05) contents of the soils with elevations.



Figure 3.4: Layer wise variation in (a) TN and (b) TOC contents in the Himalayan soils at different elevations (mean $\pm 1\sigma$).

During the present study, the litter inputs through broadleaf species present at the higher elevations were greater as compared to the small litter inputs through pines and shrubs present at the lower elevations (Table 3.1). The trees present at 3000 m (mainly *Rhododendron arboreum*) provided high litter quantity compared to other elevations, which partially decomposed at ambient low temperature conditions to generate light fractions of organic matter resulting into higher nutrients concentrations (Figure 3.3) and TN and TOC contents (Figure 3.4) in the soils.

3.3 Gross nitrogen transformation processes and elevation

Similar to nutrients concentrations, N transformation rates showed a mixed trend with elevation for the top soils, whereas no particular trend was observed for the bottom soils (Figure 3.5). In general, the gross N mineralization rates showed a decreasing trend from the upper to the lower elevations with significantly higher rates in the top soils of 3000 m asl elevation ($F_{4,10} = 6.25$, p < 0.01; Figures 3.5a & 3.5b). Although gross nitrification rates showed no particular trend with elevation (Figures 3.5c & 3.5d), these rates were significantly higher in the bottom soils of 1500 m asl elevation compared to other elevations. Similar to gross nitrification rates, with relatively higher values in the top soils at the upper compared to the lower elevations, NH_4^+ consumption rates showed no significant difference with elevation (Figures 3.5e & 3.5f). NH_4^+ immobilization rates also showed no significant variation across the elevation gradient. However, NH_4^+ immobilization rates exhibited a decreasing trend from the upper to the lower elevations in the top soils (Figures 3.5g & 3.5h).

As observed during the present study, both increase (Knoepp and Swank, 1998; Nottingham et al., 2015) and decrease (Kitayama et al., 1998; Zhang et al., 2012; Yuan et al., 2016) in rates of N transformation processes with elevation have been observed elsewhere in the world. Change in elevation is often associated with different vegetation types owing to differences in temperature, precipitation, and soil types. Despite the change in vegetation type, many studies have reported linear relationships of soil C and N dynamics with elevation (Kitayama et al., 1998; Zhang et al., 2012; Yuan et al., 2016).

The lack of consistently increasing or decreasing trend in nutrients concentration and N transformation rates with elevation indicated important contribution of factors other than elevation in regulating nutrient dynamics in the region. As mentioned previously, presence of diverse tree species at different elevations can contribute to different quantity and quality of organic matter with variable nutrients, TN, and TOC contents, which could be a potential factor to affect the nutrient dynamics at different elevations. Nutrient dynamics in montane soils have also been shown to be primarily regulated by edaphic factors resulting in different vegetation and nutrient turnover (Yang et al., 2015). Different slope facing, sunlight availability, and micro-climatic conditions at the sampling locations result in higher control of edaphic factors over climate in montane systems, which may be at play during the present study as well. In a previous study, Kitayama et al. (1998) have also reported significantly different N mineralization rates with elevation due to the different nature of the geological substrate (sedimentary and ultrabasic rocks). Our results showing inconsistent change in rates of N transformation processes with elevation were in agreement with a previous study which showed insignificant increase in N transformation processes in soils of different elevations incubated under laboratory conditions (Marrs et al., 1988).



Figure 3.5: Gross N transformation rates (mean $\pm 1\sigma$) at high and low incubation temperature conditions in the Himalayan soils: (a) gross N mineralization rates in top soils, (b) gross N mineralization rates in bottom soils, (c) gross nitrification rates in top soils, (d) gross nitrification rates in bottom soils, (e) NH₄⁺ consumption rates in top soils, (f) NH₄⁺ consumption rates in bottom soil layer, (g) NH₄⁺ immobilization rates in top soils, and (h) NH₄⁺ immobilization rates in bottom soils.
3.4 Gross nitrogen transformation rates and temperature

Temperature is an important abiotic factor which regulates N availability and its cycling in soils. In mountainous terrains, temperature becomes a function of elevation and can shape the microbial potential for N transformations different from that in the plains. This can affect C sequestration capacity of plants present at different elevations by affecting production and use of available nutrients, especially the ones which are produced through biotic pathways, such as N. The findings of this study clearly indicated a significant increase in gross N transformation rates when the incubation temperature was raised from low (10 °C) to high (23 °C). Rates of all the N transformation processes, i.e., gross N mineralization, gross nitrification rates, NH4⁺ consumption, and NH4⁺ immobilization were significantly higher (p < 0.05) for the high temperature compared to the low temperature incubation condition, especially in the top soil layers (Figure 3.5). This indicted a major control of temperature on overall N transformation rates in the Himalayan soils. Increase in gross N transformation rates with increase in temperature has been observed in other studies as well, which is largely attributed to increase in microbial activity due to high temperatures (Andersen and Jensen, 2001; Shaw and Harte, 2001; Niboyet et al., 2011; Jansen-Willems et al., 2016).

During the high temperature condition, the potential for NH₄⁺ consumption in the Himalayan soils was relatively higher than gross N mineralization rates, whereas the reverse was observed for low temperature condition (Figure 3.6). This indicated the sensitivity of these two processes towards temperature change and relative importance of temperature conditions on retention of N in soils. An increase in NH₄⁺immobilisation to gross N mineralization ratio at high temperature compared to low temperature condition indicated an increase in N immobilization potential of microbes due to the increase in temperature (Figure 3.6). The residence times of both NH₄⁺ and NO₃⁻ were significantly higher (p < 0.05) for the low compared to the high temperature condition (Figures 3.7). The high residence time of nutrients during the low compared to the high temperature conditions also indicated that nutrients turnover is accelerated by increase in temperature, possibly due to efficient microbial activity at high temperature. It is possible that low temperature condition during the present study may be lower than the required optimum temperature for efficient microbial functioning, resulting in the accumulation of nutrients in soils (Figures 3.7).



Figure 3.6: Ratios of NH_4^+ consumption rates: gross N mineralization rates and NH_4^+ immobilization rates: gross N mineralization rates at both high and low incubation temperature conditions in the Himalayan soils. The dashed line represents 1:1 ratio.



Figure 3.7: Residence time of nutrients (mean $\pm 1\sigma$) at high and low incubation temperature conditions in the Himalayan soils: (a) NH₄⁺ residence time in top soils, (b) NH₄⁺ residence time in bottom soils, (c) NO₃⁻ residence time in top soils, and (d) NO₃⁻ residence time in bottom soils.

Table 3.2: Correlation matrix indicating squared Pearson correlation coefficient (r^2) for N transformations and related parameters. Sections above and below the inclined line represent correlations at high and low incubation temperature, respectively. The abbreviations are - MR: gross N mineralization rates, A-CR: NH₄⁺ consumption rates, NR: gross nitrification rates, A-imm: NH₄⁺ immobilization rates, A-RT: NH₄⁺ residence time, N-RT: NO₃⁻ residence time, TOC: total organic carbon, TN: total nitrogen. Significance of correlations are reported as follows: ***: p < 0.001; **: p < 0.05; NS: Not significant.

	MR	A-CR	NR	A-imm	A-RT	N-RT	$\mathrm{NH_4}^+$	NO ₃ -	TOC	TN
MR		0.63 ***	0.16 *	0.75 ***	NS	NS	0.72 ***	0.25 **	0.75 ***	0.78 ***
A-CR	0.61 ***		0.60 ***	0.87 ***	NS	NS	0.50 ***	0.30 **	0.65 ***	0.69 ***
NR	NS	0.50 ***		0.25 **	NS	0.28 **	0.20 *	0.47 ***	0.19 *	0.34 ***
A-imm	0.67 ***	0.76 ***	NS		NS	NS	0.51 ***	NS	0.73 ***	0.66 ***
A-RT	0.19 *	NS	NS	NS		NS	NS	NS	NS	NS
N-RT	NS	NS	0.29 **	NS	NS		NS	NS	NS	NS
$\mathrm{NH_4}^+$	0.78 ***	0.61 ***	0.17 *	0.61 ***	NS	NS		0.35 ***	0.56 ***	0.58 ***
NO ₃ -	0.39 ***	0.36 ***	0.51 ***	NS	NS	NS	0.35 ***		0.20 *	0.40 ***
TOC	0.66 ***	0.65 ***	0.20 *	0.63 ***	NS	NS	0.56 ***	0.20 *		0.84 ***
TN	0.72 ***	0.69 ***	0.33 ***	0.53 ***	0.13 *	NS	0.58 ***	0.40 ***	0.84 ***	

The N transformation processes appeared to be tightly coupled at the high temperature than the low temperature condition as observed through stronger correlations between different N transformation processes such as, NH₄⁺ consumption, gross mineralization, gross nitrification, and NH₄⁺ immobilization under high temperature (Table 3.2) suggesting effective cycling of N due to efficient microbial functioning.

Soil C and N are used as a reliable index to measure soil quality and microbial efficiency of any ecosystem (Knoepp et al., 2000; Louis et al., 2016). Gross N mineralization rates correlated positively with TN ($R^2 = 0.78$, p < 0.001) and TOC ($R^2 = 0.75$, p < 0.001) contents of soils at high temperature condition, which remained significant with lesser degree of correlation for low temperature condition (Figure 3.8a & 3.8b). Similar to gross N mineralization, gross nitrification rates also showed significant positive relationships with soil TN ($R^2 = 0.34$, p < 0.001; Figure 3.8c) and TOC ($R^2 = 0.19$, p < 0.05; Figure 3.8d) contents. Though the rates of gross N mineralization and nitrification correlated well with TN and TOC contents of soils under both high and low temperature conditions, changes in N transformation rates with respect to TOC were not as strong as those for TN (Table 3.3). This indicated the availability of N to be relatively important regulator of N transformation processes in the Himalayan soils. Although well correlated at both temperature conditions, stronger correlations of TOC and TN with gross N mineralization and nitrification rates existed at the high temperature condition indicating the effective transformation of soil organic matter to inorganic nutrients at high temperature (Figures 3.8a-d). As evident from significantly higher slope during the high temperature compared to the low temperature condition (Table 3.3), it appeared that increase in temperature significantly enhanced N mineralization potential in soils for a given TN and TOC content. Gross nitrification rates also increased for a given TN and TOC at the high temperature compared to the low temperature condition (Figures 3.8c & 3.8d). However, contrary to N mineralization, this increase was not significant as observed from statistically similar slopes for two temperature conditions (Table 3.3). This indicated that microorganism associated with nitrification were relatively less sensitive to temperature increase than those involved in N mineralization.

Gross nitrification rates were positively correlated to gross N mineralization rates at high temperature condition ($R^2 = 0.16$, p < 0.05) which was not observed during low temperature condition (Figure 3.8e; Table 3.2). Weak correlations and similar slope of gross nitrification with gross N mineralization rates (Figure 3.8e) along with weak correlations between gross nitrification - NH₄⁺ concentrations (Table 3.2) at both high and low temperature conditions also supported relatively lesser sensitivity of nitrifying microbes to temperature.



Figure 3.8: Relationships between (a) gross N mineralization rates and TN, (b) gross N mineralization rates and TOC, (c) gross nitrification rates and TN, (d) gross nitrification rates and TOC, and (e) gross nitrification rates and gross N mineralization rates at both high and low incubation temperature conditions in the Himalayan soils (n = 30). The p-value indicates difference in the slopes.

Table 3.3: Regression equations for nitrogen transformation rates at different incubation temperatures (high: 23 °C and low: 10 °C). The data includes both top and bottom soils (n = 30). Significance of correlations are reported as follows: ***: p < 0.001; **: p < 0.01; *: p < 0.05; NS: Not significant.

Dependent Variable	Independent Incubatio variable temperati		Equation	\mathbb{R}^2	p-value for slope	
N Mineralization rates (Min)	TN	High $Min = (1.16 \pm 0.12) \times$		0.78***	0.001	
	TN	Low	$Min = (0.64 \pm 0.07) \times TN + 0.31$	0.72***	< 0.001	
	TOC	High	$Min = (0.09 \pm 0.01) \times TOC + 1.52$	0.75***	0.001	
	TOC	Low	$Min = (0.05 \pm 0.01) \times TOC + 0.73$	0.66***	< 0.001	
Nitrification rates (Nit)	TN	High	Nit = $(0.37 \pm 0.10) \times \text{TN} + 0.14$	0.34***	0.12	
	TN	Low	Nit = $(0.20 \pm 0.05) \times TN + 0.01$	0.33***	0.12	
	TOC	High	Nit = $(0.02 \pm 0.01) \times \text{TOC} + 0.56$	56 0.19*		
	TOC	Low	Nit = $(0.01 \pm 0.01) \times \text{TOC} + 0.22$		0.30	
Nitrification	N Mineralization	High	Nit = $(0.19 \pm 0.08) \times Min + 0.41$	0.16*	0.00	
Nitrification	N Mineralization	Low	Nit = $(0.16 \pm 0.08) \times Min + 0.23$	0.13 ^{NS}	0.80	

The results of the present study were in contrast to a previous study which showed increase in net nitrification rates during high temperature incubations (Grundmann et al., 1995). However, the present results were consistent with a previous study which showed increase in gross N transformations due to the heating of soils, where the gross nitrification remained unaffected (Shaw and Harte, 2001). Overall, given the increase in N mineralization and immobilization along with the insignificant response of nitrification under high temperature condition, it appears that warming of soils present in colder montane climate can lead to significant production of NH₄⁺, which may eventually get consumed through immobilization rather than nitrification.

3.5 Variation in nitrogen transformation rates with depth

Most of the parameters measured during the present study such as, NH_4^+ concentrations, TN and TOC contents, gross N mineralization, NH_4^+ consumption, NH_4^+ immobilization, and gross nitrification rates were significantly higher in the top layers compared to the bottom suggesting higher microbial activity in the top soils (Figures 3.3, 3.4, 3.5, & 3.7). The surface soils are known to be microbially more active compared to deeper layers due to high organic matter concentration, greater fine root activity, and proximity to forest floor (Bolton et al., 1993; Winkler et al., 1996; Kellman et al., 2014; Shang et al., 2015). Changes in these parameters, along with other biotic and abiotic factors, are more likely to affect the top soils and can result in higher variability in N transformation rates and nutrients pools present in the top soils compared to the bottom, which was also observed in our results. Unlike parameters mentioned above, NO_3^- concentrations and NO_3^- residence time did not vary significantly with depth (Figures 3.3 & 3.7) suggesting nitrification to be relatively less affected by surrounding conditions during the present study.

3.6 Conclusion

This study aimed to investigate the impact of change in temperature and elevation on gross N transformation rates in soils collected in depths from the Himalayas. Increase in temperature significantly enhanced the rates of gross N mineralization, NH₄⁺ consumption, and NH₄⁺ immobilization, whereas gross nitrification rates did not show significant increase. This finding partially agreed with our initial hypothesis which suggested a significant increase in rates of all N transformation processes with increased temperature. Increase in rates of both gross N mineralization and NH_4^+ immobilization at high temperature indicated the potential for acceleration in both production and consumption of N in the Himalayan soils due to increase in temperature. Also, NH_4^+ immobilization was the major pathway for NH_4^+ consumption under increased temperature condition. Contrary to our other hypothesis, there was no consistent change in process rates and pools related to N transformations with elevation indicating the microbial activity in soils of this tropical montane ecosystem to be mainly controlled by the edaphic factors at different elevations. Except NO_3^- concentrations and its residence time in soils, a significant decrease in N pool and turnover rates was observed in the bottom soils compared to the top indicating the top soils to be relatively more microbially active.

Chapter 4

Nitrogen cycling in semi-arid soils

Like other ecosystems, the availability of N is inextricably linked to plant productivity and C dynamics in drylands as well. Covering ~ $1/3^{rd}$ of the Earth's land area, drylands play an important role in regulating global C cycle by storing 46% of global C and contribute up to ~ 40% of net primary productivity (Grace et al., 2006; Prăvălie, 2016). However, drylands are facing a global threat of desertification, particularly in semi-arid regions (~ 13.6% of the world's dryland; WAD 2018), which can drastically affect their biological productivity (Verón et al., 2006; Verstraete et al., 2009). Globally, areas undergoing desertification are predicted to increase with rapid and extreme rainfall as well as drought events (Knapp et al., 2008; Dai 2012). Such changes can substantially affect normal functioning, productivity, and elemental biogeochemistry of dryland ecosystems.

Increase in soil salinity is a major consequence of desertification, which is likely to increase in the future due to frequent droughts and sea-level rise. Salinity can substantially alter biodiversity and community composition of soil microorganisms as it is considered stress for microbes (Rietz and Haynes, 2003). Several studies have evaluated the effects of salinity on soil N transformation rates as availability of N is inextricably linked to biological productivity. Majority of these studies have either measured the net rates (Pathak and Rao, 1998; Khoi et al., 2006; Bai et al., 2012) or conducted laboratory experiments with amended salinity (Lodhi et al., 2009; Akhtar et al., 2012; Zeng et al., 2013; Gao et al., 2014). In general, these studies indicated the negative effect of salinity on N mineralization (Fang et al., 2005; Yuan et al., 2007; Lin et al., 2016). Additionally, short-lived adverse effects (Khoi et al., 2006), salinity threshold (Pathak and Rao 1998; Zeng et al., 2013), and increased N mineralization (Noe et al., 2013) due to salinity change have also been reported. However, to the best of our knowledge, studies considering effects of natural salinity change on gross N transformations at ecosystem level are non-existent in tropical settings and remain poorly understood.

Similar to salinity, scarcity of water is also a major characteristic of semi-arid drylands which can limit productivity, nutrients availability, and microbial activity in these regions (Reichman et al., 1966; Stark and Firestone 1995; Homyak et al., 2017; Yang et al., 2017). Low and unevenly distributed soil moisture results in the formation of relatively nutrient-rich soil microsites compared to its nutrient-poor surroundings (Schlesinger et al., 1996; Chen and Stark 2000). The microsite formation results in patches of microbial activity, thereby creating pockets of mineralization and immobilization within the soils (Chen and Stark, 2000). If sufficient moisture is available, nutrients get transported from the N-rich to the N-poor microsites creating a homogeneous nutrient environment. Nutrients availability as a function of moisture is well-known in bacteria dominated forested and mesic environments (Stark and Firestone, 1995). However, in the arid and semi-arid ecosystems, which are characterized by drought-tolerant fungi (Zak et al., 1995; Vishnevetsky and Steinberger 1997; Schimel et al., 2007), this mechanism is not well-understood.

The arid and semi-arid ecosystems characterized by low soil moisture and poor organic content can show a significant change in nutrient dynamics with vegetation shift due to change in the quality of detrital inputs (Vinton and Burke, 1995; Evans et al., 2001). As the organic inputs determine the flow of inorganic N in soils, the quality of organic matter controls the soil N availability (Chen and Stark, 2000). Not just the quality of litter, but several other factors also regulate N transformations and its availability in soils. To understand differences in the N cycle, Booth et al. (2005), in a meta-analysis, compared N cycling rates across various climatic regimes, vegetation-types, and land-uses. Their study highlighted the lack of data related to N transformation rates in desert ecosystems and tropical

climate, which restricted our understanding towards cycling of N in such environments. Based on their work, studies were conducted in the desert and arid ecosystems to validate the controls on gross N transformation rates with a focus on cold deserts and Mediterranean climate (Homyak et al. 2017, Yang et al. 2017, Kou et al. 2018). However, the continued absence of such studies in tropics limits our understanding of the N cycle in tropical arid and semi-arid climate.

In view of the above, this study focused on N dynamics in semi-arid soils of western India by quantifying the rates of N transformations. For this purpose, ¹⁵N isotope dilution experiments were performed in soils of different land-types, which included salt flat, grassland, seasonal wetland, and agricultural field. The specific objective of the study was to identify the effect of salinity, vegetation, and moisture availability on N transformation rates to identify potential controls on the cycling of N in tropical semi-arid soils. Given that the selected land-types could be characterized by high salinity-high moisture-low vegetation (salt flat) and low-salinity-low moisture-high vegetation (grassland, seasonal wetland, and agricultural field) conditions, it was hypothesized that soils experiencing high salinity-high moisture-low vegetation not not rates.

4.1 Study area and sampling

Soil sampling for the present study was conducted in the Kutch district ($22^{\circ} 81' - 24^{\circ} 66' N, 68^{\circ} 31' - 71^{\circ} 48' E$) of the state of Gujarat, India, during January 2017 (Figure 4.1). Kutch forms the south-western continuation of the arid belt of north-western India including the Thar Desert (Maurya et al., 2003). The region falls under high aridity (aridity index > 40%) and experiences mean annual precipitation of 32 cm (< 15 rainy days), mean annual temperature of 26 °C, and mean annual humidity < 15% (Gupta, 2015). Soils of this region are of Aridisol and Entisol order (Kolay, 2007). The soils sampling was performed at five different land-types (Figure 4.1), which included two locations in salt flat and one each in wetland, grassland, and an agricultural field (n = 30, refer Chapter 2 for sampling procedure).



Figure 4.1: Sampling in different land-types across the semi-arid region of the Kutch, India. Grey shaded region indicates the geographical location of the Kutch in India. SF-1: coastal salt flat, SF-2: inland salt flat, WL: wetland, GL: grassland, and AF: agricultural field.

Situated in a region called the *Rann* of Kutch, one location in the salt flat was influenced by the adjacent Arabian Sea (coastal), whereas the other largely exhibited the effect of long dry summers (inland) (Glennie and Evans, 1976; Chatterjee and Ray, 2017). The grassland location was in *Banni* grassland, where grasses are grouped into coarse-low perennial and coarse-pioneer colonizing categories (Patel et al. 2012). The agricultural field was fertilized with urea, diammonium phosphate, and farm yard manure during the time of sampling. The wetland (*Chhari Dhand*), where the sampling was performed, was located between the *Banni* grassland and the *Rann*. Out of the five land-types, salt flat showed no visible sign of vegetation, while others (wetland, grassland, and agricultural field) showed presence of different plant species (Figure 4.2).

Following methods described in Chapter 2, the sampled soils were analyzed for various physico-chemical properties and experiments were conducted to measure gross N transformation rates. For the purpose of discussion, the study area has been divided into two groups: (i) saline, high-moisture, and non-vegetated soils represented by inland and coastal salt flat, and (ii) non-saline, low-moisture, and vegetated soils represented by wetland, grassland, and agricultural field.



a. Salt flat-1 (coastal)



c. Wetland



b. Salt flat-2 (inland)



d. Grassland



e. Agricultural field

Figure 4.2: Photographs of sampling locations in different land-types across the semi-arid region of the Kutch, India.

4.2 Soil physical properties

The soils of the study area showed large variability in moisture content with significantly higher (p < 0.001) moisture in the salt flat compared to others (Table 4.1). The average bulk density of soils was 1.5 g cm⁻³ and did not vary significantly among the land-types (Table 4.1). The soils were alkaline and showed a significant negative relationship with soil moisture ($R^2 = 0.41$, p < 0.001). Soil pH was significantly different among land-types (p < 0.001; Table 4.1), with the highest in the grassland and the lowest in the salt flat. Salinity was significantly higher (p < 0.001; Table 4.1) in soils of the salt flat, which was approximately thirty times greater compared to the rest of the land-types.

Table 4.1: Physical properties of soils with different land-type. The values are represented as mean $\pm 1\sigma$ including both depths (n = 6), except for bulk density which was measured for top soils only (n = 3). The parameters differing significantly (ANOVA) across land-types are indicated by * (p < 0.001).

	рН *	Salinity *	GWC (%) *	Bulk density
	pm	Samity	0,000 (70)	$(g \text{ cm}^{-3})$
Coastal salt flat	7.98 ± 0.07	18.36 ± 4.74	29.12 ± 3.16	1.44 ± 0.06
Inland salt flat	7.88 ± 0.06	15.91 ± 7.30	25.30 ± 6.22	1.54 ± 0.02
Wetland	8.34 ± 0.23	0.79 ± 0.38	21.64 ± 4.15	1.40 ± 0.12
Grassland	8.50 ± 0.30	0.33 ± 0.29	1.61 ± 0.71	1.55 ± 0.02
Agricultural field	8.15 ± 0.10	$0.26\ \pm 0.26$	11.97 ± 2.97	1.56 ± 0.13

4.3 Nitrogen transformation processes and related parameters

The TOC and TN contents of soils were significantly different across the landtypes (Table 4.2) with a strong positive relationship between them ($R^2 = 0.82$, p < 0.001; Table 4.3). Gross N mineralization rates in the top soils were statistically different across the land-types (p < 0.001; Table 4.2) with the highest average rate in the agricultural soils. Gross nitrification rates in vegetated (non-saline) soils were significantly higher than in non-vegetated saline soils (p < 0.01) with the highest average rate for the agricultural soils. The rates declined significantly with depth for vegetated soils (Table 4.2). NH₄⁺ immobilization showed similar trends with significantly higher rates in vegetated (non-saline) soils compared to nonvegetated (saline) soils (Table 4.2). Similar to nutrients production rates, NH₄⁺ consumption rates were also significantly different across the study area with the highest average consumption rate in the agricultural soils compared to the rest of the land-types (p < 0.001; Table 4.2). NO_3^- consumption rates were low and did not show significant change across land-types. Negative NO₃⁻ consumption rates were also observed at a few locations, which were considered zero during statistical analyses because negative values for gross N transformation rates are theoretically not possible. Negative values for gross N transformation rates have been observed elsewhere as well, which have been attributed to the lack of dilution in ¹⁵N atom % excess during small incubation time (Stiles, 2012), uneven labelling of nutrient pools at natural abundance level (Habteselassie et al., 2006), or due to preferential consumption of applied N (Watson and Mills, 1998; Verchot et al., 2002).

Both NH₄⁺ and NO₃⁻ concentrations in soils of the study area showed no significant variation with depth. However, both were significantly different across land-types (p < 0.05; Table 4.2) with the highest concentrations in the agricultural and grassland soils, respectively. Overall, average NO₃⁻ concentration was higher than average NH₄⁺ concentration in the study area, particularly due to high and variable NO₃⁻ in the grassland soils (Table 4.2). The average mean residence time of NO₃⁻ was also higher compared to that of NH₄⁺ (Table 4.2).

4.4 Gross nitrogen transformation rates and salinity

Gross N mineralization rates showed significant decrease with increase in salinity ($R^2 = 0.36$; p < 0.001; Figure 4.3a). Similar to N mineralization, gross nitrification rates also showed significant decline with increase in soil salinity ($R^2 = 0.21$, p < 0.05; Figure 4.3b). The gross N transformation rates in saline soils were significantly lower compared to non-saline soils indicating poor microbial potential for N transformations under high salinity condition (Figure 4.3). Reduction in N transformation rates due to high salinity and moisture conditions has been observed elsewhere, which was attributed to shift in the composition of microbial fauna due to salinity change (Pathak and Rao, 1998; Rath and Rousk, 2015; Yan et al., 2015; Rath et al., 2016).



Figure 4.3: Variations in (a) gross N mineralization rates (bars; mean $\pm 1\sigma$) and (b) gross nitrification rates (bars; mean $\pm 1\sigma$) with salinity (line) across different land-types; where, SF-1: coastal salt flat, SF-2: inland salt flat, WL: wetland, GL: grassland, T: top soils and B: bottom soils.

	Top Soils $(0, 20 \text{ cm})$						Pottom Soils (20, 40 cm)					
			Top Solis (0	- 20 CIII)		Bottom Solis (20 - 40 cm)						
	Coastal salt flat	Inland salt flat	Wetland	Grassland	Agricultural field	Coastal salt flat	Inland salt flat	Wetland	Grassland	Agricultural field		
Total organic carbon *	2.75	0.73	10.21	2.75	5.89	1.93	1.45	3.32	2.01	2.52		
$(mg C g^{-1})$	(2.82)	(0.74)	(6.38)	(0.69)	(2.73)	(0.45)	(0.26)	(0.69)	(0.54)	(0.70)		
Total nitrogen **	0.20	0.11	1.63	0.46	0.84	0.17	0.32	0.42	0.30	0.46		
(mg N g^{-1})	(0.01)	(0.01)	(0.64)	(0.06)	(0.30)	(0.02)	(0.12)	(0.04)	(0.06)	(0.08)		
Gross N mineralization rates ***	0.18	0.23	2.23	3.13	5.16	0.28	0.14	0.79	0.57	2.94		
$(mg N kg^{-1} d^{-1})$	(0.04)	(0.13)	(0.25)	(1.71)	(0.94)	(0.12)	(0.10)	(0.31)	(0.47)	(0.31)		
Gross nitrification rates *	0.18	0.25	1.28	1.67	3.45	0.35	0.31	0.42	1.02	0.76		
$(mg N kg^{-1} d^{-1})$	(0.12)	(0.17)	(0.21)	(1.39)	(1.54)	(0.13)	(0.26)	(0.30)	(1.00)	(0.45)		
NH ₄ ⁺ immobilization rates ***	IC	0.06	1.75	2.79	5.27	IS	0.10	0.75	0.57	3.57		
$(mg N kg^{-1} d^{-1})$	15	(0.06)	(0.50)	(2.43)	(0.76)		(0.09)	(0.74)	(0.99)	(0.71)		
NH ₄ ⁺ consumption rates ***	0.04	0.29	3.03	3.95	8.71	0.14	0.26	1.11	0.93	4.34		
$(mg N kg^{-1} d^{-1})$	(0.07)	(0.23)	(0.29)	(1.94)	(2.26)	(0.17)	(0.08)	(0.59)	(1.06)	(0.30)		
NO ₃ ⁻ consumption rates ^{NS}	IC	0.18	1.16	1.00	0.35	IC	0.41	0.41	0.04	0.16		
$(mg N kg^{-1} d^{-1})$	15	(0.32)	(0.93)	(1.58)	(0.60)	15	(0.31)	(0.56)	(0.08)	(0.28)		
NH ₄ ⁺ concentration *	1.18	1.35	1.67	2.32	6.91	0.96	1.04	1.54	1.27	1.80		
$(mg N kg^{-1})$	(0.12)	(0.27)	(0.35)	(0.66)	(3.59)	(0.11)	(0.03)	(0.27)	(0.12)	(0.39)		
NO ₃ ⁻ concentration *	1.40	0.57	3.08	20.40	6.44	1.48	1.25	0.91	15.92	2.02		
(mg N kg ⁻¹)	(0.63)	(0.25)	(0.26)	(28.84)	(1.91)	(0.20)	(0.33)	(0.31)	(12.10)	(0.22)		
NH ₄ ⁺ residence time ***	7.42	9.76	0.76	1.19	1.32	4.10	14.87	2.27	3.31	0.63		
(days)	(2.19)	(7.53)	(0.19)	(1.25)	(0.58)	(2.24)	(3.01)	(1.08)	(1.82)	(0.19)		
NO ₃ ⁻ residence time ^{NS}	17.80	3.79	2.77	8.33	2.29	5.98	4.68	5.23	25.99	3.46		
(days)	(23.06)	(3.74)	(0.52)	(7.01)	(1.26)	(1.96)	(2.70)	(3.76)	(26.60)	(1.99)		

Table 4.2: Different parameters related to nitrogen transformations with depth in the study area. Mean values are reported with the standard deviation (1σ) in the parentheses. IS = Insignificant. Parameters differing significantly across the land-types are indicated as * for p < 0.05, ** for p < 0.01, and *** for p < 0.001; whereas the non-significant variations are indicated as NS.

Table 4.3: Linear correlation coefficient (r) matrix for N transformation rates, soil N and C pools and moisture content. The values with asterisk show the significant correlation. The level of significance is represented as * for p < 0.05, ** for p < 0.01, and *** for p < 0.001. TN: total nitrogen, TOC: total organic carbon, MR: gross N mineralization rates, A-CR: NH₄⁺ consumption rates, NR: gross nitrification rates, N-CR: NO₃⁻ consumption rates, A-imm: NH₄⁺ immobilization rates, GWC: gravimetric water content, Sal: Salinity.

	TN	TOC	MR	A-CR	\mathbf{NH}_{4}^{+}	NR	N-CR	NO ₃ -	A-imm	pН	GWC	Sal
TN	1											
TOC	0.91***	1										
MR	0.48**	0.39*	1									
A-CR	0.47**	0.39*	0.98***	1								
$\mathbf{NH_{4}^{+}}$	0.30	0.30	0.73***	0.82***	1							
NR	0.41*	0.39*	0.68***	0.74***	0.86***	1						
N-CR	0.32	0.31	0.07	0.08	0.21	0.48**	1					
NO ₃ -	0.03	0.02	0.01	0.02	0.15	0.50**	0.56**	1				
A-imm	0.40*	0.31	0.98***	0.96***	0.67***	0.56**	-0.09	-0.10	1			
pН	0.19	0.21	0.36	0.29	0.04	0.08	0.02	0.11	0.37*	1		
GWC	-0.09	-0.03	-0.46*	-0.42*	-0.20	-0.35	-0.07	-0.51**	-0.47**	-0.64***	1	
Sal	-0.41*	- 0.30	- 0.60***	-0.57***	-0.34	- 0.46*	- 0.23	- 0.29	-0.58***	- 0.59***	0.73***	1

The reduction in N transformation rates may also be indicative of microbial adaptation during stress as under high saline conditions microbes deploy most of their energy in accumulating osmolytes (Yan et al., 2015), thereby resulting into low gross N transformation rates. During the present study, the most affected process due to increase in soil salinity was N mineralization as the increase in salinity creates microbial stress decreasing both mineralization and soil respiration rates (Bai et al., 2012; W. Zeng et al., 2013).

4.5 Soil nitrogen dynamics and moisture content

Increased salinity can inhibit soil evaporation and hence increase the water content of soils (Zeng et al., 2013; Zhang et al., 2013), which was also reflected in our study. Additionally, the coastal salt flat was located at a supra-tidal zone where maximum evaporation did not occur due to the difference in moisture between pore water and atmosphere (Geng and Boufadel, 2015). Wetland and agricultural soils also showed relatively higher moisture content compared to grassland due to their proximity to large water bodies and irrigation water supply, respectively. Of all the land-types, grassland soils reflected the actual moisture level for the semi-arid climate as these soils did not receive water from external sources.

In the present study, the gross N mineralization rates decreased significantly with increasing soil moisture ($R^2 = 0.21$, p < 0.05), indicating reduction in N mineralization at high moisture level (Figure 4.4a). The decrease in microbial activity with increasing soil moisture have been reported in the Mediterranean region (Parker and Schimel, 2011; Suvillian, 2012; Schaeffer et al., 2017), which is quite similar to the tropical climate. Similarly, NH₄⁺ consumption rates also showed negative relationship with soil moisture ($R^2 = 0.18$, p < 0.05; Figure 4.4b). Rates of NH₄⁺ immobilization showed negative correlation with soil moisture ($R^2 = 0.22$, p < 0.01; Figure 4.4c); whereas rates of nitrification, i.e., the other NH₄⁺ consumption process, showed no significant correlations of moisture contents with both NH₄⁺ consumption and immobilization rates suggested a pivotal role of soil moisture on NH₄⁺ consumption pathways. However, no

significant relation between moisture content and nitrification rates (Figure 4.4d) suggested limited effect of soil moisture on nitrification. It appeared that decrease in soil moisture stimulated NH4⁺ immobilization with little effect on nitrification as NH₄⁺ consumptive process. On the other hand, alkaline soils with low moisture conditions are known to be optimum for nitrification (Jiao et al., 2016), releasing H⁺ as a by-product in the soils (Sahrawat, 2008). Being in the semi-arid climate, soil pH of the study area averaged ~ 8 with relatively higher in soils with low moisture, which was also reflected in the inverse relationship ($R^2 = 0.41$, p < 0.001) between soil moisture and pH (Figure 4.4e). During the present study, soils with relatively lower moisture (i.e., non-saline soils particularly grassland) showed relatively higher buildup of NO₃⁻ pool compared to soil with high moisture (Figure 4.4f), potentially due to favorable nitrification conditions. This was evident by a negative correlation of NO_3^- concentration with soil moisture (R^2) = 0.26, p < 0.01; Figure 4.4f). In contrast, relatively lower NO_3^- pool in saline soils suggested either lower NO_3^- production due to low substrate (NH_4^+) on account of low N mineralization rates or increased NO3⁻ loss by denitrification under high soil moisture conditions.

In low moisture conditions, as experienced by grassland soils during the present study, development of 'Resource Island' is a common phenomenon where nutrients tend to accumulate in specific areas such as under shrub canopy. Abundance and movement of nutrients to the surroundings in such cases are restricted by moisture availability in soils (Schlesinger et al., 1996; Schade and Hobbie, 2005). The development of 'Resource Island' was supported by significantly higher and variable NO_3^- concentrations in the grassland soils compared to other land-types. Due to lack of moisture, soils of semi-arid regions tend to accumulate NO_3^- because of minimal microbial immobilization in the absence of sufficient detrital C (Davidson et al., 1991; Booth et al., 2005; Hooker and Stark 2008). The average TOC and TN of grassland soils in the present study were also low supporting the above argument. The observed variations in moisture contents during the present study emphasize the need for optimum moisture conditions for healthy microbial functioning as both low and high



moisture contents could adversely affect nutrient dynamics in the semi-arid ecosystems.

Figure 4.4: Variation in (a) gross N mineralization rates, (b) NH_4^+ consumption rates, (c) NH_4^+ immobilization rates, (d) gross nitrification rates, (e) pH, and (f) NO_3^- concentrations with gravimetric water content (moisture) across different land-types.

4.6 Nitrogen transformation rates and vegetation

In general, the soils with high salinity were also devoid of vegetation, thus limited by the supply of organic matter. Both gross N mineralization and gross nitrification rates were significantly higher in vegetated land-types compared to non-vegetated, indicating higher microbial activity in the presence of organic inputs (Figure 4.5). Even within the vegetated soils, gross N transformation rates (particularly mineralization, nitrification, and NH_4^+ consumption) were higher in the top soils compared to the bottom (Figure 4.5). On the contrary, no significant difference in the N transformation rates was observed with depth in non-vegetated soils, corroborating the significance of vegetation in controlling the nutrient dynamics in soils (Figure 4.5).



Figure 4.5: Gross N transformation rates (mineralization and nitrification) with depth at vegetated and non-vegetated land-types (mean $\pm 1\sigma$).

Gross N mineralization rates were positively correlated with TN ($R^2 = 0.23$, p < 0.01; Figure 4.6a) and TOC ($R^2 = 0.15$, p < 0.05; Figure 4.6b) contents in the soils. Similarly, gross nitrification rates showed a positive relationship with TN ($R^2 = 0.17$, p < 0.05; Figure 4.6c) and TOC ($R^2 = 0.15$, p < 0.05; Figure 4.6d). These correlations indicated significant control of organic matter inputs on nutrient production processes. A significant positive correlation between TN and gross N mineralization rates (p < 0.05) supported a previous study which advocated for control of mineralization on productivity due to the abundance of TN (Yang et al., 2017). The TOC and TN contents in soils of the vegetated (nonsaline) land-types showed a significant decline with depth, which was absent for non-vegetated (saline) soils. The TOC and TN in vegetated soils were higher indicating internal nutrient recycling of litter to be the major nutrient source for microbes and plants (Fontaine et al., 2003), which was absent in non-vegetated saline soils (Table 4.2).



Figure 4.6: Variations in (a) gross N mineralization rates with total nitrogen, (b) gross N mineralization rates with total organic carbon, (c) gross nitrification rates with total nitrogen, and (d) gross nitrification rates with total organic carbon in vegetated and non-vegetated soils.

The land-types compared in this work showed a cumulative negative effect of high salinity, high moisture, and low vegetation on gross N transformation processes. However, the backward stepwise regression analysis indicated salinity (p < 0.01) to be the most important factor affecting N transformation rates.

4.7 Major controls on soil nitrogen availability and nutrient dynamics

During the present study, gross N mineralization rates and gross nitrification rates were positively correlated with NH₄⁺ concentrations ($R^2 = 0.54$, p < 0.001) and NO₃⁻ concentrations ($R^2 = 0.25$, p < 0.01), respectively (Figure 4.7). The significant positive correlations of nutrients (NH₄⁺ and NO₃⁻) concentration with gross N mineralization and gross nitrification indicated major control of these processes on production and availability of nutrients in the system (Figure 4.7). The rates of gross nitrification were also positively correlated with NH₄⁺ concentrations ($R^2 = 0.74$, p < 0.001; Figure 4.8), suggesting the production of NO₃⁻ to be significantly dependent on the availability of substrate (NH₄⁺) in soils.



Figure 4.7: Relationships of (a) NH_4^+ concentrations with gross N mineralization rates and (b) NO_3^- concentrations with gross nitrification rates.

Nitrification rates showed a positive relationship with N mineralization rates ($R^2 = 0.46$, p < 0.001; Figure 4.9), and NH₄⁺ consumption ($R^2 = 0.55$, p < 0.001; Table 4.3). Of the two NH₄⁺ consumption processes, NH₄⁺ immobilization was dominant in soils showing high mineralization (Figure 4.9). Up to 95% variation in NH₄⁺ immobilization rates was explained by N mineralization rates (Figure 4.9). This indicated that the availability of NH₄⁺ is dependent on both its production (mineralization) and consumption (NH₄⁺ immobilization or nitrification; Figure 4.9) pathways.



Figure 4.8: Relationship between gross nitrification rates and NH_4^+ concentrations in soils of the study area (n = 30).



Figure 4.9: Relationships between gross mineralization and two possible NH_4^+ consumptive processes, i.e., gross nitrification or NH_4^+ immobilization in soils (n = 30).

Despite higher gross N mineralization rates in vegetated soils, comparable NH₄⁺ concentrations in vegetated and non-vegetated soils indicated relatively

higher consumption of NH_4^+ in vegetated soils. The higher NH_4^+ immobilization and nitrification along with the build-up of NO_3^- in vegetated soils compared to non-vegetated ones corroborated the above observation. Eventually, the build-up of NO_3^- along with its lower consumption rates compared to NH_4^+ resulted in longer residence time for NO_3^- than NH_4^+ in the study area. Negative values for NO_3^- consumption rates at a few locations during the present study indicated insignificant potential for NO_3^- immobilization. Lower consumption of NO_3^- and its build-up in semi-arid climate, as in the present study, may be a potential source for episodic N_2O emissions due to denitrification after occasional rain events.

4.8 Conclusion

Studies evaluating the effects of aridity and its consequences on N transformation processes in tropical drylands are scarce. Results of the present study showed a significant negative effect of salinity, lack of vegetation, and high soil moisture on N transformation rates in a natural semi-arid ecosystem. This negative effect was due to the collective response of land-type characteristics as land-type showing relatively higher salinity (salt flat) also showed no vegetation with high soil moisture. Overall, the cumulative effect of vegetation, moisture, and salinity appeared to be the key regulator of microbial activity and N transformations in semi-arid tropical soils with salinity being the most important factor. Under the similar climatic conditions, soils with vegetation, low salinity, and optimum moisture conditions exhibited relatively higher N transformation rates compared to non-vegetated soils with high salinity and moisture level. As predicted by climate change models, occasional inundation of coastal regions by seawater is likely to increase in future due to global warming (Mimura, 2013; Zickfeld et al., 2017). This may lead to an increase in soil salinity and moisture level along with loss of vegetation. Under such a scenario, as observed during the present study, the processes associated with cycling of N may get severely altered with significantly reduced rates. Prolonged exposure of vegetated fertile soils to such conditions can make them susceptible to desertification.

Chapter 5

Nitrogen transformations in tropical humid forest soils

Tropical forests are well known for their rich biodiversity and high productivity (Whitmore, 1998; Malhi, 2012). These forests act as a net C sink and contribute approximately 25% of the global and 55% of the terrestrial C sequestration, thereby acting as a buffer to the rising atmospheric CO₂ concentrations (Townsend et al., 2011; Wright et al., 2011). However, the views on the role of tropical forests as stable C sink are changing due to increased disturbances and deforestation which can turn these forests into a potential source of C, adversely affecting the climate (Stephens et al., 2007). The disturbances to forested ecosystems can also greatly affect biogeochemical cycling of other elements, such as N, which often acts as a growth-limiting nutrient for plants and controls the ecosystem's C sequestration capability.

Tropical forests are often associated with nutrient paradox. Some studies suggest these forests to be nutrient-rich as compared to temperate forests because of their high primary productivity potential (Vitousek, 1984; Vitousek and Sanford, 1986). On the contrary, there are other studies which indicate tropical forests to be nutrient-limited because of high soil acidity (Walker and Syers, 1976), high leaching losses (Vitousek et al., 2010), tendency of soils to form laterites (Jenny, 1941), and highly weathered nature (Porder et al., 2005). It has

also been suggested that tropical soils can produce large quantities of nutrients by internal recycling of litter, but the amount of available nutrients is largely controlled by abiotic factors such as temperature, precipitation, length of dry season, and light availability (Bai et al., 2012; Grau et al., 2017). In general, productivity in tropical forests is primarily controlled by the availability of P, whereas N controls the same in temperate forest soils (Hedin et al., 2009; Cleveland et al., 2011; Pajares and Bohannan, 2016). Biological N fixation (BNF) is a prominent process in tropical forests which contributes significantly towards the availability of N in these soils (15 - 36 kg N ha⁻¹ yr⁻¹) compared to temperate forest soils (7 - 27 kg N ha⁻¹ yr⁻¹; Hedin et al., 2009; Pajares and Bohannan, 2016). Studies conducted in N-poor temperate ecosystems suggested that plants are not only effective in competing with microorganisms for inorganic N, but can also absorb organic N to fulfill their nutritional requirements (Schimel and Bennett, 2004). The tendency of plants to take organic N for their nutritional needs gets diminished with the increasing amount of mineral N, particularly in tropical climate where mineral N is easily accessible (Chen and Stark, 2000; Hall and Matson, 2003). However, the pools of available mineral N and its cycling in tropical forest soils, particularly in the Indian subcontinent, are not well understood due to lack of studies (Booth et al., 2005).

In light of the above, we investigated gross rates of N transformations in tropical forest soils of the Western Ghats, one of the world's "hottest biodiversity hotspots" in India (http://whc.unesco.org/en/list/1342/). The main objective of this work was to quantify the production and consumption processes of mineral N forms in the Western Ghats to better understand the nutrient dynamics in tropical forested soils of the Indian subcontinent. Given the tropical climate and dense mixed forest, we hypothesized that N cycling in the forest soils of Western Ghats should be tightly coupled with higher rates of gross N mineralization and nitrification as compared to their temperate counterparts. We also hypothesized that soils of the Western Ghats hold large nutrient pools as the tropical forests receive high N through BNF, litter inputs, and N deposition (Phoenix et al., 2006; Hedin et al., 2009; Reed et al., 2011). The specific aims of the present study were to: (a) quantify the gross rates of N mineralization and nitrification in soils of the

Western Ghats, (b) examine the variation in process rates with depth in soil profiles, (c) investigate the relationship between production and consumption of mineral N forms, and (d) explore the potential role of external N sources on soil N dynamics.

5.1 Study area and sampling

The soil sampling during this study was carried out in a mixed broadleaf tropical forest during September, 2016 at the Bhagwan Mahavir Wildlife Sanctuary located in the northern part of the Western Ghats, Goa, India. The Western Ghats are a 1600 km long chain of mountains which runs parallel to the western coast of peninsular India (8 °N to 21 °N). The climate of the sampling region is tropical hot and humid with daily temperature ranging from 30 °C (May) to 25 °C (January) and an annual average rainfall of 3000 mm spread largely over five months (June - October). The soils in the region are mainly lateritic and acidic (CGWB Report, 2013) with Inceptisols, Ultisols, Alfisols, and Entisols as major soil types (Bhattacharyya et al., 2013). The vegetation in the Western Ghats primarily comprises of tropical evergreen to semi-evergreen and moist mixed deciduous trees, including tall plants, woody plants, undergrowth, flowering species, and the climbers (Datar and Lakshminarasimhan, 2013). The plants in the sampling region belong primarily to Burseraceae, Myristicaceae, Garcinia, Calophyllaceae, Ebenaceae, Fabaceae, Gretaceae, Apocynaceae, Asparagus, Rubiaceae, Careya, Dilleniaceae, Fabaceae, Orchidiaceae, Cyperaceae, Zingiberaceae, and Asteraceae families.

Soil samples were collected from five different locations within 240 km² area of the Sanctuary (Figure 5.1). The sampling locations were identified by the names of nearby tribal areas and were named as Tambdi Surla (W1), Kaskond (W2), Nandran (W3), Dholbag (W4), and Maida (W5) (Figure 5.1 & 5.2). Apart from regular three sites, additional sampling was conducted under young teak plantation at W2. The sampling at each location was conducted at three sites and two depth intervals (see Chapter 2 for sampling procedure) except at W5, where only two sites were sampled due to torrential rain.



Figure 5.1: Sampling locations at the Bhagwan Mahavir Wildlife Sanctuary. Grey shaded region in the inset is the location of the sanctuary within the state of Goa, India.



Figure 5.2: Photographs of sampling locations in the humid tropical forests at the Bhagwan Mahavir Wildlife Sanctuary.

The samples were tested for GWC, WFPS, total porosity, bulk density, pH, nutrients concentration, TOC, and TN contents in soils. Soils were also subjected to ¹⁵N isotope dilution experiments to measure gross rates of N transformation processes.

5.2 Physical properties of soils of the Western Ghats

The bulk density and porosity of the top soil layers of the study area were significantly different among the sampling locations (p < 0.05; Table 5.1) and averaged 1.33 ± 0.13 g cm⁻³ and 0.49 ± 0.05 , respectively. The average GWC in soils of the study area ranged from 21 - 32% with an overall average of ~ 24% (Table 5.1). The average WFPS showed variation between 60 - 72% with an overall average of 65% (Table 5.1). Despite these variations, there was no significant difference (p > 0.05) in both GWC and WFPS among the sampling locations. Soils of the study area were acidic throughout (Table 5.1).

Table 5.1: Physical properties (mean $\pm 1\sigma$) of soils in the top layer at each location. The levels of statistical significance of differences of each parameter across sampling locations are indicated by * (p < 0.05) or ** (p < 0.001).

Location	Bulk density * (g cm ⁻³)	Total porosity *	WFPS (%)	GWC (%)	pH **
W1	1.32 ± 0.03	0.50 ± 0.01	63.60 ± 12.28	24.39 ± 5.67	5.41 ± 0.37
W2	1.40 ± 0.13	0.47 ± 0.05	66.16 ± 3.40	22.54 ± 3.64	4.31 ± 0.22
W3	1.39 ± 0.11	0.48 ± 0.04	61.29 ± 9.75	21.27 ± 4.54	5.23 ± 0.19
W4	1.45 ± 0.02	0.45 ± 0.01	72.30 ± 4.32	22.67 ± 1.91	4.12 ± 0.64
W5	1.11 ± 0.10	0.58 ± 0.04	60.10 ± 0.26	31.77 ± 4.84	4.51 ± 0.12

5.3 Production of nutrients

The average gross N mineralization rate was significantly higher (p < 0.001) in the top soils ($2.6 \pm 1.1 \text{ mg N kg}^{-1} \text{ d}^{-1}$) compared to the bottom ($1.1 \pm 0.6 \text{ mg N kg}^{-1} \text{ d}^{-1}$; Figure 5.3a). Similar to N mineralization, gross nitrification rates were also significantly higher (p < 0.01) in the top soils (average ~ $1.7 \pm 1.1 \text{ mg N kg}^{-1} \text{ d}^{-1}$) compared to the bottom (average ~ $0.5 \pm 0.5 \text{ mg N kg}^{-1} \text{ d}^{-1}$; Figure 5.3b). Except W4, which exhibited the highest average gross N mineralization rate in the top soils, all other locations showed comparable N mineralization rates in the top layer (Figure 5.3a). Gross nitrification rates in the top soils were significantly different among the sampling locations (p < 0.05; Figure 5.3b); whereas no such difference was observed for the bottom layers. In general, the gross N mineralization rates did not show significant variation among the sampling locations (top: p = 0.41; bottom: p = 0.23). Overall, both gross N mineralization and nitrification rates exhibited high variability in the top soils compared to the bottom with the average rates of both the processes reducing to approximately half in the bottom layers (Figure 5.3).



Figure 5.3: (a) Gross N mineralization rates and (b) gross nitrification rates in the top and bottom soil layers at the sampling locations (mean $\pm 1\sigma$).

Significantly higher gross N mineralization and nitrification rates in the top soils of the study area compared to the bottom layers indicated higher microbial activity in the top soils (Figure 5.3). This was also supported by high TOC and TN contents in the top layer than the bottom (Table 5.2). Showing higher variability for the top layers than the bottom, both TOC (p < 0.01) and TN (p < 0.05) contents were significantly higher in the top soils (Table 5.2). The TOC and TN contents of soils were positively correlated with each other ($R^2 = 0.69$, p < 0.001; Table 5.3). Both TOC ($R^2 = 0.48$, p < 0.001) and TN ($R^2 = 0.61$, p < 0.001) showed positive correlation with NO₃⁻, which was absent in case of NH₄⁺ and gross N mineralization rates (Table 5.3).

Sampling location	Total organic carbon (mg C g ⁻¹)	$\begin{array}{ccc} \mbox{`otal organic carbon} & \mbox{Total nitrogen} \\ \mbox{(mg C } g^{-1}) & \mbox{(mg N } g^{-1}) \end{array} \qquad NC$		Gross nitrification: gross N mineralization	Gross nitrification: NH4 ⁺ immobilization
Top layer (0-20 cm)					
W1-T	25.25 ± 10.08	2.23 ± 0.34	0.30	1.14	1.43
W2-T	31.77 ± 19.63	2.65 ± 1.43	2.16	0.80	1.48
W3-T	26.09 ± 14.26	2.18 ± 0.59	0.24	0.40	0.53
W4-T	18.94 ± 0.54	2.22 ± 0.53	0.73	0.34	0.28
W5-T	51.04 ± 9.44	5.40 ± 0.11	1.91	0.94	0.94
Bottom layer (20-40 cm)					
W1-B	11.56 ± 2.73	1.75 ± 0.37	0.32	1.27	0.57
W2-B	12.44 ± 5.26	1.99 ± 0.79	0.22	0.18	0.11
W3-B	10.02 ± 3.19	1.48 ± 0.44	0.24	1.12	0.87
W4-B	8.56 ± 3.25	1.65 ± 0.10	0.10	0.60	1.63
W5-B	30.71 ± 2.15	3.22 ± 0.13	0.13	0.45	0.55

Table 5.2: Total organic carbon, total nitrogen, ratios of mineral nitrogen (NO₃⁻: NH₄⁺), gross nitrification to gross immobilization, and gross nitrification to NH₄⁺ immobilization in the top and bottom layers of the soil at each location.

Table 5.3: Correlation matrix (r) for nitrogen transformation processes and associated parameters during the present study. Significantly correlated relations are shown with asterisk such that * is for p < 0.05, ** is for p < 0.01, and *** is for p < 0.001. TN: total nitrogen, TOC: total organic carbon, MR: gross N mineralization rates, A-CR: NH₄⁺ consumption rates, A-imm: NH₄⁺ immobilization rates, NR: gross nitrification rates, and N-CR: NO₃⁻ consumption rates.

	TN	TOC	$\mathrm{NH_{4}^{+}}$	NO ₃ -	MR	A-CR	A-imm	NR	N-CR
TN	1								
TOC	0.83 ***	1							
$\mathbf{NH_4^+}$	0.16	0.27	1						
NO ₃ -	0.78 ***	0.69 ***	- 0.30	1					
MR	0.30	0.34	0.31	0.24	1				
A-CR	0.39 *	0.43 *	0.28	0.24	0.75 ***	1			
A-imm	0.17	0.18	0.37 *	-0.02	0.68 ***	0.84 ***	1		
NR	0.46 *	0.50 **	-0.08	0.48 **	0.34	0.54 **	0.07	1	
N-CR	0.14	0.22	- 0.09	0.14	0.07	0.37 *	0.11	0.68 ***	1
The TOC and TN contents of soils showed a significant positive relationship with gross nitrification rates and NH_4^+ consumption (Table 5.3). As evident by high soil TOC and TN contents, higher microbial activity in upper layers of soil profile compared to deeper layers is well known in different agricultural and forest soils around the world (Kellman et al., 2014; Fang et al., 2015; Zhang et al., 2016). This is potentially due to higher organic matter concentration, greater fine root activity, and closer proximity to the forest floor (Bolton et al., 1993; Winkler et al., 1996; Kellman et al., 2014).

The gross N mineralization rates in soils of the Western Ghats were in the range of other tropical and temperate forests (Table 5.4). On the other hand, the gross nitrification rates in the present study were comparable to tropical forests but were higher than that in many temperate forest soils (Davidson et al., 1991; Goulding et al., 1998; Neill et al., 1999; Stottlemyer and Toczydlowski, 1999; Silver et al., 2001; Bengtsson et al., 2003; Venterea et al., 2004; Silver et al., 2005; LeDuc and Rothstein, 2007; Zhang et al., 2013). This overlap in N transformation rates with temperate forests could be due to a relatively broader range for gross N mineralization and nitrification rates in temperate forest soils compared to tropical (Booth et al., 2005). In general, large differences have been observed between gross rates of N mineralization and nitrification in temperate forests (Hart et al., 1994; Stottlemyer and Toczydlowski, 1999; Perakis and Hedin, 2001; Carmosini et al., 2002; Venterea et al., 2004). However, this difference was relatively smaller during the present study. The gross nitrification: gross N mineralization ratios in soils of the Western Ghats were relatively higher (average ~ 0.7; Table 5.2) compared to many temperate soils (< 0.4; Table 5.4). This suggested potentially higher conversion efficiency of available NH₄⁺ to NO₃⁻ in soils of the Western Ghats compared to several temperate soils.

5.4 Consumption of nutrients

Consumption rates for both NH_4^+ and NO_3^- exceeded their production rates. However, this difference was significant only in the case of NH_4^+ . The NH_4^+ consumption rates were significantly higher in the top soils than the bottom (p < 0.01; Figure 5.4a) but did not show significant variation among the sampling



locations (p > 0.05). The NO₃⁻ consumption rates showed no statistical difference with depth or among the sampling locations (Figure 5.4b).

Figure 5.4: (a) NH_4^+ consumption rates and (b) NO_3^- consumption rates in the top and bottom soil layers at the sampling locations (mean $\pm 1\sigma$).

Similar to production rates, the gross consumption rates of nutrients were also higher in the top soils than the bottom corroborating relatively higher microbial activity in the top layers (Figure 5.4). In general, gross consumption rates of both NH₄⁺ and NO₃⁻ in forest soils of the Western Ghats were higher than their production rates through N mineralization and nitrification, respectively (Figures 5.3 & 5.4). Higher potential for consumption than production of N has been observed in other studies as well (Davidson et al., 1991; Perakis and Hedin, 2001; Carmosini et al., 2002; Bengtsson et al., 2003; LeDuc and Rothstein, 2007), which has largely been attributed to addition of inorganic N to nutrient-poor soils during the isotope dilution experiments. It has been observed that the addition of tracer during isotope dilution method stimulates only consumption rates of nutrients, while the production rates remain unaffected (Davidson et al., 1991). However, unlike temperate soils, which are generally N poor, soils of the Western Ghats showed high nitrogenous nutrients. Therefore, the potential for higher N consumption through artificial stimulation due to tracer addition is unlikely during the present study.

Forest type	Study area	Gross N mineralization rates	Gross nitrification rates	Reference	
	, , , , , , , , , , , , , , , , , , ,	$(mg N kg^{-1} d^{-1})$	$(mg N kg^{-1} d^{-1})$		
Tropical humid mixed forest	India	1.6 - 3.9	0.6 - 2.9	Present study	
Temperate deciduous forests	Sweden	3.2 - 53.1	0.0 - 0.7	Bengtsson et al., 2003	
Mixed Boreal forests	Canada	80.8 ± 10.3	20.26 ± 0.35	Carmosini et al., 2002	
Temperate deciduous woodland	United Kingdom	2.46	0.55 Goulding et al., 1998		
		Birch: 5 - 8	Birch: 0 - 0		
Boreal forest	Canada	Spruce: 7 - 9	Spruce: 0 - 2	Stottlemyer and Toczydlowski, 1999	
		Alder: 3 - 4	Alder: 0.5 - 1		
Mixed conifer	California	36.53	0.97	Davidson et al., 1991	
Temperate hardwood		0.2 - 1	0.007 - 0.008	Venteres et al. 2004	
Temperate pine stands	USA	0.15 - 0.75	0.007 - 0.12	Venterea et al., 2004	
Mixed temperate forests	Japan	4.22 ± 3.59	0.98 ± 0.68	Urakawa et al., 2016	
Temperate jack pine forests	Michigan	2.02 ± 0.26	0.18 ± 0.05	LeDuc and Rothstein, 2007	
Humid tropical forests	Rondônia	3.70	2.59	Neill et al., 1999	
Humid subtropical forests	Southern China	1.52 - 9.20	0.06 - 1.97	There at al. 2012	
Temperate forests Northern China		1.02 - 2.29	0.07 - 4.53	Zhang et al., 2015	
Old growth tropical forest	Costa Rica	7.94 ± 0.99	5.09 ± 2.02	Silver et al., 2005	
Wet tropical forest	Puerto Rico	4.8 ± 0.9	0.57 ± 0.12	Silver et al., 2001	

Table 5.4: Gross N transformation rates in the present and other representative studies around the world.

During the present study, average NO_3^- consumption was generally lower than average NH_4^+ consumption in the study area (Figure 5.4). Significantly higher (p < 0.05) consumption of NH_4^+ than NO_3^- in both the soil layers (Figure 5.4) suggested a relatively higher potential for assimilation of NH_4^+ by microbes in the studied region. In general, the microbial efficiency for immobilization of NH_4^+ is higher compared to NO_3^- due to its reduced form as the uptake of NO_3^- is an energetically expensive process and needs to be reduced in NH_4^+ before its incorporation into amino acids (Bengtson and Bengtsson, 2005). Microbes, therefore, prefer NH_4^+ as a nutrient source, which was also supported by the present study.

The two processes which accounted for NH_{4}^{+} consumption in the present experimental conditions were nitrification and NH_{4}^{+} immobilization. During the present study, consumption of NH_{4}^{+} through microbial immobilization was generally higher than that through nitrification (Table 5.2). Regression analyses showed significant positive relationships for NH_{4}^{+} consumption with gross nitrification and NH_{4}^{+} immobilization (Table 5.3). On comparing the two, NH_{4}^{+} immobilization explained 70%, whereas nitrification could explain only 30% variability in the NH_{4}^{+} consumption, indicating NH_{4}^{+} immobilization as the major nutrient consumption process in forest soils of the Western Ghats. It appears that, rather than getting nitrified, a larger fraction of NH_{4}^{+} is consumed by heterotrophic bacteria as nitrifying bacteria are inferior competitors to heterotrophic microorganisms (Bengtson and Bengtsson, 2005; Verhagen et al., 1995). This was also supported by NO_{3}^{-} : NH_{4}^{+} ratios in these soils (Table 5.2), where the lower ratios indicated the presence of conservative N cycling (Zhang et al., 2013).

The nutrients consumption rates showed significant positive correlation with their production rates, i.e., NH₄⁺ consumption rates with gross N mineralization rates ($R^2 = 0.56$, p < 0.001; Figure 5.5a) and NO₃⁻ consumption rates with gross nitrification rates ($R^2 = 0.47$, p < 0.001; Figure 5.5b). Significant positive correlation between gross production and consumption rates of nutrients (p < 0.05) indicated dependence of nutrients consumption on their production, i.e.,

increased production of NH_4^+ and NO_3^- led to increase in potential for their consumption.



Figure 5.5: Relationships between (a) gross NH_4^+ consumption and gross N mineralization rates and (b) gross NO_3^- consumption and gross nitrification rates in soils collected from the Western Ghats (n = 30).

Higher rates of gross NH₄⁺ consumption than gross N mineralization during the present study were independent of ambient NH₄⁺ concentrations. Large variability in both NH₄⁺ and NO₃⁻ concentrations were observed throughout the study area as well as within sites of the sampling locations (Figure 5.6). Despite the variability, nutrients concentrations showed no statistical difference among sampling locations (p > 0.05). On average, concentrations of NH₄⁺ were similar to NO₃⁻ in the top layers, but were significantly higher than NO₃⁻ in the bottom soils (p < 0.001). Although higher average NO₃⁻ consumption than nitrification was also observed for the study area, site-specific gross NO₃⁻ consumption rates revealed relatively higher consumption at sites with lower ambient NO₃⁻ concentrations, which were largely in the bottom soils (Figures 5.4b & 5.6b). This may be due to stimulation in NO₃⁻ poor bottom soils.



Figure 5.6: (a) NH_4^+ and (b) NO_3^- concentrations (mean $\pm 1\sigma$) in the top and bottom soil layers at different sampling locations.

Relatively higher nutrients consumption rates compared to their production suggested nutrient conservation mechanism at play in the Western Ghats. Different views exist regarding the status of nutrients in the tropical soils (Jenny, 1941; Vitousek, 1984; Vitousek and Sanford, 1986; Porder et al., 2005; Vitousek et al., 2010) suggesting them to be neither rich nor poor in terms of nutrient availability as these ecosystems receive pulsed nutrient loads due to continuous drying-wetting cycles (Lodge et al. 1994). Nutrients in tropical forest soils are relatively higher during hotter conditions due to optimal microbial activities, whereas in the wetter conditions the nutrients are lost via leaching or denitrification creating low nutrient supply (Lodge et al., 1994). Whenever there is high nutrient input to these systems, microbes tend to store active forms of N by immobilizing it in their body to use it later during low nutrient conditions (Singh et al., 1989). Such immobilized N in the microbial body is easily accessible and requires less energy compared to generation of new reactive N from decomposition of the organic matter (Singh et al., 1989). This mechanism makes the system stable in terms of nutrient supply and productivity. Relatively higher nutrient consumption rates compared to production during the present study (Figure 5.3 & 5.4) suggested that nutrient conservation mechanism by microbes may be active in the Western Ghats.

5.5 Availability of nutrients

The NH₄⁺ concentrations in the Western Ghats were relatively higher when compared to other forest soils of the Indian subcontinent (Devi et al., 2006; Tripathi and Singh, 2012; Bhuyan et al., 2014; Singh and Singh, 2014). Despite the high potential for NH₄⁺ consumption, presence of large NH₄⁺ pool in soils of the Western Ghats pointed towards additional sources of N in the system. The average NO₃⁻ concentrations in the top soils at two locations (W2 and W5) were anomalously high (> 27 mg kg⁻¹), even exceeding the respective NH₄⁺ concentrations (Figure 5.6). Barring the above-mentioned two locations, where average NO₃⁻ was relatively higher; NO₃⁻: NH₄⁺ ratios were < 1 throughout the study area (Table 5.2). Overall, NO₃⁻ concentrations were significantly higher in the top soils compared to the bottom (p < 0.001); however, no significant difference in NH₄⁺ concentrations were found with depth.

No significant relationships were observed for gross N mineralization rates with NH₄⁺ concentrations ($R^2 = 0.10$, p > 0.05; Figure 5.7a). However, gross nitrification rates showed significant positive relationships with NO₃⁻ concentrations ($R^2 = 0.23$, p < 0.01; Figure 5.7b).



Figure 5.7: Relationships between (a) NH_4^+ concentrations and gross N mineralization rates and (b) NO_3^- concentrations and gross nitrification rates in soils collected from the Western Ghats (n = 30).

Absence of correlation between NH4⁺ concentrations and gross N mineralization rates (Figure 5.7a) and a weak correlation between NO₃⁻ concentrations and gross nitrification rates (Figure 5.7b) also suggested the presence of N sources other than through biogeochemical N cycling. As the sampling was performed during the end of the Indian summer monsoon (June -September), wet deposition of N may be a potential nutrient source. Although direct measurements of N deposition are not available at the study site; N deposition rates in the Western Ghats are known to be the highest (13 kg N ha⁻¹ yr⁻¹ during 1990) among the world's biodiversity hotspots, which are expected to increase with time (Phoenix et al., 2006). This was also evident from another study conducted 100 km south of the sampling region, which indicated increasing pattern in the wet deposition of NH₄⁺ than NO₃⁻ in this region of the Western Ghats (Kulshrestha et al., 2014). Other than atmospheric deposition, BNF can be a significant source of N in the Western Ghats as BNF is well known to contribute large quantities of N in tropical forests (Jenny, 1950; Cleveland et al., 1999). However, rates of BNF in the Western Ghats are not known. According to a recent study, chemical weathering of bedrocks can also contribute significantly towards the supply of N to the Earth's surface (Houlton et al., 2018). However, the amount of N addition through this newly discovered pathway to soils of the Western Ghats remains unknown. To fully understand the additional sources of N to soils of the Western Ghats, quantification of sources mentioned above is desirable.

The NH₄⁺ concentrations in the bottom soils at few study sites were higher than those in the top, which may be due to its increased adsorption on clay particles with depth (Jenkinson et al., 2008). High NH₄⁺ concentrations are known to contribute towards soil acidity (Hicks et al., 2008) and may be one of the reasons for observed acidic nature of the studied soils (Table 5.1). The lowering of pH may further cause accumulation of NH₄⁺ in soils as the acidic pH conditions inhibit NH₄⁺ oxidation to NO₃⁻ by autotrophic nitrifiers (Li et al., 2017) leading to high NH₄⁺ and low NO₃⁻ concentrations in the soils.

The mean residence time for both NH_4^+ and NO_3^- was highly variable in the study area (Figure 5.8) with no significant difference across the sampling

locations. The residence time of NH_4^+ was significantly higher in the bottom soils (average ~ 15 ± 7 days; Figure 5.8a) than the top (average ~ 7 ± 5 days; Figure 5.8a), whereas NO_3^- showed comparable mean residence time in both top (average ~ 11 ± 9 days; Figure 5.8b) and bottom layers (average ~ 9 ± 9 days; Figure 5.8b). Despite active N transformations in soils of the Western Ghats, long residence time for both NH_4^+ and NO_3^- was observed due to high concentrations of available nutrients in the system (Figures 5.6 & 5.8). Owing to anomalously high concentrations at two locations (W2 & W5; Figure 5.6b), NO_3^- showed relatively higher average mean residence time than NH_4^+ in the top layers.



Figure 5.8: Mean residence time for (a) NH_4^+ and (b) NO_3^- in the top and bottom layers of the soils (mean $\pm 1\sigma$).

At the locations where surface NO_3^- was very high, residence time was significantly longer, implying major control of nutrient concentrations on the residence time. Although longer residence time of nutrients in soils suggested the Western Ghats to be N-rich, gross nitrification to NH_4^+ immobilization ratios (< 1) at the majority of locations (Table 5.2) indicated these forests to be not saturated with N (Li et al., 2017). Nitrification to NH_4^+ immobilization ratio > 1 suggests the system to be N saturated while ratio < 1 indicates N limited system (Zhang et al., 2013; Li et al., 2017). Irrespective of the amount of nutrients available, higher NH_4^+ immobilization compared to gross nitrification reaffirmed nutrient conservation mechanism in soils of the Western Ghats.





Figure 5.9: Box model of nitrogen dynamics in (a) top and (b) bottom soil layers at the Western Ghats. The pool sizes are in mg N kg⁻¹ and rates are in mg N kg⁻¹d⁻¹. The difference in the size of boxes in the diagram indicates relative pool size (not to scale), whereas the thickness and direction of arrows are indicative of the relative magnitude and direction of processes.

The N dynamics of the top soils showed higher rates of production and consumption for NH_{4^+} which reduced to almost half in the bottom soils. The NH_{4^+} consumption was higher than the production in both layers of the soil. A similar pattern was observed for NO_3^- production and consumption; however, the

magnitude was relatively smaller compared to that of NH_4^+ . The pool size of NH_4^+ did not show significant change with depth, whereas the NO_3^- pool decreased significantly in the bottom layer. Contrary to one of our hypotheses, the cycling of N did not appear to be tightly coupled in the Western Ghats, whereas the presence of large N pool in soils supported our second hypothesis that soils of the Western Ghats hold large nutrient pools. This large nutrient pool in soils makes the Western Ghats susceptible to N loss via leaching or denitrification. Overall, this indicates that despite the potentially high microbial activity, the N cycle in the soils of tropical forests is more open for N loss as compared to its temperate counterparts.

5.6 Conclusion

Isotope dilution experiments were performed in forest soils of the Western Ghats in southern India to investigate nutrient dynamics in a tropical forested ecosystem. Similar to most forests, soils of the Western Ghats also showed a reduction in rates of gross N mineralization and nitrification with depth, corroborating earlier findings for higher microbial activity in top soils. These forest soils also showed higher potential for consumption of nutrients than their production with relatively higher NH_4^+ consumption compared to NO_3^- , indicating NH_4^+ as a preferred microbial nutrient, possibly due to higher energy requirement during assimilation of NO₃⁻. High NH₄⁺ immobilization in this nutrient-rich ecosystem indicated conservative N cycle at play in the Western Ghats, where microbes tend to store the inorganic N into their body for later utilization. Interestingly, despite higher consumption, accumulation of N was observed in soils of the Western Ghats leading to the possibility of significant external sources of N, such as atmospheric N deposition and BNF, which needs to be quantified. High residence time of nutrients due to its accumulation in soils makes the Western Ghats susceptible for N loss, potentially in the form of N₂O, exact estimation of which is desirable.

Chapter 6

Nitrogen dynamics in differently managed forest soils

In the past few decades, increased demand for resources by humans has led to an immense shift in land-use pattern. This has also popularized the practice of converting naturally grown forests into commercialized forest plantations, which accounted for almost 116 million hectares of land in Asia at the end of the last century (Nsabimana et al., 2008). In this regard, conversion of forests into rubber plantation is a common practice in humid tropics as the tropical climate provides ideal rainfall and temperature condition for the healthy growth of rubber plants. The natural rubber (Hevea brasiliensis), a native of Amazon of South America, was bought to Asia in the 18th century (Dove, 2003). Like many other Asian countries, India also became a large producer of rubber due to favorable geological and agro-climatic conditions. Although the Indian subcontinent encompasses regions with different climate, the southern part of peninsular India offers optimum rainfall and temperature condition for rubber cultivation. At present, India is the 4th largest producer of natural rubber in the world (Mathew, 2006) with ~ 93% of its production in the southern state of Kerala alone (Rajasekharan and Veeraputhran, 2002). The average rotation period for rubber plants is 30 years and the plantation in Kerala has now entered its third cycle (Philip, 2015).

Although mono-cropping system, like rubber, does not disturb many of the above and below ground processes such as water flow regulation and C sequestration, long term mono-plantation may alter the elemental biogeochemical cycles (Kooch et al., 2016). In a naturally grown forest, the litter inputs by different plants provide different C: N ratios in soils, maintaining a balance between C and N cycle. However, this biogeochemical balance in monoplantation can get disturbed due to the lack of species diversity. Change in quality of litter can also change soil properties such as pH, exchangeable base cations, and nutrient cycling (Kooch et al., 2016). The mono-plantation can notably reduce organic C, total N, P, and potassium content of the soils (Bargali et al., 1993). A previous work on the conversion of Hyrcanian forests areas to pine plantation and agricultural land also showed disruption in soil natural activities which significantly affected soil quality and nutrient supply (Asadiyan et al., 2013). Among the nutrients, N is highly vulnerable to land-use and vegetation change as the availability of N in soils is largely governed by aerobic and anaerobic microbial processes, which are influenced by the change in soil properties and litter quality. Therefore, in order to develop proper forest management policies, it is important to understand the cycling of N in soils undergoing different forestation practices and land-use changes.

In this regard, this chapter discusses the effects of forest type alteration on gross N mineralization rates in tropical soils. To fulfill the objectives of the present study, isotope dilution experiments were performed to measure and compare the gross rates of N mineralization in soils of a managed, an unmanaged (naturally grown), and a converted (rubber plantation) tropical forest of Kerala, India. The central hypothesis of this study was that the conversion of mixed tree forests to monoculture plantations diminishes the rates of N transformation in soils.

6.1 Study area and sample collection

The soil samples were collected from seven sites across three differently managed forests (three sites each in the managed and unmanaged forest and one in the rubber plantation) in Nilambur region of Kerala, India (Figure 6.1a). The study area experienced similar climatic conditions and differed mainly in forest management practices. The soil samples were collected in August 2015, when the average humidity and temperature across the region was 60% and 30 °C, respectively. The Nilambur region of Kerala, which includes the study area, is covered with a mixture of tropical moist deciduous and evergreen forests with bamboo (Poaceae), rosewood (Dalbergia), (Tectona), Mahogany teak (Swietenia), Choropin, orchids, and ferns as major species. The sample collection sites in the managed and unmanaged forests also showed the above-mentioned species with dominance of teak. The soil sampling in the managed forest was performed beneath three different tree species viz. Tectona grandis, Tetrameles nudiflora, and Schleichera oleosa (Figure 6.1b), whereas it was conducted randomly in the unmanaged forest (Figure 6.1c). At both managed and unmanaged forests, the three sampling sites were around 20 m apart forming vertices of a triangle. Lastly, the soil sampling was performed from a rubber estate (Hevea brasiliensis) used for commercial rubber production (Figure 6.1d). This rubber estate showed mono-culture of rubber with no other plant species; therefore, soil sampling at this location was limited to one site beneath rubber canopy.



Figure 6.1: (a) Sampling region in the Indian state of Kerala, (b) managed forest, (c) unmanaged forest, and (d) rubber plantation. The white band across the southwest part of India represents the Western Ghats. The black circle indicates the sampling region.

The collected samples were tested for soil properties such as pH, conductivity, TN, TOC, δ^{15} N, δ^{13} C, and nutrients concentrations, as explained in Chapter 2. Soils were subjected to ¹⁵N isotope dilution experiment for the measurement of gross N mineralization rates, NH₄⁺ consumption rates, and NH₄⁺ residence time. Detailed methodology is discussed in Chapter 2.

6.2 Soil physical properties

Soils collected from the managed and unmanaged forests showed slightly acidic nature (pH ~ 6.3), whereas soils from the rubber plantation were highly acidic with a pH of 4.8 (Table 6.1). The conductivity of soils from the rubber plantation was significantly higher (121 mV) compared to the managed and unmanaged forest soils (< 45 mV; Table 6.1).

Characteristics	Managed forest	Unmanaged forest	Rubber plantation		
Location	11°16' N, 76°12' E	11°17' N, 76°20' E	11°15' N, 76°18' E		
рН	6.2	6.3	4.8		
Conductivity	44 mV	36 mV	121 mV		

Table 6.1: Characteristics of soils and sampling sites.

The observed high conductivity and low pH in soils of the rubber plantation indicated the effect of fertilizer addition (Haynes and Naidu, 1998). The demand for fertilizers is known to increase in the mono-cropping system like rubber as loss of natural biodiversity leads to imbalanced nutrient supply. This eventually results in loss of fertility in such systems and subsequent addition of fertilizers to these infertile soils increases the soil acidity (Bünemann et al., 2006). The increase in acidity and loss of fertility in soils due to the conversion of natural forests to monoculture has been observed in other regions of the world as well (Zhang et al. 2007). Although the addition of fertilizers to such soils improves its fertility in short-term, in the long run it further deteriorates the soil quality (Zhang et al., 2007) by decreasing soil pH and microbial biomass (Ma et al., 2018; Yang et al., 2018).

6.3 Ambient nutrients (mineral N) concentration

Overall, the soils showed a higher average concentration of NO_3^- (4.25 ± 1.63 mg N kg⁻¹) than NH₄⁺ (2.23 ± 0.78 mg N kg⁻¹) in the study area. The concentration of NO_3^- was the highest in the managed forest, followed by the unmanaged forest and rubber plantation (Table 6.2; Figure 6.2). The average NO_3^- concentration in the managed forest was significantly higher (p < 0.05) than the unmanaged forest and rubber plantation. A relatively larger pool of NO_3^- compared to NH_4^+ in the study area suggests that these forests are not limited by N as the presence of higher NO_3^- in any natural system indicates ample supply of N. When present in sufficient quantity, the nitrogenous nutrient demand by both microbes and plants is normally fulfilled through NH_4^+ , which is the preferred form of N. This allows the remaining pool of NO_3^- in soils (Schimel and Bennett, 2004).



Figure 6.2: Mineral N (nutrients) concentrations in soils of the study area (mean $\pm 1\sigma$).

A trend reverse to NO₃⁻ was observed for NH₄⁺ concentrations where the highest NH₄⁺ was in the rubber plantation followed by the unmanaged and managed forest (Table 6.2; Figure 6.2). The NH₄⁺ concentration in the rubber plantation was significantly higher (p < 0.05) from the other two forest types. However, NH₄⁺ concentrations in the managed and unmanaged forests were not significantly different from each other. Presence of higher NH₄⁺ than NO₃⁻ in rubber soils indicated the addition of fertilizers to the plantation. High NH₄⁺ concentrations are known to increase soil acidity (Hicks et al., 2008) and may be one of the reasons for observed low pH in the rubber soils. The lowering of pH may further enhance the accumulation of NH_4^+ in soils as acidic pH condition inhibits NH_4^+ oxidation to NO_3^- by autotrophic nitrifiers (Li et al., 2017). An increase in NH₄⁺ and decrease in NO₃⁻ concentrations of soils following the conversion of tropical forest to rubber and tea plantations has also been observed elsewhere, with the most pronounced effect in plantations that are more frequently fertilized (Li et al., 2012). The nitrification in rubber soils is also inhibited due to the production of monoterpenes (alpha- and beta-pinene) from rubber contributing to higher NH₄⁺ pool in soils (White, 1991; Allen et al., 2015; Wang et al., 2016). In a previous study, production of monoterpenes was shown to be correlated with dissimilatory nitrate reduction to ammonia and NH4⁺ immobilization, thereby suggesting the role of monoterpenes in triggering N retention mechanism causing low NO_3^- and high NH_4^+ content in soils under rubber trees (Allen et al., 2015). This has also been linked to low N mineralization rates in soils occupied by rubber plantation.

6.4 Gross N mineralization and NH₄⁺ consumption rates

The highest average gross N mineralization rate in the study area was in soils of the managed forest, whereas the lowest was in the rubber plantation (Figure 6.3). The gross N mineralization rates in all three forest types were significantly different ($F_{2,18} = 17.13$, p < 0.05) from each other. Considering the forests with mixed species, rates of N mineralization among all three sampling sites of the managed forest were comparable, whereas large variability was observed for the unmanaged forest sites indicating the effect of management practices on the soils. Despite the similarity in tree composition (mixed forest with the dominance of teak), the relative increase in gross N mineralization rates from unmanaged to the managed forest (Table 6.2) also indicated the effect of forest management on soil N mineralization. The gross N mineralization rates decreased considerably in the rubber plantation compared to mixed forests (i.e., managed and unmanaged forests). This indicated that forest type alteration from a mixed forest regime to a mono-culture might slowdown the microbial activity and hence dampen the efficient cycling of nutrients, which agrees with the central hypothesis of the present study. The microbial processes controlling C cycle, and in turn the N cycle, have been linked to forest vegetation. Previous studies have found the dependence of soil microbial biomass on the diversity of vegetation cover (Bargali et al., 2018), absence of which affected the microbial community (Bargali et al., 2018).



Figure 6.3: Rates of gross N mineralization and NH_4^+ consumption in the study area (mean $\pm 1\sigma$).

Similar to gross N mineralization, NH_4^+ consumption rates were also the highest and the lowest for soils of the managed forest (2.20 mg N kg⁻¹ d⁻¹) and rubber plantation (0.85 mg N kg⁻¹ d⁻¹; Figure 6.3), respectively. However, NH_4^+ consumption rates differed significantly only between the managed forest and the rubber plantation soils. The NH_4^+ consumption to gross N mineralization ratio was < 1 in all soils indicating N mineralization of organic matter to be one of the principal processes in the study area.

Parameters	Managed forest	Unmanaged forest	Rubber plantation
NH4 ⁺ (mg N kg ⁻¹)	1.92 (0.86)	2.15 (0.29)	3.45 (0.18)
NO_{3}^{-} (mg N kg ⁻¹)	5.61 (1.65)	3.41 (0.31)	2.66 (0.66)
Total nitrogen (mg N g ⁻¹)	0.78 (0.23)	0.54 (0.10)	1.49 (0.02)
Total organic carbon (mg C g ⁻¹)	4.41 (1.20)	4.28 (1.31)	7.96 (0.86)
δ ¹³ C (‰)	-26.42 (0.55)	-27.41 (0.50)	-26.43 (0.02)
δ ¹⁵ N (‰)	5.55 (1.51)	4.54 (0.53)	5.47 (0.91)
Gross N mineralization rate (mg N kg ⁻¹ d ⁻¹)	3.71 (0.35)	2.20 (1.07)	1.08 (0.08)
NH_4^+ consumption rate (mg N kg ⁻¹ d ⁻¹)	2.20 (1.41)	1.39 (0.27)	0.85 (0.09)
NH ₄ ⁺ residence time (days)	1.06 (0.62)	1.61 (0.43)	4.09 (0.45)

Table 6.2: Different parameters related to N cycling in soils of the study area. The values are reported as mean $\pm 1 \sigma$ (in parentheses).

6.5 Total nitrogen and total organic carbon contents in soils

In the rubber plantation, both TN and TOC contents were significantly higher (TN: 1.49 mg N g⁻¹; TOC: 7.96 mg C g⁻¹) compared to the managed and unmanaged forest soils (Table 6.2; Figures. 6.4a & 6.4b). No significant difference was found in TN and TOC contents of soils of the managed and unmanaged forest. The unmanaged forest soils showed the lowest TN (0.54 mg N g^{-1}) and TOC (4.28 mg C g^{-1}) concentrations, while the managed forest soils showed intermediate values (TN: 0.78 mg N g⁻¹; TOC: 4.41 mg C g⁻¹). In general, high N mineralization rates are associated with high ambient TN and TOC contents (Booth et al., 2005). However, during the present study, gross N mineralization and NH₄⁺ consumption rates in soils of the rubber plantation were the lowest despite higher TN and TOC contents. This implies that conversion of natural forests to mono-plantation may lower the microbial activity even under high organic matter availability. This observation also supports a previous study which reported that increased soil acidity, as observed in the rubber plantation during the present study, can increase nutrients and organic matter in soils and protect it from decomposition (Turrión et al., 2009; Michopoulos et al., 2019), resulting in relatively high TN and TOC contents in acidic soils compared to the ones with less acidity. Additionally, the conversion of native forests to mono-crop cultivation increases the proportion of recalcitrant fraction and reduces the fraction of labile organic matter in soils (Guimarães et al., 2013); thereby generating the need for the addition of organic fertilizers with higher labile fractions.



Figure 6.4: (a) Total nitrogen and (b) total organic carbon contents (mean $\pm 1\sigma$) in three differently managed soils of Kerala.

Therefore, the addition of organic fertilizer to rubber plantations along with high acidity of these soils can increase the TN and TOC contents, but may not essentially improve the microbial functioning of soils. Thus, it appears that the change in tree species composition of naturally grown forests can affect microbial N cycling in soils depending on the forest type alteration. It has been shown that soil fauna responsible for N release from the litter mass is sensitive to change in plant species and can significantly affect litter mass loss and the associated N release dynamics (Peng et al., 2019).

6.5.1 Stable isotopic compositions of soils

The δ^{15} N of bulk soil and δ^{13} C of soil organic matter were similar for the managed forest and rubber plantation, whereas the unmanaged forest soils showed significantly decreased values for δ^{13} C (F_{2,11} = 6.89, p < 0.05; Figure 6.5). The higher δ^{15} N and δ^{13} C in soils of the managed forest might have resulted due to relatively higher mineralization of organic matter in these soils. The decomposition of organic matter by microbiological processes preferentially consumes lighter isotope resulting in remaining organic matter to be enriched in the heavier isotope, which was reflected in soils of the managed forest. Similar isotopic composition despite lower N mineralization rates in the rubber soils may be due to the addition of organic fertilizer to the plantation.



Figure 6.5: $\delta^{15}N$ and $\delta^{13}C$ of organic matter in soils with different management practices (mean $\pm 1\sigma$).

6.6 Residence time of ammonium

Except for the rubber plantation, residence time of NH_4^+ in soils of the study area was less than 2 days. The rubber plantation showed the highest NH_4^+ residence time of approximately 4 days due to higher NH_4^+ concentrations and lower consumption rates in its soils compared to others (Figure 6.6) resulting in the build-up of its pool. This also corroborated the slowdown of soil microbial N cycle in the rubber monoculture compared to other locations.



Figure 6.6: Mean residence time of NH_4^+ in differently managed soils (mean $\pm 1\sigma$).

6.7 Conclusion

Gross N mineralization and NH₄⁺ consumption rates were measured in soils of differently managed forests to assess the effects of forest type alteration and management practices on N cycling in tropical soils. While the soils of the managed forest showed spatial homogeneity in gross N mineralization rates, the unmanaged forest showed large variability indicating the effect of forest management practices on N cycling. Change in tree species composition from mixed to mono-culture of rubber plants significantly diminished the gross N mineralization and NH₄⁺ consumption rates in soils suggesting slowdown of microbial activity. It appeared that the conversion of mixed forested ecosystems to mono-culture cultivation adversely affected the efficient cycling of nutrients within the system. This slowdown of nutrient cycling did not appear to improve despite higher TN and TOC contents through fertilizer addition. The findings of this study emphasize that the fertilizer addition in mono-plantation cropping is not a desirable option; rather intercropping different tree species such as rubber-teakcoffee etc. may be better to naturally maintain the health of the ecosystem. Our study also underscores the need for more such investigations in different ecozones of India to develop a proper understanding of various aspects of N cycling.

Chapter 7

Organic matter content and its stable isotopic composition in soils of India

For decades, scientists have used soil organic matter content as an index to measure soil quality (Laishram et al., 2012; Bünemann et al., 2018). Soil organic matter consists of various components including living organisms, decomposed plant and animal residues, and microorganisms which are capable of determining physical, chemical, and biological health of soils of an ecosystem (Signor et al., 2018). The TOC and TN are the two important constituents of soil organic matter which provides information regarding ecosystem productivity and soil fertility, respectively (Avramidis et al., 2015; Zhang et al., 2019). Soil inorganic N, which is an important growth-limiting nutrient in many ecosystems, is mainly composed of NH₄⁺ and NO₃⁻ and can be directly consumed by plants and microorganisms. NH4⁺ is mainly produced by organic matter mineralization and is a preferred nutrient over NO₃, which if left unconsumed, is oxidized to NO₃ via nitrification. As the processes regulating TOC and TN contents in soils are microbially mediated, any change in abiotic factors such as temperature, bulk density, pH, and soil moisture that can affect microbial functioning can significantly alter the TOC and TN availability in an ecosystem (Tietema et al., 1992; Tian et al., 2016). Apart from these, situations such as precipitation, flood, and drought can contribute to loss of C and N from soils. As all of these factors regulating availability of C and N in soils can broadly be categorized under different

climates, many studies have been conducted to understand spatial distribution of TOC and TN in soils of different climates (Batjes, 1996; Jobbágy and Jackson, 2000; Prusty et al., 2009; Tian et al., 2010; Liu et al., 2012).

Despite large number of studies addressing distribution of TOC and TN in soils, it is difficult to identify their production and loss rates and/or pathways in different soils due to complex interaction of belowground processes and their dependence on numerous factors, such as shift in microbial biodiversity and mineral surface (Billings and Richter, 2006). Stable isotope analysis of C (δ^{13} C) and N (δ^{15} N) can be an excellent tool to study the sources, sinks, and biogeochemical processes related to C and N availability in any ecosystem (Ehleringer et al., 2000; Robinson, 2001). For instance, soil organic matter which has undergone high microbial processing possess enrichment in ¹³C and ¹⁵N (Natelhoffer and Fry, 1988). Such isotopic enrichment, resulting from fractionation during microbial decomposition processes, is often observed in older soils present in deeper soil layers (Natelhoffer and Fry, 1988; Kramer et al., 2003; Staddon, 2004). Another reason behind high δ^{13} C and δ^{15} N signatures in certain soils could be the use of isotopically enriched organic matter as substrate (Dijkstra et al., 2006). This is especially evident in regions where the concentration of C is already low in the pool and further degradation of remineralized organic matter leaves behind a more enriched substrate (Diochon et al., 2009). Other than the decomposition process, δ^{13} C of soil organic matter is mostly used to identify vegetation pattern and its shift in a region (Kohn, 2010; Cernusak et al., 2013). The δ^{15} N signatures, on the other hand, indicate the 'openness' of the N cycle (Hobbie and Ouimette, 2009). N is available in soils through a chain of microbial processes which decide the flow of N in an ecosystem. If the N is lost from the system during any of the steps in the N cycle or other, the lighter isotope of N (¹⁴N) preferentially leaves, making the remaining pool enriched in ¹⁵N. Important processes affecting δ^{15} N signatures of soil N are atmospheric deposition, fertilizer application, mineralization, NH₃ volatilization, nitrification, assimilation, and denitrification.

Temperature and precipitation variations across different climates can result in variable vegetation inputs and microbial activity which can affect the soil organic matter composition and patterns of δ^{13} C and δ^{15} N (Von Fischer et al., 2008; Ma et al., 2012; Zhang et al., 2015; Wang et al., 2017). For instance, Amundson et al. (2003) showed that soils and foliage from colder climate are depleted in ¹⁵N and ¹³C compared to that from the regions present in warmer climate. Similarly, Martinelli et al. (1999) have shown higher $\delta^{15}N$ in tropical soils compared to temperate soils. However, the variation in δ^{13} C and δ^{15} N of soil TOC and TN contents in different ecozones of a particular climate is not well studied, particularly in tropical countries like India. Most of the work regarding stable isotope studies in India are focused on identifying the origin and distribution of aquatic (marine, estuarine, and lacustrine) sediments (Bouillon et al., 2003; Ramaswamy et al., 2008; Gireeshkumar et al., 2013; Ray et al., 2015; Mukherjee et al., 2019). There are limited studies on the isotopic composition of terrestrial sediments of India (Agrawal et al., 2014; Basu et al., 2015, 2019); however, these works do not address the biogeochemical processes related to organic matter transformations in the contemporary soils. Due to the continued absence of data regarding soil organic matter, its isotopic signatures (especially δ^{13} C and δ^{15} N), and controls of different parameters on C and N availability in Indian soils, this work aimed to study and compare TOC and TN contents in soils of different ecozones of India. Isotopic measurements of δ^{13} C and δ^{15} N in bulk soils were performed to identify potential causes for the observed patterns in TOC and TN distribution and their potential transformations. We hypothesize patterns in our results to be similar to spatial studies conducted in different parts of the world due to major control of temperature and precipitation in determining the composition of soil organic matter over broader climatic classifications.

7.1 Study area and sample collection

Based on Köppen climate classification system, soil samples were collected from three different climatic zones of India: (i) tropical montane region, (ii) semi-arid ecosystems, and (iii) tropical humid forests. The detailed description of sampling locations is provided in Chapter 3, Chapter 4, and Chapter 5 for tropical montane, semi-arid ecosystems, and humid tropical forests, respectively, as an aliquot of same soils which were used for N transformation experiments were analyzed for natural stable isotopic compositions. Sampling strategy followed at each location and the methods employed in sample analyses (physical properties, TOC and TN content, organic C stock, isotopic measurement, and nutrient analysis) are discussed in Chapter 2.

7.2 Soil physical properties

Bulk density of soils was measured in the top soils only, where it showed significant differences (p < 0.001) among soils of semi-arid, humid, and montane climate zones (Figure 7.1a), with the highest bulk density in the semi-arid soils. Soil pH also varied significantly (p < 0.001) in both the layers of soils collected from different ecozones (Figure 7.1b). However, it showed no significant difference with depth at a particular climate zone. The GWC of soils was significantly higher (p < 0.001) in the humid soils compared to the semi-arid soils in both the soil layers (Figure 7.1c). The montane soils showed intermediate GWC values in the top layers. Within a particular climate zone, soils showed no significant difference in the moisture content with depth.



Figure 7.1: (a) Bulk density (for top soils: 0-10 cm), (b) pH, and (c) gravimetric water contents in soils of different climatic zones of India (mean $\pm 1\sigma$). Top soils: 0-20 cm, Bottom soils: 20-40 cm. Values indicated with the same alphabet are not significantly different at p < 0.05. Significant variation in top soils is indicated by uppercase letters and in bottom soils is represented by lowercase letters.

In a previous work on Chinese forest soils, bulk density was shown to correlate inversely with the plantation density (Duan et al., 2019). Similar results obtained in our work indicated role of organic matter inputs and high plantation density in maintaining the low bulk density of soils present in forested humid and montane climate compared to the semi-arid climate (Figure 7.1a). Lower bulk density in the forested soils also indicated the capacity of roots to penetrate deeper

into the ground (Bonfante et al., 2019). On the other hand, significantly high bulk density in the semi-arid soils was associated with vegetation consisting of small roots (such as grasses) which could not go deeper.

Our results for soil pH (Figure 7.1b) were in agreement with previous findings that soils of semi-arid climate are characterized as alkaline whereas acidic soils are common in the humid climate (Weil and Brady, 2016). The occurrence of acidic or alkaline soil pH in a specific type of climate can be attributed to buffering by two important minerals, i.e., calcite (CaCO₃) and gibbsite (Al(OH)₃) which shows a pH of 8.2 and 5.1, respectively, under typical laboratory conditions (Slessarev et al., 2016). In the humid climate, where precipitation exceeds evaporation, mobile Ca⁺⁺ ions are removed through leaching resulting in accumulation of relatively immobile Al⁺⁺⁺, thereby buffering soil pH towards acidic (Slessarev et al., 2016). Contrary to this, in the arid climates, where evaporation exceeds precipitation, low leaching rates result in precipitation and accumulation of dissolved Ca⁺⁺ as CaCO₃, buffering the soil pH towards alkaline (Slessarev et al., 2016).

Our results showed significantly higher GWC in the humid soils which is expected due to high precipitation in the region (Figure 7.1c). However, there was no significant difference in GWC in soils from the semi-arid and the montane region. This unexpected similarity in GWC of soils from the semi-arid and the montane climate can be attributed to the characteristic of sampling locations. As three out of five sampling locations in the semi-arid climate were under the influence of external water source (Chapter 4), they did not represent the true moisture content of the region. Therefore, the relationship between GWC and soil properties are not discussed in the present study.

7.3 Total organic carbon, soil organic carbon stock, and total nitrogen contents

Both TOC and TN contents in soils showed significant variations among the climatic zones (Figure 7.2a & 7.2b), with significantly lower contents in the semiarid climate for both the soil layers. Despite large variations in TOC and TN contents in the top layers of the montane soils, these soils showed significantly higher TOC and TN contents compared to the soils from other climatic zones. However, the bottom soils of the montane climate showed TOC and TN contents similar to soils of the humid climate. Soils from both montane and humid climate showed significantly higher TOC and TN contents in the top soils compared to the bottom soils (Figure 7.2a & 7.2b). Variation of TOC and TN contents with depth at individual sampling locations of each climate zone of the present study is given in Table 7.1. Soil organic C stock was similar in soils of the humid and the montane climates, whereas it was significantly lower in the semi-arid soils (Figure 7.2c). Overall, the TOC and TN contents of the soils showed a significant positive correlation between them (Figure 7.3a).



Figure 7.2: (a) Total organic carbon contents, (b) total nitrogen contents, and (c) organic carbon stock in soils of different climatic zones of India (mean $\pm 1\sigma$). Values indicated with the same alphabet are not significantly different at p < 0.05. Significant variation in top soils is indicated by uppercase letters and in bottom soils is represented by lowercase letters. Values which are significantly different with depths of same climate zones are indicated by star mark with a horizontal line over the bars. Soil organic carbon stock is given for a depth of 0-10 cm.

A reduction in soil TOC and TN contents with depth was observed in the humid and the montane climates (Figure 7.2a & 7.2b) which can be attributed to reduced vegetation input and microbial activity in the deeper soils (Kellman et al., 2014; Fang et al., 2015; Zhang et al., 2016). Meanwhile, high salinity in the semiarid soils, as observed during the present study, can reduce porosity and microbial activity in these soils resulting in reduced organic C content in the soils (Yu et al., 2014). The reduction in TOC and TN contents in the semi-arid soils can also be attributed to reduced water availability and vegetation cover in these soils as it can reduce photosynthesis, N fixation, and microbial activity along with enhancing soil drying and erosion (Li et al., 2007; Reynolds et al., 2007; Vicente-Serrano et al., 2012; Jiao et al., 2016). The montane soils showed significantly higher TOC and TN contents compared to other climate zones (Figure 7.2a & 7.2b), probably due to lower rates of organic matter decomposition in colder temperature (Kirschbaum, 1995; Wu et al., 2003) resulting in accumulation of high TOC and TN contents in the montane soils. Higher TOC and TN in soils of the humid climate compared to the semi-arid climate (Figure 7.2a & 7.2b) can be attributed to high precipitation, leading to high vegetation productivity in these regions (Jobbágy and Jackson, 2000; Callesen et al., 2003). The positive relationship between soil TOC and TN contents was well expected as both are derived from organic matter and have closely linked production and consumption pathways. This relationship was supported by previous works as well (Cheng et al., 2016; Wang et al., 2016). As organic C stock is a function of TOC and bulk density, it resulted in similar soil organic C stocks in soils of the montane and humid climates (Figure 7.2c). Despite the use of different methods for calculation of soil organic C stock, our results were in agreement with the previous studies conducted in these regions (Chhabra et al., 2003; Krishnan et al., 2007; Lo Seen et al., 2010; Gahlod et al., 2019).

7.4 Stable isotopic compositions of carbon and nitrogen in soils

The soils from different climate zones showed different δ^{13} C signatures in both the soil layers, with significantly higher δ^{13} C in the semi-arid soils compared to the humid soils (Figure 7.3b). Soils from the montane climate showed a large range of δ^{13} C values which overlapped with δ^{13} C of soils from both semi-arid and humid climates. Contrary to δ^{13} C, the δ^{15} N signatures showed no significant variation in soils collected from different climatic zones (Figure 7.3b). Though the δ^{13} C values were significantly higher in the deeper soils compared to the top soils at individual sampling site (Table 7.1), altogether δ^{13} C showed no significant variation with depth in soils of any of the climatic zone. Similarly, δ^{15} N signatures of the soils showed no significant difference with depth in the semi-arid and humid soils. However, soils from the montane climate were significantly enriched ^{15}N in in the deeper layers compared to the top soils.

Table 7.1: General soil characteristics along with total organic carbon, total nitrogen, and their isotopic compositions in soils of different climate zones of India (mean $\pm 1\sigma$). TOC: total organic carbon, TN: total nitrogen, [NH₄⁺]: NH₄⁺ concentration, [NO₃⁻]: NO₃⁻ concentration, and GWC: gravimetric water content.

Soil depth	Sampling location	TOC	$\delta^{13}C$	TN	$\delta^{15}N$	$[NH_4^+]$	[NO ₃ ⁻]	pН	GWC
Top soils	W1	25.3 ± 10.1	-27.6 ± 0.8	2.2 ± 0.3	5.7 ± 0.7	16.2 ± 7.0	4.8 ± 2.7	5.6 ± 0.2	22.8 ± 3.1
	W2	31.8 ± 19.6	-27.3 ± 0.4	2.6 ± 1.4	4.3 ± 0.5	12.7 ± 9.8	27.3 ± 22.4	4.5 ± 0.2	22.7 ± 8.8
	W3	26.1 ± 14.3	-26.2 ± 1.4	2.2 ± 0.6	4.3 ± 0.5	18.8 ± 3.4	4.5 ± 0.8	5.4 ± 0.2	22.9 ± 5.3
	W4	18.9 ± 0.5	-23.2 ± 1.8	2.2 ± 0.5	10.2 ± 4.6	19.7 ± 15.6	12.8 ± 20.7	4.0 ± 0.2	30.8 ± 5.4
	W5	51.0 ± 9.4	-26.9 ± 0.6	5.4 ± 0.1	6.7 ± 1.1	20.1 ± 16.8	38.4 ± 9.6	4.6 ± 0.1	45.8 ± 1.5
Bottom soils	W1	11.6 ± 2.7	-26.3 ± 1.1	1.8 ± 0.4	7.3 ± 0.6	8.4 ± 4.9	2.6 ± 1.8	5.2 ± 0.4	22.8 ± 3.1
	W2	12.4 ± 5.3	-26.5 ± 0.1	2.0 ± 0.8	5.7 ± 0.7	16.5 ± 3.6	3.7 ± 3.1	4.2 ± 0.1	45.2 ± 32.8
	W3	10.0 ± 3.2	-25.3 ± 0.3	1.5 ± 0.4	6.5 ± 0.3	6.9 ± 2.4	1.7 ± 0.3	5.1 ± 0.1	29.1 ± 9.6
	W4	8.6 ± 3.3	-24.4 ± 0.4	1.7 ± 0.1	13.9 ± 3.6	12.1 ± 2.9	1.5 ± 0.3	4.2 ± 1.0	30.9 ± 3.3
	W5	30.7 ± 2.2	-26.1 ± 0.4	3.2 ± 0.1	7.6 ± 0.4	30.9 ± 2.8	3.9 ± 0.8	4.4 ± 0.0	45.1 ± 3.7

(a) Soils of humid climate

(b) Soils from arid climate

Soil depth	Sampling location	TOC	δ ¹³ C	TN	δ^{15} N	$[NH_4^+]$	[NO ₃ ⁻]	pН	GWC
Top soils	SF-1	2.8 ± 2.8	-21.60 ± 1.6	0.2 ± 0.0	7.1 ± 1.1	1.2 ± 0.1	1.4 ± 0.6	8.0 ± 0.0	27.1 ± 0.8
	SF-2	0.7 ± 0.7	-23.4 ± 1.3	0.1 ± 0.0	7.3 ± 0.7	1.4 ± 0.3	0.6 ± 0.3	7.8 ± 0.0	21.7 ± 6.3
	WL	10.2 ± 6.4	-24.7 ± 3.6	1.6 ± 0.6	5.6 ± 1.5	1.7 ± 0.3	3.1 ± 0.3	8.2 ± 0.2	22.6 ± 5.5
	GL	2.8 ± 0.7	-19.3 ± 0.7	0.5 ± 0.1	9.2 ± 0.3	2.3 ± 0.7	20.4 ± 28.8	8.6 ± 0.3	1.2 ± 0.1
	AF	5.9 ± 2.7	-24.9 ± 0.2	0.8 ± 0.3	8.2 ± 0.1	6.9 ± 3.6	6.4 ± 1.9	$8.2\pm.01$	13.7 ± 3.7
Bottom soils	SF-1	1.9 ± 0.4	-20.8 ± 0.1	0.2 ± 0.0	17.0 ± 12.3	1.0 ± 0.1	1.5 ± 0.2	7.9 ± 0.1	31.2 ± 3.4
	SF-2	1.4 ± 0.3	-22.5 ± 0.5	0.3 ± 0.1	6.4 ± 0.9	1.0 ± 0.0	1.3 ± 0.3	7.9 ± 0.0	28.9 ± 4.3
	WL	3.3 ± 0.7	-24.1 ± 0.6	0.4 ± 0.0	7.0 ± 0.8	1.5 ± 0.3	0.9 ± 0.3	8.5 ± 0.1	20.7 ± 3.2
	GL	2.0 ± 0.5	-18.2 ± 0.6	0.3 ± 0.1	9.2 ± 0.4	1.3 ± 0.1	15.9 ± 12.1	8.4 ± 0.3	2.0 ± 0.8
	AF	5.5 ± 0.7	-23.4 ± 0.2	0.5 ± 0.1	9.6 ± 0.2	1.8 ± 0.4	2.0 ± 0.2	8.1 ± 0.1	10.3 ± 0.5

(c) Soils from montane climate

Soil depth	Sampling location	TOC	$\delta^{13}C$	TN	$\delta^{15}N$	$[\mathrm{NH_4}^+]$	[NO ₃ ⁻]	pН	GWC
Top soils	3000 m	69.4 ± 37.3	-25.5 ± 0.1	6.2 ± 3.0	2.2 ± 0.7	27.0 ± 10.8	4.7 ± 5.2	4.4 ± 0.2	35.3 ± 10.0
	2500 m	47.6 ± 17.4	-21.1 ± 1.3	2.9 ± 1.1	6.4 ± 0.6	10.7 ± 5.0	0.8 ± 0.2	6.1 ± 0.6	18.7 ± 0.6
	2000 m	66.2 ± 33.6	-26.2 ± 0.4	5.7 ± 2.4	4.6 ± 0.3	16.6 ± 4.0	2.1 ± 2.2	5.1 ± 0.8	15.4 ± 0.8
	1500 m	25.5 ± 8.4	-22.9 ± 2.3	2.9 ± 0.7	7.9 ± 0.2	14.5 ± 2.2	3.7 ± 1.7	6.7 ± 0.1	37.2 ± 2.7
	1000 m	30.2 ± 10.6	-21.9 ± 0.5	3.5 ± 0.2	7.1 ± 0.1	14.1 ± 2.7	3.3 ± 0.3	6.3 ± 0.1	6.4 ± 1.5
Bottom soils	3000 m	15.0 ± 13.8	-26.1 ± 0.4	1.6 ± 0.4	6.0 ± 1.4	6.6 ± 2.5	1.3 ± 0.2	4.5 ± 0.4	18.5 ± 4.4
	2500 m	12.7 ± 7.7	-20.5 ± 1.4	1.5 ± 0.7	7.7 ± 0.9	8.1 ± 2.5	0.5 ± 0.2	6.2 ± 0.9	19.5 ± 1.3
	2000 m	13.0 ± 6.0	-25.2 ± 0.4	1.7 ± 0.5	6.7 ± 0.8	3.8 ± 1.6	0.8 ± 0.4	4.8 ± 0.4	17.3 ± 0.9
	1500 m	15.9 ± 7.1	-22.4 ± 0.3	1.9 ± 0.8	8.2 ± 0.4	13.1 ± 0.7	2.5 ± 0.1	6.4 ± 0.2	38.0 ± 3.9
	1000 m	13.4 ± 7.4	-20.7 ± 0.7	1.5 ± 0.4	7.2 ± 0.6	11.0 ± 0.8	2.2 ± 0.6	6.3 ± 0.0	3.7 ± 1.2
Table 7.2: Correlation matrix (r^2) for the measured variables in top soil layers of three climatic zones (humid, arid, and montane). n = 45 for each parameter except bulk density (n = 15). TOC: total organic carbon, TN: total nitrogen, $[NH_4^+]$: NH_4^+ concentration, $[NO_3^-]$: NO_3^- concentration, GWC: gravimetric water content, and BD: bulk density. Significantly correlated values are indicated by * (p < 0.05), ** (p < 0.01), and *** (p < 0.001), whereas non-significant correlations are denoted by ns.

	TOC	$\delta^{I3}C$	TN	$\delta^{15}N$	$[NH_4^+]$	[NO ₃ ⁻]	pН	GWC	BD
ТОС	1								
$\delta^{I3}C$	0.12 *	1							
TN	0.85 ***	0.13 *	1						
$\delta^{15}N$	0.26 ***	0.28 ***	0.23 ***	1					
$[NH_4^+]$	0.37 ***	0.11 *	0.34 ***	0.11 *	1				
[NO ₃ ⁻]	0.02 ns	0.04 ns	0.04 ns	0.00 ns	0.02 ns	1			
рН	0.32 ***	0.25 ***	0.33 ***	0.12 *	0.49 ***	0.06 ns	1		
GWC	0.12 ***	0.10 *	0.14 *	0.04 ns	0.12 *	0.04 ns	0.18 **	1	
BD	0.68 ***	0.03 ns	0.72 ***	0.18 **	0.25 ***	0.00 ns	0.21 **	0.06 ns	1

There was a significant positive correlation between $\delta^{15}N$ and $\delta^{13}C$ of the soils collected from different climatic zones (Figure 7.3b). The $\delta^{15}N$ and $\delta^{13}C$ showed a significant negative correlation with TN and TOC contents of the soils, respectively (Figure 7.3c & 7.3d).



Figure 7.3: Relationship between (a) total organic carbon and total nitrogen, (b) δ^{13} C and δ^{15} N, (c) δ^{15} N and total nitrogen, and (d) δ^{13} C and total organic carbon in soils of different climatic zones of India (n = 90 for total organic carbon, total nitrogen, and δ^{13} C; n = 89 for δ^{15} N).

Independent of climate zone, the δ^{13} C signature of the soils (- 17‰ to - 28‰; average = - 23.8‰) indicated the dominance of C3 vegetation. Soils showing enrichment in ¹³C were mostly present in the semi-arid climate, whereas

the soils from the humid climate were significantly depleted in ¹³C (Figure 7.3b). The spread in δ^{13} C of the montane soils over both humid and semi-arid soils suggested that climatic factors hold no major control in shaping the isotopic composition of montane soils. It has previously been shown that edaphic rather than climatic factors regulate the δ^{13} C isotopic composition in high altitude soils (Yang et al., 2015), as observed in the present study.

Though there was no significant difference between the two, soils from the semi-arid climate were relatively enriched in ¹⁵N compared to the humid climate (Figure 7.3b) indicating N cycle to be more open in the semi-arid climate and conservative in the humid climate (Amundson et al., 2003; Wang et al., 2014). Similar to that observed in δ^{13} C composition, the δ^{15} N values in the montane soils were also spread over humid and semi-arid soils, indicating higher control of edaphic factors over climatic factors in shaping the isotopic composition and nutrient dynamics in the montane soils, supporting the conclusions drawn in Chapter 3. The δ^{15} N values showed an increase with depth and were relatively higher in semi-arid soils compared to the humid and montane soils (Table 7.1). Such differences in δ^{15} N of soils can occur due to decreased retention of N in soils which leads to preferential loss of lighter N isotope (¹⁴N) from the system (Mitchell et al., 2001).

Relatively higher δ^{15} N and δ^{13} C in the semi-arid soils compared to soils of other climate zones might have resulted from disproportionately higher mineralization of limited organic matter available in these soils. The decomposition of organic matter by microbiological processes preferentially consumes the lighter isotope leaving the remaining organic matter to be enriched in heavier isotope (Ågren et al., 1996). Due to the unavailability of fresh organic inputs in the semi-arid soils, decomposition of already isotopically enriched organic matter can further enrich the bulk soils in heavier isotopes. Similar patterns for high δ^{15} N and δ^{13} C in arid climate than humid climate or low precipitation than high precipitation regions have been reported previously (Aranibar et al., 2004; Wang et al., 2010). The negative correlation observed between δ^{13} C and TOC content of soils (Figure 7.3d) was in agreement with previous studies and indicated the importance of trees or C3 plants over grasses in maintaining TOC stock of soils. (Rosen et al., 2009; Bai et al., 2012). Similar to δ^{13} C and TOC, the δ^{15} N of soils was inversely related to TN content (Figure 7.3c), possibly due to woody vegetation present in the humid climate. As the tree density increases in the humid climate, the possibility for higher abundance of N-fixer species also increases which would provide ¹⁵N depleted organic matter to soils of the humid climate compared to the semi-arid climate (Bai et al., 2013). Additionally, the contribution of atmospheric N deposition in the humid climate during the present study can be a possibility for lower δ^{15} N as N inputs through atmospheric deposition are generally depleted in ¹⁵N (Ansley et al., 2006).

7.5 Mineral nitrogen content in soils

The NH₄⁺ concentrations were significantly lower in soils of the semi-arid climate in both the layers, whereas soils of the humid climate showed the highest NH4⁺ concentrations among the studied climate zones (Figure 7.4a). This was expected due to low nutrient availability and turnover rates in soils of the semi-arid climate. Low organic inputs to the semi-arid soils can also result in continuous remineralization of available organic matter resulting in similar nutrient composition in soil depths. NH₄⁺ concentrations showed no variations with depth in the humid and semi-arid climate zones. However, NH₄⁺ was significantly higher in the top layers compared to the bottom layers in the montane climate soils. NH_4^+ and NO_3^- concentrations at each sampling location of the three climate zones included in this study are presented in Table 7.1. The NH₄⁺ concentrations were significantly correlated with TN, TOC, pH, and GWC of the soils (Table 7.3). In the top soil layers, NH_4^+ also showed a positive correlation with soil bulk density (Table 7.2). Contrary to NH4⁺, NO3⁻ concentrations in soils showed no correlation with any other variable measured during the present study (Table 7.2 & 7.3). NO_3^- concentrations were significantly different in depths for soils of the humid climate only (Figure 7.4b). In the top soil layers, NO₃⁻ concentrations of the humid soils were significantly higher than the semi-arid soils, whereas the montane soils showed a broad range of NO₃⁻ concentrations in the top soils, overlapping with both humid and semi-arid soils (Figure 7.4b). For the bottom

soils, NO₃⁻ concentrations showed no significant difference for different climatic zones.



Figure 7.4: (a) NH_4^+ and (b) NO_3^- concentrations in soils of different climatic zones of India (mean $\pm 1\sigma$). Values indicated with the same alphabet are not significantly different at p < 0.05. Significant variation in top soils is indicated by uppercase letters and in bottom soils is represented by lowercase letters. Values which are significantly different in depths of same climate zones are indicated by star mark with a horizontal line over the bars.

 NO_3^- was the dominant nutrient over NH_4^+ in the semi-arid soils (Table 7.1) during the present study (Figure 7.4b), probably due to its accumulation in soils with low moisture which could result in restricted movement of NO_3^- in soils creating its hotspots (Schlesinger et al., 1996; Schade and Hobbie, 2005). The dominance of NO_3^- over NH_4^+ in calcareous soils has been demonstrated previously (Breemen et al., 1982). Soils of semi-arid regions also tend to accumulate NO_3^- under low moisture conditions because of minimal microbial immobilization in absence of sufficient detrital C (Davidson et al 1991, Booth et al 2005, Hooker and Stark 2008). Despite being present in a rainfall influenced region, the humid soils showed large concentrations of both NH_4^+ and NO_3^- indicating the presence of external sources of N, such as atmospheric N deposition. In a region influenced by rainfall, one expects low NO_3^- concentrations due to its high mobility and losses through leaching, which was not observed in the humid soils during the present study. Conversely, NO_3^- concentrations were

significantly higher in the top compared to the bottom soil layers in the humid climate, supporting the possibility of external N source. Higher NH_4^+ in the top than the bottom layers of the montane soils can be attributed to high litter inputs in these soils which tend to stay in the top layers due to slow rates of decomposition under cold temperatures (Kirschbaum, 1995; Wu et al., 2003). Concentrations of NH_4^+ correlated well with TN, TOC, and other soil properties indicating major control of these variables in NH_4^+ availability of any ecosystem. NO_3^- showed no correlation with any of these variables.

Table 7.3: Correlation matrix (r^2) for the measured variables in both the soil layers of three climatic zones (humid, arid, and montane). n = 90 for each parameter except $\delta^{15}N$ (n = 89). TOC: total organic carbon, TN: total nitrogen, [NH_4^+]: NH_4^+ concentration, [NO_3^-]: NO_3^- concentration, and GWC: gravimetric water content. Significantly correlated values are indicated by * (p < 0.05), ** (p < 0.01), and *** (p < 0.001), whereas non-significant correlations are denoted by ns.

	ТОС	$\delta^{I3}C$	TN	$\delta^{15}N$	$[NH_4^+]$	$[NO_3]$	pН	GWC
ТОС	1							
$\delta^{I3}C$	0.12 ***	1						
TN	0.86 ***	0.16 ***	1					
$\delta^{^{15}}N$	0.15 ***	0.14 ***	0.14 ***	1				
$[NH_4^+]$	0.39 ***	0.14 ***	0.42 ***	0.06 *	1			
[NO ₃ ⁻]	0.04 *	0.01 ns	0.06 *	0.00 ns	0.00 ns	1		
pH	0.19 ***	0.33 ***	0.27 ***	0.04 ns	0.41 ***	0.01 ns	1	
GWC	0.05 *	0.13 ***	0.07 *	0.00 ns	0.13 ***	0.00 ns	0.15 ***	1

7.6 Variation in soil carbon and nitrogen with soil physical properties

Soil pH showed a significant correlation with TOC and TN contents of the soils (Figure 7.5a & 7.5b). Among the nutrients, NH_4^+ showed a significant negative relationship (Figure 7.5c) while NO_3^- showed no correlation with soil pH. Bulk density, measured in the top soils only, showed significant correlations with TOC, TN, and NH_4^+ contents of the soils (Figure 7.6). Similar to pH, soil NO_3^- concentrations showed no correlation with bulk density. GWC of soils also showed a significant, but weak correlation with soil TOC and TN contents (Table 7.2). Soil moisture showed a negative correlation with soil pH (Table 7.2).



Figure 7.5: Relationship of (a) total organic carbon, (b) total nitrogen, and (c) NH_4^+ concentrations with soil pH of different climatic zones of India (n = 90).



Figure 7.6: Relationship of (a) total organic carbon, (b) total nitrogen, and (c) NH_4^+ concentrations with bulk density of soils from different climatic zones of India (n = 45).

In the present study, the negative relationship between TOC and soil pH was driven by lower (acidic) pH and high organic C in the humid soils while higher (basic) pH and low organic C in semi-arid soils (Figure 7.5a). Similar results showing low organic C driven by alkaline pH in arid soils have been reported previously (Yu et al., 2014; Jiao et al., 2016). Furthermore, a negative correlation between pH and soil moisture (Table 7.2) indicated major control of precipitation in shaping the pH of the soils. It appears that accumulation of organic matter content in the soil also leads to its acidic nature whereas high inorganic C content, as observed during decarbonation of the semi-arid soils during the present study, can contribute to increased soil alkalinity. Similar observations showing the relation of soil pH to available organic or inorganic C

has been reported elsewhere (Ji et al., 2014). As evident from the negative relation of soil pH and organic C, it is also possible that when compared to arid regions, acidic soils from the humid regions tend to store more organic C by inhibiting its decomposition in acidic environment, thereby minimizing the loss of C from soils (Shi et al., 2012). Meanwhile, in semi-arid climate, high salinity and pH holds the potential to destroy the structure of soil aggregates, resulting in reduced TOC and TN content in soils (Duchicela et al., 2013). The NH₄⁺ concentrations exhibited an inverse relationship with the soil pH which was in agreement with the trend of high ammonification at lower pH and high nitrification favored at higher pH (Watros et al., 2018). Although the NO_3^- concentrations showed no relationship with soil pH, increase in NH4⁺ concentrations at low pH supports the above argument (Figure 7.5c). Bulk density of the soils showed negative correlation with TOC contents of the soils. These results were in agreement with Yu et al. (2014) and Whalen et al. (2003) who attributed this relationship to increased stability of soil aggregates due to increased soil porosity. Decrease in NH₄⁺ concentrations associated with increase in both bulk density and pH indicated degradation of land in semi-arid climate which resulted in barren soils with less nutrients (Li et al., 2015).

7.7 Conclusion

The soils from different climate zones of India were analyzed for the distribution of TOC and TN contents and their isotopic compositions. Results indicated relative enrichment in heavier isotopes of C and N in soils from semi-arid climate compared to other climates, which can be attributed to low organic matter contents with relatively disproportionate mineralization in these soils. Relatively higher precipitation and organic matter inputs in soils of the humid climate led to higher concentrations of TOC and TN with lower δ^{13} C and δ^{15} N values compared to soils from other climate. This indicated efficient organic matter turnover and tightly coupled N cycle in the humid climate. Soils of montane climate showed a spread over both semi-arid and humid climate suggesting higher controls of edaphic factors over the climate in organic matter turnover in these soils. Despite the climatic controls, soil properties like pH appeared to be an important indicator of soil C and N contents. Soil bulk density appeared to be a major variable deciding soil C storage capacity. Overall, as evident from isotopic signatures and organic C stock in soils of different ecozones, it appears that change in climatic conditions experienced by soils has the potential to significantly decide their organic matter dynamics.

Chapter 8

Summary and scope for future works

This thesis aimed to quantitatively understand the biogeochemical cycling of N in terrestrial ecosystems of India by measuring gross rates of N transformation processes in soils of different climatic zones. To the best of our knowledge, the results presented in this thesis are the first such quantitative estimates of gross N transformation rates in soils of the Indian subcontinent providing better insights into N and C dynamics in the soils of the region. Major findings of this thesis are summarized below.

8.1 Nitrogen dynamics in montane soils of the Himalayas

Rates of gross N transformation processes were measured at two temperature ranges in soils collected from different elevations in the Himalayas. Following major observations were made:

- Increase in temperature significantly enhanced the rates of gross N mineralization, NH₄⁺ consumption, and NH₄⁺ immobilization.
- Change in temperature showed no significant effect on gross nitrification rates in tropical montane soils of the Himalayas.
- NH₄⁺ immobilization was the major pathway for NH₄⁺ consumption under increased temperature condition.

- There was no consistent change in process rates and pools related to N transformations with elevation.
- Rather than climatic, edaphic factors regulated N biogeochemistry at different elevations of the Himalayas.
- N pool and turnover rates decreased significantly in the bottom soils compared to the top soils.
- NO₃⁻ concentrations and its residence time in soils did not vary with soil depths.

8.2 Nitrogen cycling in semi-arid soils

An attempt to understand nitrogen dynamics in soils collected from different landtypes in the semi-arid climate of Kutch was made by measuring gross rates of N transformations. Following are the important findings of the study:

- The highest rates of N transformation processes were in the agricultural soils.
- The average NO₃⁻ concentration was the highest in the grassland soils, which were characterized by low moisture conditions.
- Wetland soils showed the highest organic matter (TN and TOC) contents among the studied land-types.
- Soil moisture showed a significant negative correlation with rates of gross N mineralization, NH4⁺ consumption, and NH4⁺ immobilization rates, whereas gross nitrification rates showed no significant relationship with soil moisture.
- Gross rates of N transformations showed a significant decline with increasing salinity.
- Of the two NH₄⁺ consumption processes, NH₄⁺ immobilization was dominant in soils showing high N mineralization.
- The cumulative effect of vegetation, moisture, and salinity appeared to be the key regulator of microbial activity and N transformations in semi-arid tropical soils with salinity being the most important factor.

8.3 Nitrogen transformations in tropical humid forest soils

Rates of N transformation processes were measured in soils of the Western Ghats to understand N dynamics in tropical humid forest soils. Below are the highlights of the study:

- Rates of gross N mineralization and nitrification in the soils of the Western Ghats reduced with depth.
- The overall consumption of nutrients exceeded their production rates with higher consumption of NH₄⁺ than NO₃⁻.
- NH₄⁺ immobilization was the dominant NH₄⁺ consumption pathway in this tropical humid region.
- Despite the higher consumption, accumulation of N was observed in soils of the Western Ghats.
- High residence time of nutrients was found in soils of the Western Ghats.
- Evidence for significant contribution of external sources of N, such as atmospheric N deposition and BNF, to the soil N pool of the Western Ghats was found.

8.4 Nitrogen dynamics in differently managed forest soils

Effect of mono-plantation (rubber plantation) by replacing natural forested ecosystems on soil N dynamics was studied in soils of Kerala, India. Findings of the study are summarized below:

- NO₃⁻ dominated in soils of the managed and unmanaged forests, whereas soils under rubber plantation showed relatively higher NH₄⁺ concentration.
- TN and TOC contents were the highest under the rubber canopy.
- Despite high TN and TOC concentrations, the rates of gross N mineralization and NH₄⁺ consumption were considerably lower in soils of the rubber plantation compared to the managed and unmanaged forests.
- Soils of the managed forest showed spatial homogeneity, whereas the unmanaged forest showed large variability in gross N mineralization rates.

- Significant reduction in gross N mineralization and NH₄⁺ consumption rates was observed due to change in tree species composition from mixed to mono-culture of rubber plants.
- The residence time of NH₄⁺ was significantly higher in soils of the rubber plantations compared to the managed and unmanaged forests.

8.5 Organic matter content and its stable isotopic composition in soils of India

- Soils from the semi-arid climate were enriched in the heavier isotopes of C and N compared to soils from the humid climate possibly due to low organic matter content and relatively disproportionate mineralization.
- Soils from the humid climate showed higher concentrations of TOC and TN compared to semi-arid soils.
- Isotopic signatures of soils from the humid climate indicated efficient organic matter turnover and tightly coupled N cycle.
- Soils of montane climate showed a spread in δ^{13} C and δ^{15} N signatures over both semi-arid and humid climate suggesting higher control of edaphic factors over climate on organic matter dynamics.
- Despite the climatic controls, soil properties like pH appeared to be an important indicator of soil C and N contents.
- Soil bulk density was a major variable regulating soil C storage capacity.
- Change in climatic conditions experienced by soils under different climate has the potential to significantly control the organic matter dynamics.

8.6 Scope for future works

Although a lot of work has been carried out towards understanding the N cycling processes in the Indian soils, most of these works are based on measurements of net rates. As mentioned earlier, net rates provide limited information on N dynamics. For the first time, the works reported in this thesis are based on quantification of gross rates of N transformation processes in the Indian soils, providing a detailed understanding of N cycling in selected ecosystems of the subcontinent. Apart from

the need for detailed studies on N dynamics in the Indian soils at better spatial and temporal scales, following are the specific topics which may provide scope for the future works:

- This thesis quantifies gross rates of two important processes (N mineralization and nitrification) of the N cycle in soils from different terrestrial ecosystems of India. However, the thesis did not delve into the fate of N further in the soil N cycling. A detailed study on N loss pathways and N cycling processes such as denitrification is desirable to better understand the N cycling in the terrestrial ecosystems.
- Some of the study areas selected during the present thesis were associated with a long residence time of the nutrients in soils. These accumulated nutrients are highly susceptible to loss and can cause the production of greenhouse gases such as N₂O or can pollute nearby water bodies through leaching. A study focused on N₂O dynamics in these soils along with identification of leaching losses of nutrients on watershed-scale would help to understand the environmental impacts of excess N in soils.
- Excess N in soils of certain ecosystems, such as the Western Ghats, indicated the possibility of external N sources, other than internal cycling of litter, to the soils. Quantification of N sources to soils, such as BNF, atmospheric N deposition, and N inputs through weathering of rocks, would help in constraining sources of reactive N in such ecosystems.
- The ecozones studied in the present thesis showed excess nutrients concentrations with NH4⁺ as the dominant nutrient form in these soils. Processes responsible for NH4⁺ accumulation in the soils with the help of microbial culture experiments would be desirable to understand the role of the microbial community in shaping the nutrient profile of an ecosystem.
- Extension of the present work by studying gross N transformation rates in different seasons would help to understand the mechanism of N transformation processes and behavior of responsible microorganisms in different environmental or seasonal conditions.
- Volatile organic compounds (VOCs) appear to exert a significant control on soil N cycling in agro-forestry (particularly in rubber plantations). The

interaction between VOCs and N transformation processes needs to be explored in detail.

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