CHAPTER I General Introduction

Tropical montane forests

Tropical montane forests are mostly found at elevations ranging from 1500 – 3500 meters above sea level (masl) in large inland mountains (e.g. Andes), but could be found also at lower elevations in areas close to the coast and in insular mountains (Bubb et al. 2004; Hamilton 1995). With increasing altitude in montane areas, temperature (Walker and Flenley 1979) and amount of precipitation (Bendix et al. 2008) change. Unlike the forests found in lower elevations in the humid tropics, vegetation in the montane forests is usually characterized by the reduced stature of trees, its trunks and branches often gnarled and twisted, crowns that are dense and compact, and with sclerophyllous leaves. Stem density also increases with elevation, with lichens, mosses, bryophytes, and filmy ferns becoming common and comprising a high proportion of the biomass (Nadkarni 1984). Soil is often wet or waterlogged with thick organic layers and consequently a slow release of mineral nutrients (Edwards and Grubb 1977; Tanner et al. 1998), that points to a possible nutrient limitation in these ecosystems. In the eastern Andean tropical montane forests, organic layer thickness increase with elevation (Wilcke et al. 2002), and this has been tied to the soil N availability of this ecosystem (Wolf et al. 2012).

There are several threats to tropical montane forests, mostly anthropogenic in nature, including the increasing atmospheric nutrient deposition (which include key nutrients such as nitrogen (N) and, phosphorus (P)), that the tropics are facing today (Hietz et al. 2011; Homeier et al. 2012) mainly from fertilizer and fossil fuel use (Galloway et al. 1994, 2008) and biomass burning (Crutzen and Andreae 1990; Cochrane 2003). Biomass burning is a major contributor to the increased deposition of nutrients, including N, especially in Ecuador (Fabian et al. 2005).

Anthropogenic alterations to the global N and P cycles

All ecosystems receive N inputs from atmospheric deposition. These inputs are often small, ranging from 1 to 5 kg ha^{-1} year^{-1}, in the ecosystems downwind from pollution-free open-ocean waters (Hedin et al. 1995). Human activities are now the major source of N deposited in many

areas of the world (Chapin et al. 2011). Inputs from anthropogenic sources of N to ecosystems can be quite large, for example 10 to 20 kg ha^{-1} year^{-1} in northeastern US or 50 to 100 kg ha^{-1} yr^{-1} in northern China (Chapin et al. 2011). In Ecuadorian montane forests, the annual N deposition rate for the period 1998 to 2010 was 14 to 45 kg N ha^{-1} year^{-1} (Homeier et al. 2012). Anthropogenic activities such as land-use changes and biomass burning have accelerated the movement of N not only within, but also between ecosystems (Vitousek et al. 1997). The distribution of fixed N can range from regional (mineral N deposition on land) to global (production of GHGs) and fixed N can occur in various forms. The Haber process fixes more N than any other anthropogenic process, and projections are that it will reach 165 Tg year^{-1} by 2050 (Galloway et al. 2004). Elevated N input to terrestrial ecosystems can change the plant species composition (Vitousek et al. 1997), decrease plant diversity (Bobbink et al. 1998; Phoenix 2006), can lead to soil acidification (van Breemen et al. 1982) and declining soil fertility, pollute ground and surface waters (Aber et al. 1998; Schulze 1989), contribute to the formation of acid rain and smog through increased emissions of N$_2$O and NO (Vitousek et al. 1997).

Unlike carbon (C)and N, phosphorus (P) has only a tiny gaseous component and no biotic pathway that brings the new P into ecosystems. Until recently, available P in ecosystems were derived from organic forms and recycled tightly within terrestrial ecosystems. The physical transfers of P globally are constrained by a lack of gaseous component. P moves around the atmosphere primarily through wind erosion and runoff of particulates in rivers and streams to oceans. Global atmospheric P deposition is estimated to be around 3 Tg year^{-1} (Smil 2000; Ruttenberg 2004) of which 4.8% of this is anthropogenic in source (Mahowald et al. 2008). In Ecuadorian montane forests, the annual P deposition rate was 0.4 to 4.9 kg P ha^{-1} year^{-1} in 1998 to 2010 (Homeier et al. 2012). The major alteration to the global P cycle caused by anthropogenic activities is the acceleration of the entry of P into the biosphere from the mining of P-bearing rock such as phosphate apatite, and loss of P from soil into aquatic ecosystems (Bennett & Carpenter 2002). Because the P commonly limits production in lakes, P fertilization

of freshwater ecosystems can lead to eutrophication and associated negative consequences for aquatic organisms and humans.

Impacts of elevated nutrient deposition in tropical forests

Tropical forests are both sinks and sources of important greenhouse gases (GHGs) because they generate a third of the global net primary production (Field et al. 1998; Malhi and Phillips 2004) and is the largest natural source of a potent GHG, nitrous oxide (N_2O) (Bouwman et al. 1995). Although biogeochemical cycles in tropical forest ecosystems are highly vulnerable to climate change (Malhi & Phillips 2004), the soil N cycle that regulates the production of these trace gas fluxes as well as the availability of N to plants have rarely been studied in tropical montane forests.

Increased N inputs to tropical forest soils have been tied to increases in soil N-oxide (NO + N_2O) emissions, nitrate (NO_3^-) leaching, decrease in microbial N immobilization, and differential changes in plant productivity, soil carbon dioxide (CO_2) fluxes and soil carbon (C) dynamics (e.g. Hall and Matson 2003; Lohse and Matson 2005; Koehler et al. 2009a, 2009b; Corre et al. 2010, 2013; Cusack et al. 2011a; Wright et al. 2011). On the other hand, studies on how P addition affects soil N-cycling in tropical montane forests are rare, and to our knowledge only one study so far reported on P-addition effects on net rates of soil N cycling and N_2O emissions where net rates of soil N-cycling and soil N_2O emissions were not affected by P addition alone but only by combined N + P addition (Martinson et al. 2013). These results suggest deleterious consequences on the environment. These processes are strongly controlled by internal transformations of N in the soil, illustrating the importance of quantifying gross rates of soil N-cycling, which separate mineral N production processes from N retention processes, in order to understand the mechanisms of how changes in soil N-cycling rates with elevated N input result in changes in N losses.

Studies on retention and fates of N in tropical forests are rare because the studies conducted so far have only traced ^{15}N within days (e.g. Templer et al. 2008) but are relatively well-studied in some temperate forest ecosystems. Temperate forests generally demonstrate

4

that the soil is an important and large sink of N over a long-term period (e.g. Emmett and Quarmby 1992; Buchmann et al. 1996; Magill et al. 1997; Nadelhoffer et al. 1999). On the other hand, in a subtropical lower montane forest in Puerto Rico, NO_3^- transformation processes (i.e. nitrification with subsequent dissimilatory NO_3^- reduction to NH_4^+ and uptake of NH_4^+ by plants) play a major role of N retention during a 7-day ^{15}N-tracing study (Templer et al. 2008). A meta-analysis of ^{15}N tracing studies conducted across ecosystem types show that N retention was influenced by ecosystem type, vegetation type, mycorrhyzal type, soil C:N ratio, disturbance history and even the method of ^{15}N application (Templer et al. 2012). They also showed that above a certain threshold of added N (i.e. 46 kg N ha^{-1} $year^{-1}$) total ecosystem N retention decreased. Since studies assessing short-term N cycles mostly exclude competition from plant uptake it is also of interest to study the long-term N cycling, which involves several short-term N-cycling events and the recycling of N within the plant-soil-microbe system. This links the short-term fates of N into long-term patterns of retention in an ecosystem. Because the N cycle is closely linked with the C cycle, the fates of N in the soil influence the ability of the soil to sequester C (Cusack et al. 2011b; Templer et al. 2012). How increase in nutrient deposition affects the long-term fates of soil mineral N in tropical montane forest soils remain poorly understood.

The N cycle

The N cycle (Fig. 1) describes the movement and the transformation of N_2 into various organic or inorganic forms in various oxidation states in the atmosphere, biosphere, and geosphere interface, each of which has consequences for the ecosystem (Brady and Weil 2002). The major N transformations, which are N fixation, N assimilation, N mineralization, nitrification, and denitrification are mainly facilitated by microbes (Fig. 1).

N-fixation is the conversion of N_2 to biologically available forms (Eq. 1). N fixation can be done either by lightning or by nitrogen-fixing organisms. The energy from lightning causes nitrogen (N_2) and water (H_2O) to combine to form ammonia (NH_3) and nitrates (NO_3^-). Biological N fixation occurs when atmospheric N_2 is converted to NH_3 by an enzyme called
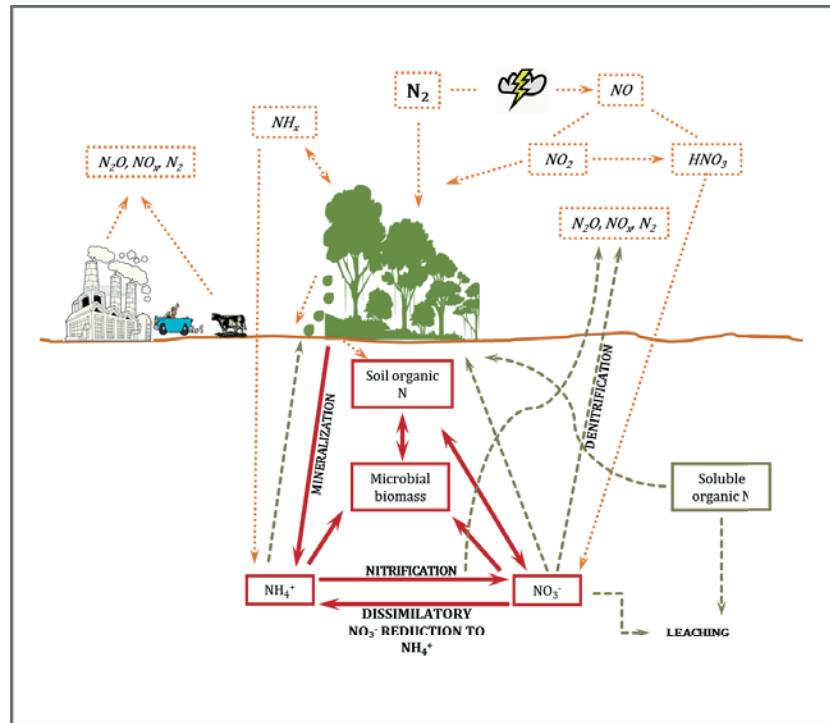
5

Fig. 1 A simplified model of the nitrogen cycle showing the N transformations in the atmosphere and N inputs to the N cycle (orange arrows), the soil N cycle (red arrows), N assimilation and losses from the soil N cycle (green arrows). (Adapted from Arnold 2008, PhD dissertation)

nitrogenase. The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2.

$$\text{Eq. 1 } N_2 + 8H + 8e^- \rightarrow 2\,NH_3 + H_2$$
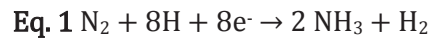
Fixed N is then converted to NH_4^+ (Eq. 2). NH_4^+ and NH_3 are closely related. NH_4^+ is a positively charged ion (cation) and is therefore attracted to negatively charged soil particles hence it is

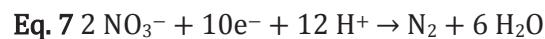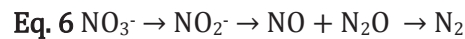$$\text{Eq. 2 } NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$
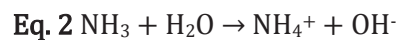
held as an exchangeable cation in soil and does not easily leach. NH_3 on the other hand is a gas that once formed can quickly escape from soil to the air. *Nitrification* is the conversion of NH_4^+ to NO_3^-, where NH_4^+ is first oxidized to NO_2^- (Eq. 3) performed by oxidizing bacteria and *Archaea*, then NO_2^- is further oxidized to NO_3^- (Eq. 4) done by the bacteria *Nitrobacter*. Oxidation of NO_2^- usually proceeds at a more rapid rate so NO_2^- is a relatively rare form of inorganic soil N. NO_3^- is a negatively charged anion and is highly soluble so it moves with water and can easily be leached. NH_4^+ and NO_3^- make up the bulk of soil inorganic N and are the principal forms available for plant growth (e.g. Walworth 2013).

$$\text{Eq. 3 } 2\,NH_4^+ + 3O_2 \rightarrow 2\,NO_2^- + 2\,H_2O + 4H^+$$

$$\text{Eq. 4 } 2\,NO_2^- + O_2 \rightarrow 2\,NO_3^-$$
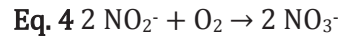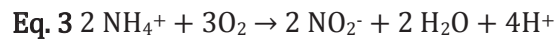
Plants *assimilate N* in the form of NH_4^+ or NO_3^- or they may also assimilate dissolved organic N (DON). The transformation of organic N into NH_4^+, when the decomposers including bacteria, fungi and protozoa use up the amino groups of dead biomass is N *mineralization*.

NO$_3^-$ can be chemically reduced to through the process of denitrification. *Denitrification* is the transformation of NO_3^- and NO_2^- to N_2 (Eq. 5). This process (Eq. 6) is anaerobic and carried out by denitrifying bacteria such as *Pseudomonas*. This process is important in that it removes fixed N (i.e. NO_3^-) from the ecosystem and returns it to the atmosphere in a biologically inert

$$\text{Eq. 6 } NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2$$

$$\text{Eq. 7 } 2\,NO_3^- + 10e^- + 12\,H^+ \rightarrow N_2 + 6\,H_2O$$

form (N_2) and also plays an important role in the removal of unwanted NO_3^- in bodies of water where NO_3^- accumulation might lead to undesirable consequences such as algal blooms (e.g. Bernhard 2012). However during denitrification, gaseous N losses occur in the form of N-oxides that may contribute to environmental pollution and global warming.

One major part of the N cycle is the soil internal N cycle (Fig. 1, red arrows) because it regulates nutrient availability to plants as well as the loss of potentially harmful N through emissions of GHGs and the leaching of NO_3^-. Beside N mineralization and nitrification, there are three other transformation processes that are important in the N cycling within the soil. First, is microbial immobilization of mineral and organic N through the incorporation into microbial biomass that is released again after the organisms die. Second, abiotic NH_4^+ and NO_3^- retention by NH_4^+ fixation of clay minerals (Davidson et al. 1991) or physical condensation reactions with phenolic compounds (Nömmik 1970; Nömmik and Vahtras 1982; Johnson et al. 2000), and NO_3^- reduction to NO_2^-, which readily reacts with soil organic matter (Smith and Chalk 1980; Azhar et al. 1986; Thorn and Mikita 2000). Third, the conversion of NO_3^- to NO_2^-, and then to NH_4^+ which is known as the dissimilatory NO_3^- reduction to NH_4^+ (DNRA) (Silver et al. 2001, 2005; Sotta et al. 2008).

The NH_4^+ produced by N mineralization has several potential fates. In addition to being absorbed by plants or microbes, NH_4^+ readily absorbs to the negatively charged surfaces of soil minerals and organic matter, reducing the concentration of NH_4^+ in the soil solution. NH_4^+ can also be oxidized mainly by bacteria to NO_2^- and NO_3^- or converted to NH_3, and lost to the atmosphere. The potential fates of NO_3^- are being absorbed by plants and microbes, exchanged on anion exchange sites, or loss from the ecosystems via denitrification or leaching. Some microbes also absorb NO_3^- and reduce it to NH_4^+ through DNRA to NH_4^+. Gaseous losses of N from ecosystems course through major processes like NH_3 volatilization, nitrification, and denitrification. These processes release N as NH_3 gas, N_2O, NO, and N_2 and the gas fluxes are controlled by the rates of soil processes and by soil and environmental characteristics that regulate diffusion rates through soils. N is lost by leaching of DON from all ecosystems and as NO_3^- from NO_3^- rich ecosystems. NO_3^- leached from terrestrial ecosystems moves into groundwater to lakes and rivers, and is subsequently lost to the atmosphere through denitrification or transported to the ocean (Chapin et al. 2011).

Hypotheses

This thesis has two major experiments. The first study investigates the impact of four years of low additions of N, P, and N + P on gross rates of mineral N production (i.e. N mineralization and nitrification) and retention (microbial immobilization of NH_4^+ and NO_3^-, and DNRA) in three montane forest soils along a 3000-m elevation gradient in the Ecuadorian Andes (Fig. 2). The hypotheses for this experiment are:

（1) in control plots, gross rates of mineral N production are closely coupled with or equal to rates of microbial N retention, and soil N-cycling rates will decrease along the 3000-m elevation gradient