THE IMPACT OF FOREST CONVERSION TO OIL PALM PLANTATION ON THE INTERNAL NITROGEN CYCLE OF TROPICAL LOWLAND SOILS

By

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ABSTRACT

This thesis seeks to quantify the effect of land use change from tropical forest to oil palm plantation on nitrogen biogeochemical cycling in Sabah, Malaysia (Borneo). Nitrogen cycling process rates and indices were examined across four forests and six oil palm plantations during the inter-monsoon and end of wet season in 2010 and 2012 respectively. Firstly, the study establishes a baseline to assess the impact of land use change along a chronosequence of forest succession. Results indicate that forests follow a trajectory of nitrogen recovery and increased "openness" to nitrogen cycling through secondary forest development. Secondly, the spatial and temporal variation of nitrogen cycling within oil palm plantations is assessed. Results show that plantation management practices result in spatial variability in soil nitrogen. Examining process rates revealed an increasing trend of N_2O emission and decreasing trends of soil organic matter content as plantations matured. However, season and soil type also affected denitrification and N_2O emission. Finally, a replicated comparison of process rates in forests and plantations on riparian and *terra firme* soils revealed that plantation establishment significantly altered rates of nitrogen cycling and resulted in greater emissions of N_2O from *terra firme* plantations.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND RATIONALE

Nitrogen (N) is an essential element for all living organisms. In natural systems, the availability of N regulates key ecosystem functions including primary production and decomposition. However, anthropogenic activities such as fertiliser use, fossil fuel combustion and cultivation of N-fixing crops have more than doubled the amount of terrestrial N, with important consequence for global nitrogen cycling (Vitousek, et al., 1997; Galloway, et al., 1995). The effect of increased N inputs include an increase in atmospheric pollutants such as nitrous oxide (N_2O) and nitric oxide (NO) (Penner, et al., 1991; Kroeze, et al., 1999; Mosier, 1998). At the ecosystem level, increased N can reduce biodiversity and accelerate nitrate leaching causing effects such as eutrophication, hypoxia, groundwater pollution and acidification of water bodies (Aber, et al., 1995; Howarth, et al., 2000; Galloway, et al., 2003; Smith, et al., 1999). Conversely, alleviation of nitrogen limitation to primary production can increase carbon sequestration from CO_2 through increased plant growth or reductions in microbial respiration (Thomas, et al., 2011; Pregitzer, et al., 2008; Janssens, et al., 2010). Hence, in order to comprehend the changes occurring to ecosystem function and to predict future response to increased N inputs, it is necessary to have a thorough understanding of N cycling processes within terrestrial ecosystems.

In soils, microbial processes are largely responsible for nitrogen cycling. Therefore, the rate of microbial transformation regulates the form of N within the soil and determines whether it

is retained in, or lost from, the system. Processing rates and N storage vary considerably across land use and ecosystem type. For example, in temperate, industrialised parts of the globe, widespread fertilisation and fossil fuel combustion have caused enhanced N deposition pushing, largely nitrogen-limited, regions towards nitrogen saturation. The "nitrogen saturation model" is widely used to describe alterations to ecosystem function that occur where nitrogen availability exceeds plant and microbial demand (Aber, et al., 1989; Aber, et al., 1998). The model, which was developed for temperate soils, predicts that losses of soil nitrogen will only increase once plant and microbial N limitation have been alleviated. However, nitrogen availability is often greater in tropical systems that are more commonly limited by nutrients such as P and base cations (Kaspari, et al., 2008; Townsend, et al., 2011; Vitousek & Farrington, 1997; Walker & Syers, 1976). Accordingly, the ability of plants to assimilate additional N and increase carbon storage in tropical areas, as predicted by this model, is likely to be limited in a system where other nutrients restrict growth. The response of microbial respiration to increased nitrogen deposition is also uncertain, (Janssens, et al., 2010).

When, or whether, nitrogen saturation occurs is primarily dependent on the initial N status of the ecosystem and the rate of N input. Until recently, the temperate-centric approach to nitrogen saturation has been justified by the observation of nitrogen pollution issues primarily within developed countries. Recently however, tropical regions of the world have experienced dramatic population increase, rapid industrial development and large-scale land use change (Lambin, et al., 2001). The result of socio-economic change is an increase in deforestation, fertiliser use, and fossil fuel combustion with attendant increases in reactive nitrogen inputs and mobility that are already having an effect on regional N deposition (Hietz, et al., 2011). Furthermore, evidence that tropical soils have greater N availability and faster

rates of microbial cycling suggests that the environmental impact of an altered nitrogen cycle may be greater in the tropics than in temperate regions (Vitousek & Sanford, 1986; Matson & Vitousek, 1987; Keller & Reiners, 1994; Martinelli, et al., 1999; Lewis, et al., 1999; Matson, et al., 1999). However, information on soil N status and processing rates within the tropical region is sparse. For example, little is known of how these systems function in their natural state and even less of how they might respond to disturbances such as land use change or additional N deposition. Consequently, there is considerable uncertainty in, *inter alia,* estimates of N_2O emission or carbon sequestration response highlighting the need for greater understanding of process rates and the changes that occur following deforestation and agricultural development. This is exemplified by the rapid expansion of oil palm plantations over the last four decades in Southeast Asia. For example, Malaysia, the world's second largest producer of palm oil after Indonesia, has seen the area planted for oil palm expand from an estimated 0.15 million ha in 1970 to 4.4 million ha by 2012 (FAOSTAT, 2013a). The, already strong, global demand for palm oil is also projected to rise in future as factors driving production diversify from food to biofuel and energy supply, (FAO, 2011a). Increased demand is likely to be met by increased production through deforestation of lowland tropical forests in biodiverse areas such as Indonesia, Papua New Guinea, the Congo and Brazil, (Fitzherbert, et al., 2008; ICCT, 2012; Turner, et al., 2011). Yet, despite a year-on-year increase in the area planted and the prediction that expansion is set to continue at an accelerated rate, little has been published on the effect of plantation establishment on soil biogeochemistry. This thesis, therefore, is a timely study on the effect of land use change from forest to oil palm plantation agriculture on N biogeochemical cycling.

In summary, this thesis focuses on the effect of land use change on microbial nitrogen transformations in the tropical lowlands of Malaysian North Borneo because of: (1) the lack

of published data on soil nitrogen cycling both in the tropical region as a whole, and specifically, in Borneo; (2) the importance of tropical nitrogen cycling to global budgets of greenhouse gas emissions and nitrogen status to carbon sequestration; and (3) the uncertainty in predicting the response of nitrogen cycling and greenhouse gas emissions to land use change, particularly that of forest replacement with large-scale plantation agriculture.

1.2 RESEARCH GAPS AND OBJECTIVES

The general paucity of data on nitrogen biogeochemical cycling within the tropics, and particularly in Southeast Asia, represents a major research gap in current knowledge. The literature review in Chapter 2 also highlights the following issues deserving of further enquiry:

1. Primary production in tropical forests is frequently assumed to be limited by nutrients other than N. Whilst old-growth forests often display symptoms of N saturation, the same is not necessarily true of secondary forests where N export has occurred through removal of vegetation, soil erosion, leaching and volatilisation. Yet, it is secondary, rather than primary, forests that are increasingly being converted to agricultural use. Therefore, it is uncertain whether differences in nitrogen cycling such as losses of N though denitrification and N_2O emissions between primary forests and agricultural land uses will also be observed where secondary forests are substituted for primary.
2. Management routines within oil palm plantations are likely to lead to heterogeneous nutrient concentrations in line with practices that impact fertilisation and organic matter returns. These discrepancies in nutrient input may affect the spatial distribution

of processes such as nitrification, denitrification or N_2O emissions, and accordingly can better inform sampling strategies within the plantation environment.

3. A few studies have reported higher emissions of N_2O from oil palm plantations when compared to forests (Ishizuka, et al., 2005; Melling, et al., 2007); however, the effect of plantation age on nitrogen cycling and gaseous N emissions is uncertain.
4. The establishment of oil palm plantations represents a major land use change within the tropics that is predicted to grow at the expense of biodiverse lowland tropical forest, yet the impact of forest replacement with plantation agriculture on N process rates remains poorly constrained.

In light of the research gaps summarised above and developed in Chapter 2, this thesis aims to:

1. Determine whether secondary forests follow a trajectory of increased nitrogen availability with the time since disturbance, and if so, to establish whether mature secondary forests represent an appropriate baseline by which to gauge the effect of land use conversion on N process rates (Chapter 4).
2. Quantify the magnitude of spatial variability for soil properties indicative of nitrogen cycling within oil palm plantations using geostatistical analysis with a view to determining the biogeochemical processes responsible for the observed spatial heterogeneity. In addition, through an examination of the range of spatial autocorrelation for each parameter, the aim is to inform optimum sampling design within oil palm plantations by identifying the minimal distance over which samples should be collected for the variables measured (Chapter 5).

3. Examine nitrogen cycling indices in plantations through a chronosequence of stand age with a view to establishing whether nitrogen loss and retention processes (e.g. N_2O emissions, denitrification and nitrate ammonification) follow predicted patterns with time since plantation establishment. The effect of wet (post monsoon) and dry (inter-monsoon) conditions is also examined with a view to determining the significance of seasonal differences that can potentially inform nitrogen cycling models (Chapter 6).
4. Compare nitrogen status and process rates in tropical forests with oil palm plantations in order to evaluate the impact of land cover change with respect to deforestation and agricultural development on N cycling (Chapter 7).

1.3 THESIS STRUCTURE

The aims of this thesis set out above frame the structure of the chapters that follow as illustrated in Figure 1-1. Following this introduction, Chapter 2 reviews the literature on nitrogen cycling in soils and identifies current research gaps. The rationale for site selection and a description of the field and analytical methods employed are described in Chapter 3. Data are presented in Chapters 4 to 7. Chapter 4 characterises the nature of the secondary forests sampled with the objective of establishing a baseline for soil nutrient and nitrogen transformation status. Chapter 5 examines spatial variation of soil variables within one plantation using geostatistical methods to map nutrient concentrations in line with management practices. Results of temporal variability in denitrification, dissimilatory nitrate reduction to ammonia (DNRA) and N_2O emissions over two seasons (wet and dry) through a chronosequence of plantation age are presented in Chapter 6. Finally, Chapter 7 draws on the conclusions of Chapters 4 to 6 to compare representative secondary forests and oil palm

plantations and their rates of nitrogen transformation with a view to addressing the principal aim, namely the effect of plantation establishment on N transformation process rates.

Finally, Chapter 8 provides a synthesis of the key findings of the research, which is then used to identify directions of future research.

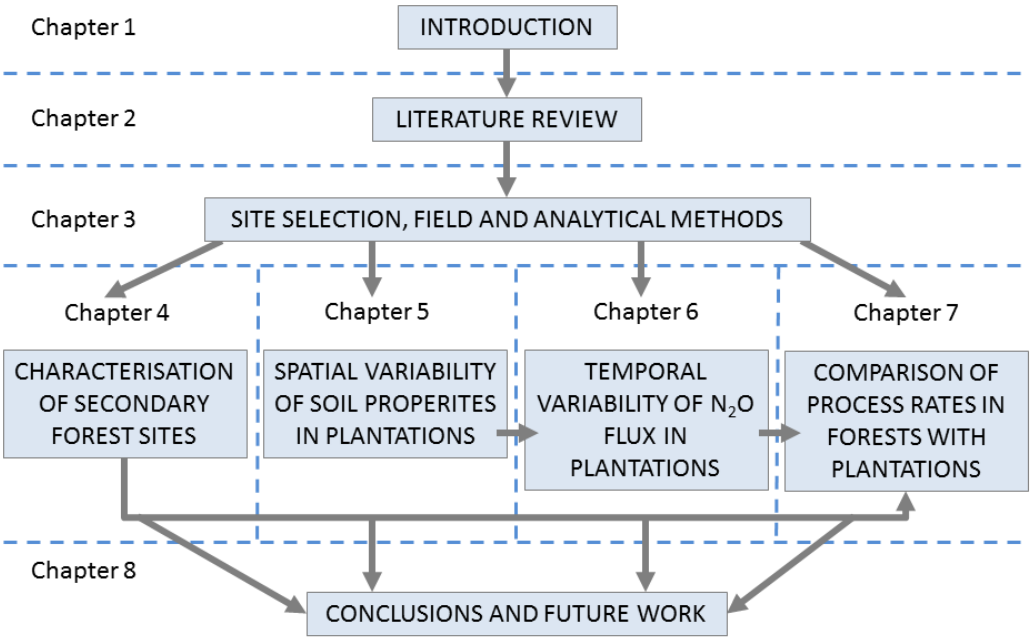


Figure 1-1: Schematic representation of the structure of the chapters presented in this thesis.

CHAPTER 2: REVIEW OF NITROGEN CYCLING IN LOWLAND, WET TROPICAL SOILS

2.1 INTRODUCTION

This chapter provides a review of current knowledge of nitrogen cycling within the lowland wet tropics. The review is organised in three parts. First, a summary of the characteristics of tropical nitrogen cycling are outlined that identify a need to study this region separately to temperate regions. Secondly, nitrogen (N) input, cycling, loss and retention pathways are reviewed with particular emphasis on tropical soils. Given the focus of this thesis on the conversion of tropical forest to oil palm plantations, we also discuss the impact on N cycling of land use change primarily for agricultural purposes. Finally, the review is summarised and aspects of tropical N cycling deserving further research are highlighted.

Due to the enormous variability in precipitation, vegetation and soil nutrient status that "the tropics" encompass, this review concentrates primarily on the lowland wet tropical zone (as defined by Vitousek & Sanford (1986)); namely, those areas between the tropics of Capricorn and Cancer (23° 28′ N and S) where precipitation is >1500mm p.a., elevations are <100 m a.s.l., monthly temperatures never fall below 18°C and monthly rainfall >100mm. Essentially the purpose of this definition is to exclude montane and dry, or seasonally-dry, tropical environments, which may function differently to the lowland wet tropics. The limited amount of research on soil N cycling in the tropical zone means it has been necessary to reference some montane, sub-tropical and temperate studies and, where appropriate, attention is drawn to the fact that these examples are conducted in environments that fall outside this definition of the lowland humid tropics.

2.2 NITROGEN CYCLING IN TROPICAL SOILS

In 1950, the renowned soil scientist Hans Jenny published the results of a study contrasting the N and C content of soils in Columbia with those in California's Sierra Nevada (Jenny, 1950). It would be the first of many publications to contemplate the reasons for N richness of some tropical soils when compared to those in more temperate climates (Jenny, 1950). On first examination, N richness appears to be in keeping with the abundance and biodiversity of tropical rainforest fauna. Yet the productivity associated with high biological activity and biomass accumulation belies the inherent infertility of most tropical soils. Intensive weathering results in a shallow, or near absent, humic layer and depletion of essential plant nutrients such as phosphorus (P), calcium (Ca) and magnesium (Mg). Conversely, these often-acid soils contain high levels of aluminium (Al) and iron (Fe), both of which directly affect plant growth or interfere with the availability of nutrients such as P.

For the most part, tropical forests are able to maintain productivity through nutrient conservation (Vitousek & Sanford, 1986; Hamdan & Burnham, 1996). Contrary to this, N appears to operate as a nutrient in excess of plant demand and, as such, large losses are often observed from old-growth forests already at the point of N saturation (Houlton, et al., 2006). When forests are cleared for agricultural development, the export of logs, burning of scrub and soil erosion liberates N stored within the soil. Post clearing, tropical soils can be N deficient and require fertilisation to maintain crop productivity over the long term such that rapid depletion of soil nutrients has led historically to small-scale swidden agricultural practices primarily of a subsistence nature. More recently, large industrial plantations employing modern N fertilisation practices have begun to dominate these regions. The result is that alterations to soil nitrogen cycling through large-scale deforestation and agricultural

expansion may have important consequences not only at the regional scale but also globally (Downing, et al., 1999; Pyle, et al., 2011; Bai, et al., 2012).

Lowland wet tropical zones are characteristically aseasonal. Daily temperatures rarely fluctuate outside 25-35°C and rainfall often exceeds 2500mm y^{-1}. Consequently, climatic constraints to plant and microbial growth are minimal with the result that biogeochemical cycling of N is perennial in the absence of alternate environmental or chemical limitation (Jordan, 1985). As such, the paradigm that tropical soils generally cycle more N at faster rates than their temperate equivalents is evidenced by (*inter alia*) high rates of N fixation (Cleveland, et al., 1999; Cusack, et al., 2009; Reed, et al., 2011); rapid mineralisation and nitrification (Arnold, et al., 2009; Corre, et al., 2010); high leaf N:P ratios (Vitousek, 1984; McGroddy, et al., 2004; Reich & Oleksyn, 2004; Townsend, et al., 2007); and enriched $\delta^{15}N$ signatures (Martinelli, et al., 1999; Nardoto, et al., 2008). Losses of N from wet humid soils through trace gas emission (Matson & Vitousek, 1990; Yienger & Levy II, 1995; Mosier, et al., 2004; Houlton, et al., 2006; Werner, et al., 2007) and leaching (Lewis, et al., 1999; Hedin, et al., 2003; Fang, et al., 2009; Brookshire, et al., 2012) also appear greater than those generally found in temperate climates.

The high potential for N loss places the tropical zone in a position of primary importance to global N budgets with important consequences for ecosystem health (Bai, et al., 2012; Downing, et al., 1999; Galloway, et al., 2008; Vitousek, et al., 1997). Under the saturation model developed for N-deficient temperate soils, nitrate mobility, and therefore N loss, follows alleviation of limitation to biological activity as N availability increases over time (Aber, et al., 1989; Aber, et al., 1998). In temperate soils, a relatively closed cycle with efficient nutrient return means there is likely to be biological capacity to assimilate additional N. However, saturation may already be a feature of tropical forests where N is more abundant

and N cycling more open (Hall & Matson, 1999; Brookshire, et al., 2012). Therefore, the capacity to absorb N may be limited, resulting in large and immediate losses as anthropogenic inputs increase (Lohse & Matson, 2005).

In the absence of artificial fertilisation, N inputs occur through biological fixation and wet and dry deposition following abiotic atmospheric fixation. Once within the soil, N is only removed through gaseous emissions from processes such as denitrification, ammonia oxidation and volatilisation or is hydrologically removed through leaching as NO_3^- or dissolved organic nitrogen (DON). Following fixation, nitrogen is transferred between soil N pools through largely biotic transformation via processes such as assimilation, mineralisation, nitrification, and dissimilatory nitrate ammonification (DNRA) (Figure 2-2). The "openness" of the N cycle is determined from the magnitude of losses and gains relative to the total N pool. In a closed cycle, N is efficiently retained within the terrestrial system through tight coupling between soil and vegetation resulting in inputs and losses that are small relative to the total soil turnover (Figure 2-1). Conversely, in an open N cycle, inputs and losses make up a large proportion of total N turnover. Nitrification in particular plays an important role in the openness of nitrogen cycling, as it impacts both gaseous and hydrological loss. Nitrification produces NO and N_2O as a by-product of NO_3^- production although NO_3^- can also be converted to N_2 (and N_2O and NO as intermediates) through the process of denitrification. Denitrification may be a significant loss pathway in N-rich wet tropical soils which are potentially a major source of global N_2O (Matson & Vitousek, 1990; IPCC Report, 2007) and NO (Davidson & Kingerlee, 1997; Yienger & Levy II, 1995). N_2O, an important greenhouse

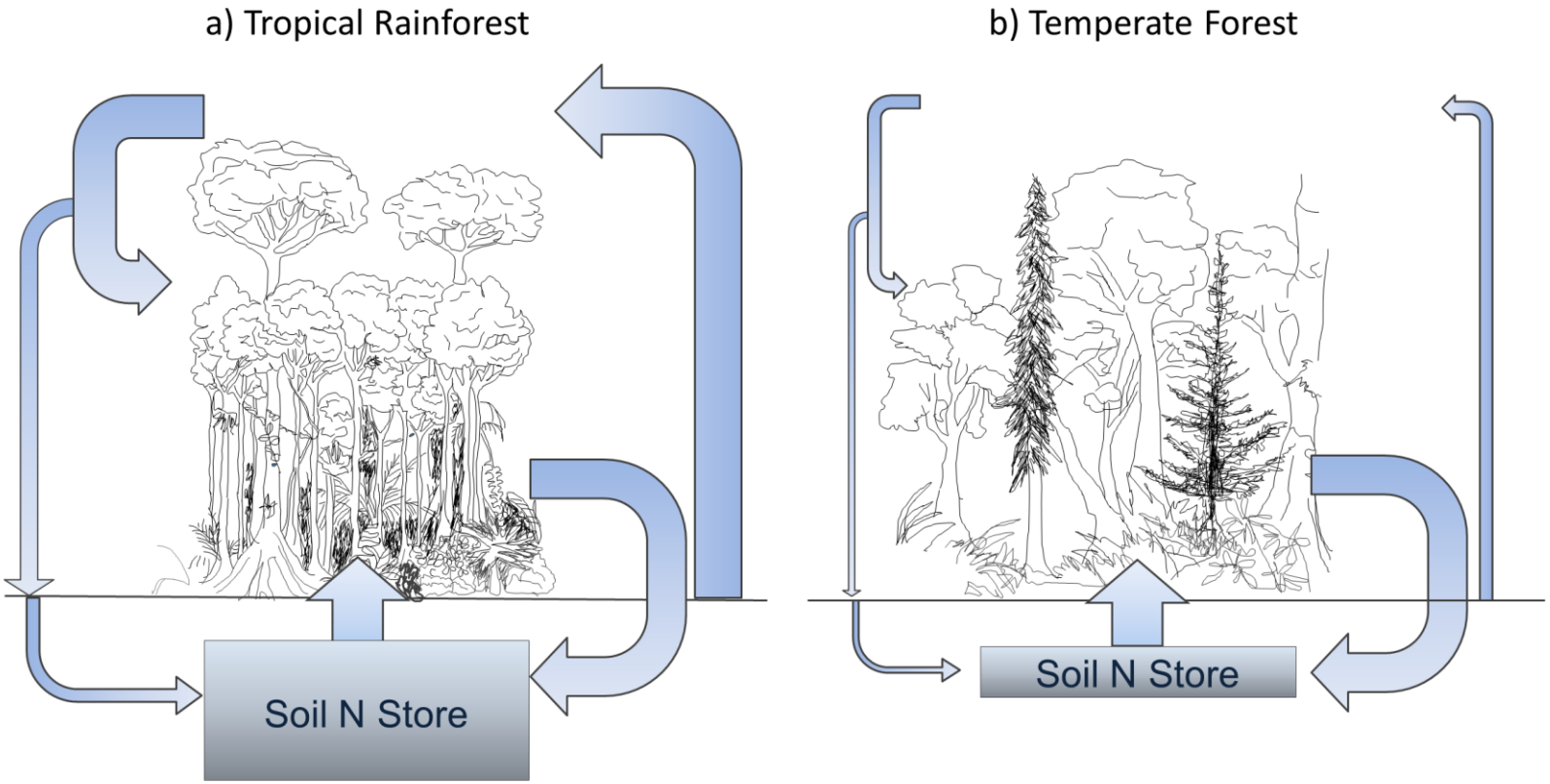


Figure 2-1: Representation of a) an “open” nitrogen cycle in a tropical forest ecosystem; and b) a “closed” nitrogen cycle in a temperate forest ecosystem. The arrows represent inputs, outputs and internal soil cycling and are approximately proportionate to the magnitude of process rates.

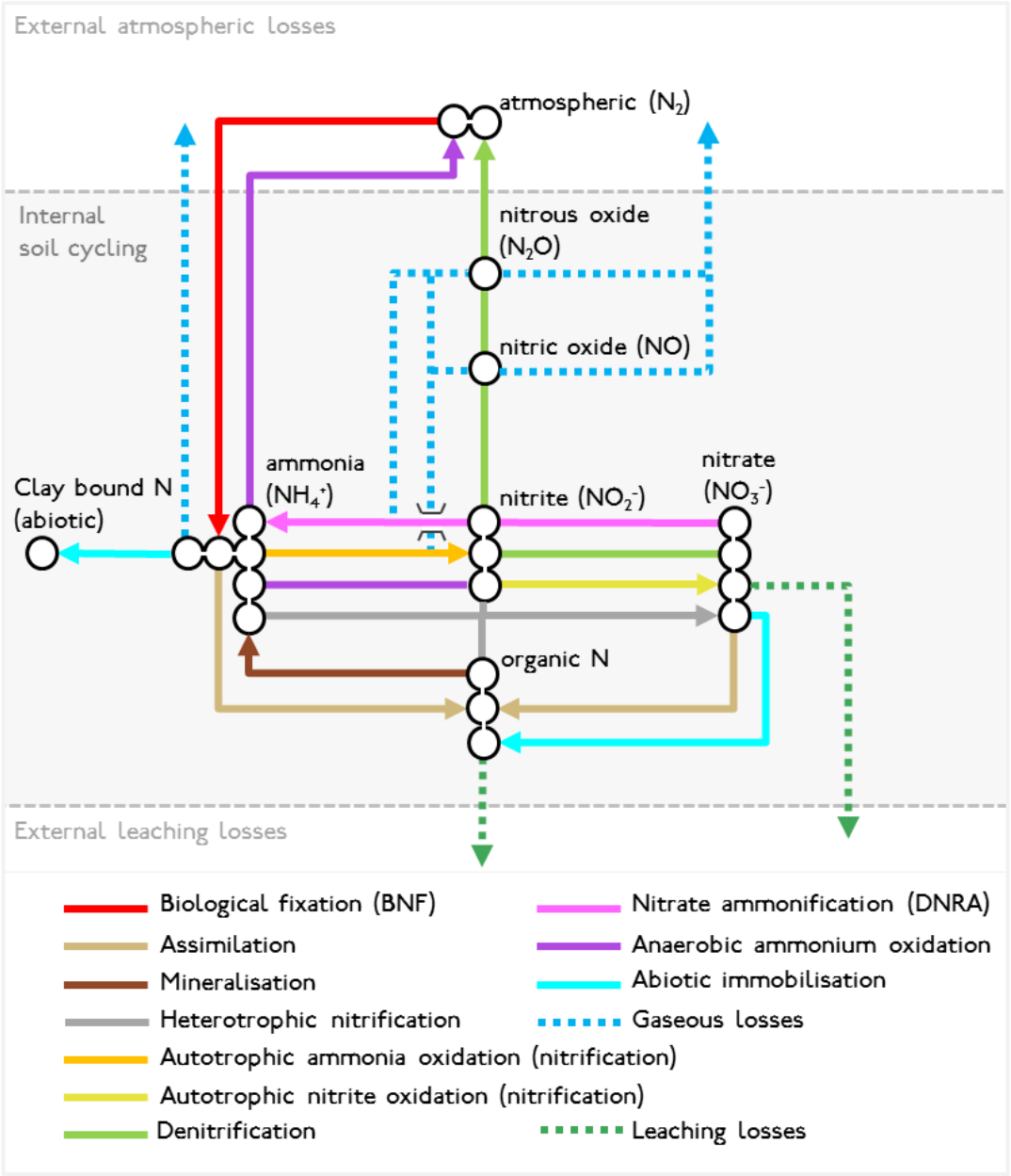


Figure 2-2: Simplified model of the nitrogen cycle showing the main soil processes (solid lines) and external losses (broken lines).

gas, depletes more stratospheric ozone per unit mass than any other emission arising from human activity, (Ravishankara, et al., 2009). Meanwhile NO is responsible for the creation of ground-level ozone through photochemical reactions in the troposphere with implications for human health and plant productivity (Reich & Amundson, 1985; Finlayson-Pitts & Pitts Jr., 1997). The mobility of the nitrate ion in solution also results in a high potential for leaching losses.

The environmental consequences of NO_3^- leaching are well documented and include, degradation of soil fertility through depletion of base cations, acidification and mobilisation of aluminium, (Likens, et al., 1996; Fenn, et al., 1998); changes to biodiversity (Gilliam, 2006; Lu, et al., 2010); eutrophication and acidification of waterbodies (Ryther & Dunstan, 1971; Smith, et al., 1999; Camargo & Alonso, 2005); and contamination of drinking water (Bouchard, et al., 1992; Goodrich, et al., 1991). The environmental impact of N loss resulting from rapid microbial turnover is likely therefore to be substantial in tropical regions where soils are already naturally rich in N. Whilst there is growing recognition of the importance of the tropics to global biogeochemical cycles, our understanding of nitrogen cycling in this region is still relatively limited as evidenced by the zone's under-representation in the literature. However, population increase and agricultural and industrial expansion highlight the need to understand the combined effect of land use change and increased N inputs on N cycling processes and loss pathways. For example, fertiliser use in Indonesia and Malaysia alone increased by 70% between 2002 and 2010: and these two countries currently account for 43% of Southeast Asian fertiliser consumption[1] (FAOSTAT, 2013a). Furthermore, Southeast Asia is expected to be responsible for 68% of the increase in global fertiliser N

[1] Countries included in the statistics for Southeast Asia fertiliser consumption include Brunei Darussalam, Cambodia, Indonesia, Malaysia, Myanmar, the Philippines, Thailand and Vietnam.

demand between 2011 and 2015 (FAO, 2011). It is foreseeable therefore, that N saturation will increase as industrialisation proceeds rapidly in a part of the world that, until recently, lagged behind temperate regions where intensive farming and industrial enterprise have tracked development. Yet the ability of plants and microbes to assimilate additional N, as predicted by the nitrogen saturation model, is most likely limited in a system where other nutrients (notably P and base cations) are likely to restrict growth (Kaspari, et al., 2008; Townsend, et al., 2011). Furthermore, the mechanisms of N retention in tropical soils may differ and there is evidence that processes such as abiotic retention and DNRA, are relatively more important in highly weathered, anoxic clay soils (Silver, et al., 2001; Davidson, et al., 2003; Lohse & Matson, 2005; Templer, et al., 2008). In addition, new pathways of nitrogen cycling are being still being discovered. Most important for tropical nitrogen cycling, are the processes of aerobic ammonium oxidation coupled to iron reduction (feammox) (Yang, et al., 2012) and DNRA (Silver, et al., 2001; Pett-Ridge, et al., 2006; Templer, et al., 2008). Consequently, the temperate model of nitrogen saturation where increased N losses only occur after decades of elevated inputs may require significant revision when considering N export from tropical forests (Aber, et al., 1989; Aber, et al., 1998).

2.3 SOIL MICROBIAL TRANSFORMATIONS

2.3.1 Fixation

Rates of nitrogen fixation were not measured in the sites sampled for this thesis. However, as biological nitrogen fixation (BNF) is the primary mechanism by which new nitrogen is conveyed to natural systems, the process warrants consideration in this review. Nitrogen fixation describes the conversion of inert N_2 gas to bio-available N (Figure 2-2). Fixation occurs largely through biotic processes, although abiotic fixation also arises during lightning

strike when N_2 combines with oxygen to form NO and NO_2 which subsequently precipitates as HNO_3 (nitric acid). Approximately 10% of global reactive nitrogen (circa 12 Tg N y^{-1}) reaches the soil in this way (Price, et al., 1997). By contrast, BNF is estimated to contribute somewhere between 44 to 128 Tg N y^{-1} to the global terrestrial input (Galloway, et al., 2004; Vitousek, et al., 2013). In agricultural systems, BNF is either replaced by the application of artificial fertilisers or exploited through planting of N-fixing crops.

BNF breaks the triple bond of the N_2 atom and combines N with H_2 to form ammonia (NH_3). A wide range of phylogenetically diverse symbiotic and free-living bacteria, cyanobacteria and Achaea are able to fix atmospheric N, although legume-rhizobial symbiosis is perhaps the best-studied BNF mechanism. For the most part, symbiotic nitrogen fixing woody species are absent from late-successional temperate forests and rates of free-living fixation are low. As such, many climax communities in Europe and North America show signs of N limitation in the absence of anthropogenic inputs. However, tropical lowland forests (at least in South America and Africa) are often abundant in canopy legumes (Crews, 1999; Sprent, 2009; Raes, et al., 2013). Southeast Asian forests by contrast are dominated by Dipterocarpaceae (Slik, et al., 2003). With the exception of BNF in flooded rice fields (not considered in this review), our knowledge of the role that free-living N-fixers play at the ecosystem level is still relatively limited. However, the few estimates of free-living fixation that have been conducted within the humid tropics suggest that rates of N fixation through this mechanism may also be significant (Cusack, et al., 2009; Cleveland, et al., 2010; Reed, et al., 2011). Consequently, high rates of N fixation are commonly surmised as the mechanism for N richness observed in many old-growth tropical forests.

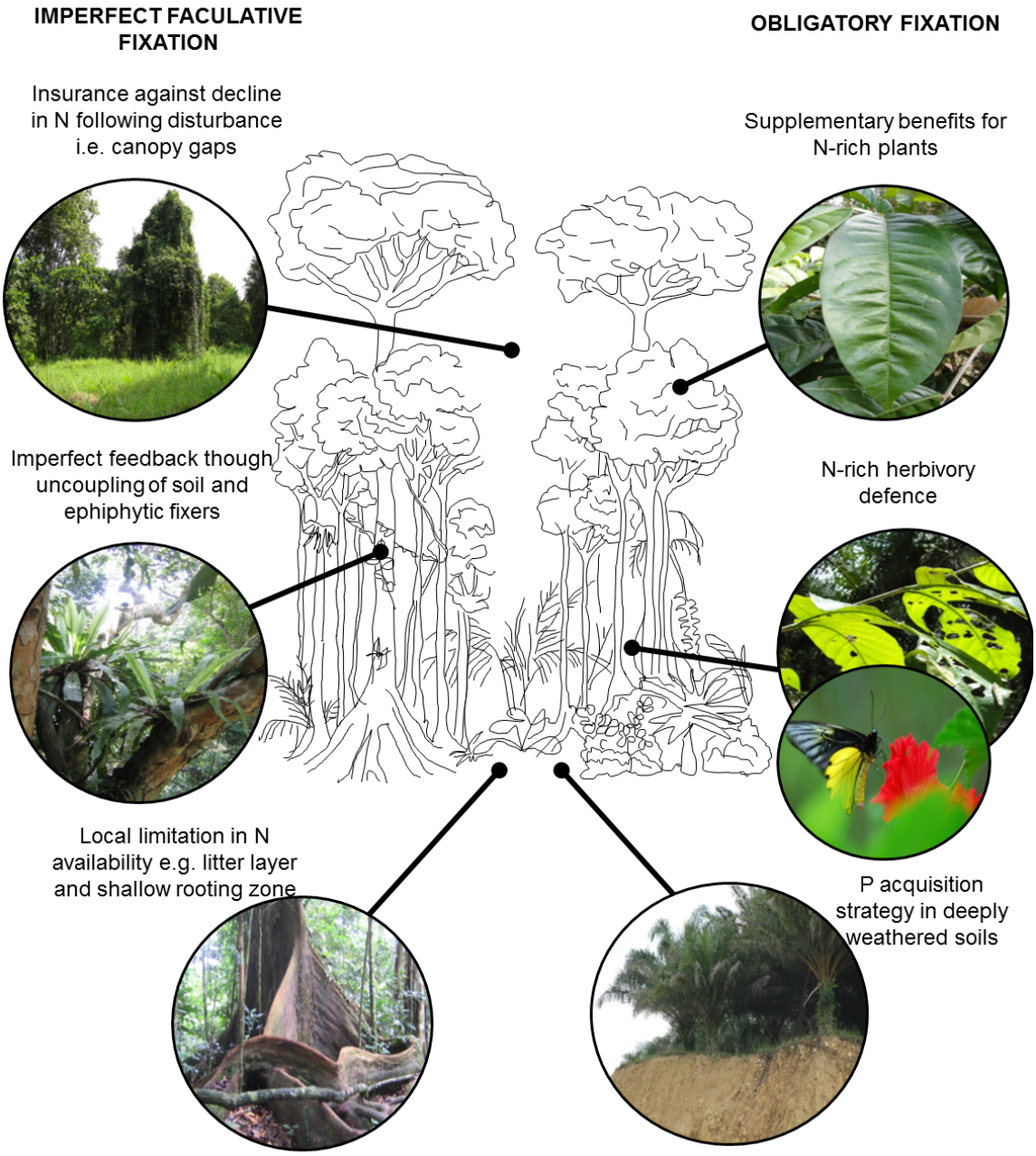


Figure 2-3: Summary of hypotheses in resolution of the "nitrogen paradox" in tropical soils.

The supposition that N fixation is responsible for N richness presupposes that fixers continue to fix even where N is abundant within the wider ecosystem. BNF is an inherently costly activity and as such, obligate continuation is inconsistent with both theoretical expectation and physiological observations of the facultative nature of the process. Accordingly, resolution of what has been termed the "nitrogen paradox" in tropical soils has sparked much recent debate (Vitousek, et al., 2002; Houlton, et al., 2008; Hedin, et al., 2009; Reed, et al., 2011) and a number of explanations have been offered to resolve this apparent incongruity. Explanations largely fall into two categories, those that propose a form of obligatory fixation to acquire supplementary benefits, or those that refer to imperfect facultative fixation (Figure 2-3).

McKey (1994) proposed that leguminous plants, which have a higher N status than non-legumes, acquire supplementary benefits from over-fixation in N-rich tropical soils. Similarly, N fixation may also be advantageous to plants that require additional N for N-rich herbivory defence mechanisms (Menge, et al., 2008). However, whilst over-fixation may occur in some soils (Pons, et al., 2007; Menge & Hedin, 2009), most field evidence points to facultative fixation of both symbiotic and free-living fixers (Crews, et al., 2000; Pons, et al., 2007; Barron, et al., 2009; Barron, et al., 2011). Enriched $\delta^{15}N$ isotopic signatures further confirm field observations that only a small number of legumes present appear to actively fix (Yoneyama, et al., 1993; Sprent, et al., 1996; Martinelli, et al., 1999; Ometto, et al., 2006; Nardoto, et al., 2013).

The large diversity of symbiotic and free living fixers with the capacity for over-fixation in tropical soils may also offer an alternative explanation based on the insurance hypothesis of Yachi & Loreau (1999). This hypothesis proposes that declines in ecosystem function caused by environmental change are stabilised by microbial biodiversity (Yachi & Loreau,

1999). Thus, the ability to activate N fixation in response to N limitation following disturbance may ensure competitive advantage for individual species whilst at the same time decreasing ecosystem (i.e. N status) variability. Studies in disturbed soils support this mechanism of facultative fixation. For example, in Panama nodulation in canopy gaps associated with low N availability and/or increased N demand from recovering vegetation was six times higher than in the surrounding mature forest matrix suggesting an ability to respond to disturbance with increased fixation (Barron, et al., 2011). Furthermore, the abundance (though not diversity) of N fixing trees persisted at similar concentrations over a chronosequence of 300 years of forest development, however active fixation declined as demand for nitrogen decreased (Batterman, et al., 2013).

Another explanation for the high N status of tropical forests proposes that spatial variability in N availability may result in regions of high fixation despite overall N-richness. This suggests that where N availability is low, or C:N ratios are high, the demand for N at a local scale could keep BNF at an over-fixation level in the wider ecosystem. Hedin et al. (2009) highlight the significance of vertical layering within tropical forests where persistent organic inputs to the litter layer and shallow rooting zone maintain N exigency. Under such conditions, heterotrophic bacteria may continue to fix even where N is non-limiting in deeper soil horizons. Furthermore, epiphytic fixers, which are numerous in tropical forests, are disconnected from the soil matrix and therefore unable to access freely available N. This may result in an imperfect feedback mechanism where facultative down-regulation is blocked by an uncoupling of soil and canopy processes (Bentley, 1987; Cusack, et al., 2009).

Houlton et al. (2008) propose a somewhat controversial third explanation: that there is an advantage in maintaining N fixation because it increases competitive advantage for P. Specifically, extracellular phosphatases responsible for the breakdown and availability of P

are N-rich (circa. 15%-N) metabolites. Therefore, in P-limited environments, such as lowland tropical zones, N richness may be employed as a P acquisition strategy. Although soil phosphatase activity is observed to be higher under leguminous plants (Keller, et al., 2013), N_2 fixation does not necessarily translate to greater investment in extracellular phosphatase and alleviation of P limitation (Batterman, et al., 2013). Our understanding of the exact mechanisms for N richness within lowland tropical environments is at present incomplete. For the most part, evidence points to the facultative nature of fixation in tropical forests. However, a large diversity of fixers with capacity for over-fixation may insure against ecosystem decline in an environment characterised by an open cycle where nitrogen losses can be rapid following disturbance. When activities such as logging and burning result in the export of N through biomass removal, leaching, erosion and volatilisation, the capacity for BNF to supply new N to the soil is instrumental to forest recovery post-disturbance (Batterman, et al., 2013; Barron, et al., 2011). In Panama, nitrogen fixing trees played a fundamental role in nitrogen accumulation over a relatively short time-scale with maximum rates of fixation being observed in 12 year old stands (Batterman, et al., 2013). However, decreased N demand, in parallel with a slowdown in biomass accumulation, decreased rates of fixation between 12 – 80 years to the point where 300 year old stands had similar rates of fixation to old growth forests. These observations raise an important point with respect to the secondary forests sampled for this study. Specifically, N limitation in early successional or highly disturbed forests is likely to result in high rates of nitrogen accumulation and conservation illustrative of a closed N cycle. Whereas late-successional forests should have greater soil and plant nitrogen, and greater N losses indicative of a more open N cycle similar to that observed in old-growth tropical forests. Therefore, prior to determining the effect of forest conversion to alterative land uses, it is important to establish whether the secondary

forests sampled for this thesis fall into the former category of a conservative and closed N cycle, or the latter category of open and leaky N cycle.

2.3.2 Mineralisation

The process of decomposing organically bound nitrogen (e.g. amino sugars, proteins, nucleic acids, and urea) to NH_3 and NH_4^+ is known as N mineralisation. Due to the biological nature of decomposition, temperature and moisture strongly influence the rate at which mineralising bacteria, fungi and protozoa operate. Whilst climatic factors are the primary regulators of NH_3 production, the quantity and quality of the decomposing substrate, soil fauna, soil aeration and texture, C:N ratios, and pH are also important (Curtin, et al., 1998; Leiros, et al., 1999; Gonzalez & Seastedt, 2001; Booth, et al., 2005). NH_3 and NH_4^+ produced by mineralisation are made available for assimilation by plants or microbes but can also be released back to the atmosphere (through volatilisation), abiotically fixed within the soil matrix, or transformed via alternate microbial pathways such as autotrophic nitrification or anaerobic ammonium oxidation (Figure 2-2). Therefore, low soil NH_4^+ concentrations do not necessarily reflect low rates of mineralisation if immobilisation and consumption processes are substantial. Where the net mineralisation rate is determined by measuring NH_4^+ accumulation after consumption, it often seriously underestimates the total amount of mineralisation taking place (i.e. the gross mineralisation rate) (Davidson, et al., 1990; Hart, et al., 1994; Neill, et al., 1999). In recent years, the limitation of net mineralisation as an estimate of N turnover has led to a shift towards measurements of gross mineralisation in the literature; the method most commonly employed being the isotope pool dilution technique (Kirkham & Bartholomew, 1954). For gross mineralisation, this approach involves enriching the soil with $^{15}NH_4^+$ and then observing dilution of the atomic % enrichment over time as ^{14}N is mineralised and added to the total NH_4^+ pool. Gross consumption of NH_4^+ is also estimated

Table 2-1: Summary of gross mineralisation and nitrification rates in tropical soils.

Location	Land Cover	Elevation (m)	Temp (°C)	Rainfall (mm y^{-1})	Min (g m^{-2} d^{-1})	Nit (g m^{-2} d^{-1})	C (g kg^{-1})	N (g kg^{-1})	C:N	Source
Panama[1]	a. Old-growth lowland forest	< 61	27.5	2650	0.89-1.11	7-26	51	3.8	13.3	Corre et al (2010)
	b. Montane forest	1200-1300	20.0	5532	0.57	2	73	5	14.5	
Ecuador[1]	a. Old-growth lowland forest	300	23	3467	1.15	Below	141	9.1	15.7	Arnold et al (2009)
	b. Montane forest	1500	18	3485	~0.72	detection	76	5.3	14.4	
Australia‡	a. Old-growth lowland forest	80	24.3	4395	n.d.	2.8-3.6	31.1	n.d.	12.1	Kiese et al (2008)
	b. Montane forest	790	20.9	1594		4.8-10.3	29.3-35.1		14.6	
Puerto Rico	Lower montane forest	n.d.	19	4500	0.54-1.69	0.32-1.13	53-92	2.7-3.8	19.6-24.5	Templer et al (2008)
Brazil[1]	Old-growth forest	n.d.	25.7	2272	3.5-8.34	0.98-2.28	20-36	1.3-2.5	15.5-14.0	Doff Sotta et al (2008)
Australia	a. Subtropical forest	428	11.2-25.6	816	0.74†	6.6†	89	0.75	11.8	Burton et al (2007)
	b. Hoop pine plantation				0.62-1.78†	2.1-2.4†	58-71	0.42-0.47	12.6-13.7	
Indonesia[1]	a. Lower montane forest	700-1100	21.0	1590	1.6-3.08	Below detection	32-59	3.2-3.8	10.0-15.5	Corre et al (2006)
	b. Corn agriculture				3.5-4		25-49	2.5-4	10.0-11.8	
	c. Agroforestry				1.3-3.5		15-32	1.2-3.1	10.3-12.5	
Costa Rica	a. Old growth lowland forest	40	25.8	~4000	4.5	2.9	22	5.2-5.3	6.9-7.3	Silver et al (2005)
	b. Forestry plantations				3.4	3.8	23	3.2	9.1-12.3	
Hawaii	a. Montane forest	850-900	20	1500	1.8	0.2-0.8	91.6	6.9	13.25	Mack and D'Antonio (2003)
	b. Forest + Grasses				0.9	0.2-0.8	102.1	6.1	16.86	
	c. Grasses				2.56	0.3-0.9	89.6	6.4	13.92	
Puerto Rico	a. Lower montane forest	Lowland	19.1	4000	4.8†	0.57†	68	n.d.	n.d.	Silver et al (2001)
	b. Palm forest	Lowland	19.5	4000	8.2†	0.59†	109			
	c. Cloud forest	>750	18.6	4000	9.6†	0.63†	139			
Brazil[1]	a. Logged forest	n.d.	25.6	2200	2.2	1.5	160	13	12.4	Neill et al (1999)
	b. Pasture				0.3-2.7	0.01-1.40	160–197	11-14	13.9-15.3	
Hawaii[2]	a. Eucalyptus plantation	450-550	21	4600	10-14†	0-1.5†	126	10	13-15	Garcia-Moniel & Binkley (1998)
	b. Albizia plantation				12-16†	0.25-0.75†			12	

[1]Soil sampled to 0-5 cm depth. [2] Soil sampled to 0-20 cm depth. All other soils were sampled between 0-10 cm depth. †Units given in mg N kg^{-1} d^{-1}. ‡BaPS technique employed.

by observing the change in the size of the NH_4^+ pool over time. The number of studies recording rates of gross mineralisation is still relatively few when compared with estimates of net mineralisation, and Table 2-1 summarises those publications reporting gross rates within the lowland tropics. Some montane (i.e. >1000m a.s.l.) sites are also included where rates at higher elevations are compared with lowland N transformations. For the most part, researchers have used the isotope pool dilution technique to derive process rate estimates. However, the barometric process separation (BaPS) technique has also been used to estimate gross nitrification rates in Australia (Breuer, et al., 2002; Kiese, et al., 2002; Kiese, et al., 2008). Although experimental difference such as incubation time, sampling depth and preservation make comparison difficult, some broad generalisations are possible: Firstly, rates of mineralisation in old-growth forests are highly variable with seasonal averages ranging from 0.3 to 8.34 g N m^{-2} d^{-1} in Brazilian Oxisols (Neill, et al., 1999; Doff Sotta, et al., 2008). Rates in Hawaiian leguminous tree plantations were even higher (up to 16 mg N kg^{-1} d^{-1}), although no figures for bulk density are available to convert these rates to an aerial basis (Garcia-Montiel & Binkley, 1998). Temporal variability in N mineralisation within old-growth tropical forests is likely to be less variable than in temperate climax communities, where low temperatures restrict microbial activity during the winter months. The strong control that temperature has over mineralisation is illustrated by the fact that rates are often, but not always, inversely related to altitude (Arnold, et al., 2009; Corre, et al., 2010). Decomposition usually increases with precipitation, however, very high rainfall can depress microbial activity and restrict oxygen diffusion into soils having a negative effect on rates of mineralisation (Holtgrieve, et al., 2006).

Some lowland forests experience “hot moments” of mineralisation activity when land is cleared and burned prior to agricultural use or when soils are wetted following a period of

drought (McClain, et al., 2003; Kiese, et al., 2008). Generally, the latter event occurs in environments that have a pronounced dry season although, even within the wet tropics, extremely high rates of mineralising activity may result with the onset of rain following periods of drier weather (Luizão, et al., 1992; Davidson, et al., 1993; Wong & Nortcliff, 1995; Eaton, et al., 2011; Luizão, et al., 1989). Temporal variability to mineralisation rates has been reported during land preparation for oil palm re-planting. Specifically, rates of net mineralisation increased following cutting, chipping and windrowing of mature palms into plantation soils prior to replanting (Khalid, et al., 1999). Spatial variability within oil palm plantations may also occur through, for example, the control of undergrowth vegetation close to the palm trunk where root density is greatest, or re-incorporation of empty fruit bunches and pruned palm fronds in plantation inter-rows. Specifically, rates of net mineralisation in a mature Amazonian plantation increased with distance from the palm trunk, which the authors attributed to increased microbial activity, the presence of undergrowth vegetation and decreased bulk density in the plantation inter-rows (Schroth, et al., 2000). High root density within a 1m radius surrounding the palm trunk also resulted in decreased soil NO_3^- concentrations and NO_3^- leaching relative to plantation inter-rows, thereby highlighting the effect of management practices on the spatial variability of nitrate (Schroth, et al., 2000). In Costa Rica, Matson et al. (1987) report an approximate 3-4 fold increase in gross mineralisation rates immediately after forest clearance and burning, however, rates returned to pre-disturbance levels within six months. Commercial forestry plantations have been responsible for an increase in gross mineralisation in sub-tropical Australian hoop pine plantations (Burton, et al., 2007), and a decrease in Costa Rican *Cordia alliodora* (Spanish elm) plantations (Silver, et al., 2005). The increased rates in hoop pine were attributed to recent disturbance and higher soil temperatures in the plantations relative to forests (Burton,

et al., 2007), whereas mineralisation rates declined in the Spanish elm plantations concurrent with a decline in microbial biomass (Silver, et al., 2005). Where pastures are established, any initial increase in mineralisation rates after forest clearance is often followed by a decline in mineralisation to rates below those of the original forest as pastures age and organic matter returns decline (Reiners, et al., 1994; Neill, et al., 1999). Conversely, replacement of montane forest with N-rich cattle pasture grass in Ecuador increased gross mineralisation rates concurrent with an improvement in litter quality and an increase in the number of fungi and gram-negative bacteria (Potthast, et al., 2012). The magnitude of change in mineralisation rates following land-use change is likely, therefore, to depend on the change to climatic factors such as increased temperatures or higher soil moisture through canopy and root biomass removal, the initial soil N status, and the nature of disruption to soil microbial populations and C and N storage. These parameters in turn are affected by alterations to soil physical properties such as compaction, aeration, and disturbance level and frequency.

2.3.3 Nitrification

Nitrification refers to the biological conversion of NH_3 or NH_4^+ to NO_2^- (nitrite) and NO_3^- (nitrate). Some heterotrophic bacteria and members of the Achaea can nitrify (Brochier-Armanet, et al., 2008; Spang, et al., 2010), although it is largely attributed to chemolitho-autotrophic bacteria in the presence of O_2 using inorganic N as the energy source for CO_2 fixation and growth. Nitrification is a major regulator of soil N export and its product, NO_3^-, is the primary inorganic N loss from soils either through denitrification or leaching.

Autotrophic nitrification is a two-step process: The first step is carried out by ammonia oxidising bacteria such as *Nitrosomonas* or *Nitrosospira* that convert ammonia to nitrite via the following pathway:

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$$

In the second step, nitrite oxidising bacteria such as *Nitrobacter* convert nitrite to nitrate through the reaction:

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

Heterotrophic nitrification, by contrast, refers to the oxidation of reduced forms of N (including organic N) and is performed by various fungi, actinomycetes and bacteria. Heterotrophic nitrification is generally considered secondary to autotrophic nitrification but can be important in some temperate (Brierley & Wood, 2001; Islam, et al., 2007; Trap, et al., 2009) and subtropical (Zhu, et al., 2013) acidic forest soils. Unlike autotrophic nitrification, which is strictly aerobic and inhibited at pH<6, heterotrophic nitrification does not require O_2 and is not impeded by low pH. Furthermore, being decoupled from cell growth, heterotrophic nitrification can proceed at a faster pace than autotrophic nitrification (De Boer & Kowalchuk, 2001). In tropical soils that, for the most part, are waterlogged and acidic, heterotrophic nitrification may account for a significant proportion of total nitrification with important implications for overall rates of N cycling and emissions of NO and N_2O. Although NO and N_2O are by-products of the nitrification process, the implications of these trace gas emissions in the context of denitrification are discussed Section 2.3.4.

Once nitrified, NO_3^- may be assimilated by plants and microorganisms, reduced though coupled iron oxidation (Davidson, et al., 2003; Weber, et al., 2006), reduced to NH_4^+ through nitrate ammonification (DNRA) or lost from the soil through denitrification and leaching (Figure 2-2). The concentration of extractable NO_3^- in soil is thus the product of gross nitrification minus consumption processes. Due to dependence on NH_4^+ production, rates of gross nitrification lag behind those of gross mineralisation. Estimated rates range from below

detection levels in many studies to >10 g N m^{-2} d^{-1} in Australian montane forests (Kiese, et al., 2008). Like mineralisation, tropical nitrification rates are sensitive to variations in temperature and moisture. Whilst rates of gross nitrification often decrease with altitude as a result of lower temperatures, the process can only occur under conditions of sufficient O_2. Thus soil moisture is often the primary regulator with nitrification rates peaking under conditions of water-filled pore space (WFPS) <65%, (Davidson, 1991; Firestone & Davidson, 1989; Bouwman, 1998). For example, in Ecuadorian montane forests, decreasing soil temperature (and soil development) with increasing altitude had a negative effect on gross N transformation rates (Arnold, et al., 2009). However, lowland sites may show very low nitrification rates particularly where high rainfall on poorly drained substrate results in anaerobic soils. This lack aeration in lowland forests was the reason for the higher nitrification rates reported for montane sites (despite lower soil temperatures) in Kiese et al. (2008).

Factors affecting rates of gross nitrification following land use change are similar to those affecting gross mineralisation on account of the microbiological propinquity of the two processes. Namely, rates are dependent on the quantity and quality of organic matter with lower C:N ratios indicative of higher quality inputs and greater nitrification (Mack & D'Antonio, 2003; Burton, et al., 2007). For example, the reduced quality of organic returns from Spanish elm and hoop pine plantations in comparison to natural forest vegetation resulted in lowered nitrification rates in Costa Rica and Australia respectively (Silver, et al., 2005; Burton, et al., 2007). Meanwhile the conversion of forest to pasture in the Amazon basin reduced gross nitrification primarily as a result of the decline in N availability (Neill, et al., 1999). Although as nitrification is dependent on net NH_4^+ production, which with vegetation removal and soil disturbance can increase, disturbance may increase nitrification

and losses of nitrate. Land use change may also affect soil microbial size and composition. In Australia, although mineralisation rates were lower in forests than hoop pine plantations, Burton et al. (2007) partly attributed higher gross nitrification rates in forests relative to plantations to disruption of heterotrophic nitrifying populations following land use change. Therefore, rates of nitrification will be affected by changes that occur to NH_4^+ availability, soil physical properties (such as aeration and temperature) and the microbial population.

2.3.4 Denitrification

Biologically mediated denitrification (as opposed to chemodenitrification) uses nitrogen oxides as an alternative electron acceptor to O_2 during respiration and accordingly, it requires anaerobisis to proceed (Knowles, 1982). The electron donor is usually provided in the form of carbon although some denitrifying organisms can use sulphur, methane or iron when carbon is not available. The direct (or proximal) controls on denitrification can therefore be summarised as: i. an oxygen-limited environment, ii. a suitable electron donor, iii. available nitrogen oxides and iv. the presence of a population of denitrifying bacteria (Wallenstein, et al., 2006). However environmental factors (or distal controls) such as pH, soil texture, moisture content and quality and quantity of aboveground biomass affect the long-term composition and structure of the denitrifying community through which the proximal controls operate (Wallenstein, et al., 2006).

Denitrification is most commonly attributed to facultative anaerobic bacteria that reduce NO_3^- or NO_2^- to N_2 through the following sequence:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

When complete (i.e. uninterrupted reduction from NO_3^- to N_2), it is the principal process by which reactive nitrogen is removed from the biosphere. Unlike nitrification, which releases

NO and N_2O as by-products during the transformation process, these gases are intermediate products of the denitrification reaction chain. The relative importance of nitrification versus denitrification to atmospheric NO and N_2O is perhaps best described through the hole-in-the-pipe model of Firestone & Davidson (1989). This conceptual model describes the emissions of both NO and N_2O from soils as a function of: (1) processes related to the flow through the pipe: i.e. rates of denitrification and nitrification (and more generally the availability of NH_4^+, NO_2^- and NO_3^-); and (2) the size of the two holes in the pipe (one each for NO and N_2O) out of which the trace gases escape. The size of the holes in the pipe are dictated by factors such as WFPS and carbon availability which, together with the microbial population, are here considered first before giving examples of denitrification rates (i.e. the flow) within a tropical context.

2.3.4.1 The holes in the pipe

Soil WFPS is an important regulatory factor for both the amount and form of gaseous N loss. The wetter the soil, the less opportunity there is for gas diffusion and the greater the potential for complete reduction to N_2 (Davidson, et al., 2000). Ordinarily there is a shift in trace gas emissions from predominantly NO release under aerobic conditions to N_2O under anaerobic conditions (Figure 2-4) (Davidson, 1991; Davidson, et al., 2000; Davidson & Verchot, 2000; Weitz, et al., 2001). Whilst rainfall is the prime regulator of soil O_2, microbial respiration can also lead to oxygen depletion in soil microsites. Where soils are relatively aerobic (i.e. WFPS <65%), nitrification is typically the primary NO and N_2O forming process but as soils become wetter, the relative importance of denitrification increases. At high soil moisture (i.e. WFPS>80%), N_2 is usually the dominant end product from denitrification. Peak NO and N_2O emissions ordinarily occur at intermediate soil moisture (i.e. 60% <WFPS< 80%), as the presence of aerobic and anaerobic microsites allow simultaneous nitrification and

denitrification. Abundant and frequent rainfall in the humid tropics should translate therefore to high WFPS placing tropical soils in the denitrification zone of emissions where N_2O and N_2 dominate (Veldkamp, et al., 1998; Veldkamp & Keller, 1997; Koehler, et al., 2012). However, high rates of evaporation and transpiration may rapidly deplete moisture and limit the length of time that soils are anaerobic. The periodicity of wetting and drying cycles therefore affects redox status and production of trace gases through alternate nitrification and denitrification (Liptzin, et al., 2011; Pett-Ridge, et al., 2006).

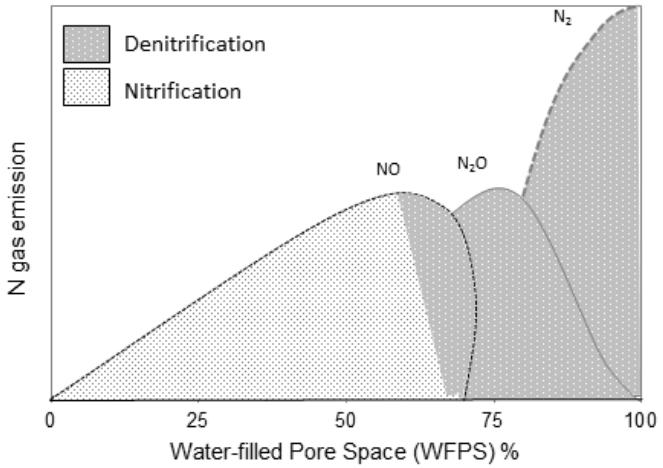


Figure 2-4: Conceptual model of the relationship between WFPS(%) and NO, N_2O and N_2 emissions from nitrification and denitrification (after Davidson (1991) in Bouwman et al 1998)).

In the presence of an oxygen-limiting environment, denitrification still requires both a suitable electron donor and available nitrogen oxides to proceed. The electron donor (usually labile carbon) is a pre-requisite of heterotrophic respiration and an important regulator of denitrification, (Nobre, et al., 2001; Garcia-Montiel, et al., 2003). In old-growth forests of the humid tropics, plant and microfauna inputs are perhaps the highest of any world biome. Hence one might expect soil carbon pools to be greater here than in any other region.

However, the same climatic controls (i.e. warm year-round temperatures and a lack of moisture stress) that favour high rates of net primary production also result in high CO_2 efflux via soil respiration (Schlesinger, 1977; Raich & Schlesinger, 1992; Pregitzer & Euskirchen, 2004). Consequently, even within tropical forests that appear to have plentiful litter returns, there is evidence of C limitation to microbial N processing (Nobre, et al., 2001; Hall & Matson, 2003; Neill, et al., 2005; van Haren, et al., 2010).

In the absence of disturbances such as deforestation, tropical soils are generally not assumed to be N-limited, (Parsons, et al., 1993; Martinelli, et al., 1999; Jenny, 1950; Vitousek & Sanford, 1986). Under the nitrogen saturation model, N status is the principal factor determining the magnitude of losses from soils (Aber, et al., 1998). Therefore, the response of tropical soils to anthropogenic N will depend on factors such as current soil nutrient status, size of the nitrifier and denitrifier community and hydrological pathways (Hall, et al., 2004; Lohse & Matson, 2005; Hall & Matson, 2003; Koehler, et al., 2012). Under N-limiting conditions, there may be additional capacity for the microbial community to assimilate nitrate rather than, for example, denitrify. However, where microbial process rates are limited by other nutrients, anthropogenic N additions might be expected to increase denitrification rates and N_2O emissions. For example, on Mount Kinabalu, Sabah, at elevations between 700-3100m, N-rich sedimentary soils generally responded with greater NO and N_2O emissions to nutrient additions than N-poor ultrabasic soils indicating possibly greater assimilation where N was limiting (Hall, et al., 2004). Similarly, in Hawaii where P, or P and N in combination, limited microbial production, N fertilisation resulted in large losses of NO and N_2O after both one-time and long-term applications, (Hall & Matson, 2003). In general, limited capacity for assimilation of additional N will increase denitrification and N_2O emissions with one recent study reporting a doubling of N_2O emissions following the 10-year addition of 125 kg N ha^{-1}

y^{-1} to lowland Panamanian rainforests (Koehler, et al., 2012). Ultimately, physical and chemical characteristics such as hydrological pathways and nutrient status may control the response of tropical soils to increasing anthropogenic N (Lohse & Matson, 2005). However, much is to be gained by increasing the number of nutrient addition experiments undertaken to determine resource limitations and fertilisation response in this region.

Until recently a lack of evidence to the contrary, coupled with the fact that denitrification genes are widespread among evolutionary distinct species of denitrifiers, has led to the assumption that the denitrifying population (be it temperate or tropical) exerts little control over denitrification rates. Advances in molecular methods now permit characterisation of the population through DNA and mRNA analyses and have begun to open up this area of research. One area of interest is the effect of community structure on ecosystem function. For example, not all microorganisms carry each of the necessary genes to reduce NO_3^- sequentially to N_2. Some bacteria and most (possibly all) fungi appear able to reduce NO_3^- to N_2O but many organisms facilitate only one step in the 4-step sequence (Shoun, et al., 1992; Takaya & Shoun, 2000; Hayatsu, et al., 2011; Shoun & Tanimoto, 1991). It is theoretically possible, therefore, that a community lacking in sufficient organisms carrying the N_2O reductase gene may produce more N_2O or have higher $N_2O/(N_2O+N_2)$ ratios than a community where N_2O reducers are abundant, (Henry, et al., 2006; Richardson, et al., 2009; Toma, et al., 2011; Philippot, et al., 2009). This structural population control of denitrification is exemplified by fungi: fungal denitrifiers appear to lack the N_2O reductase though there is increasing evidence of their importance to soil denitrification, (Laughlin & Stevens, 2002; Yanai, et al., 2007; Ma, et al., 2008; Toma, et al., 2011). Whilst there is no evidence that fungi can denitrify to N_2, the reduction of nitrate may be coupled to the reaction of nitric oxide with organic nitrogen to form N_2 through the process of codenitrification,

(Spott, et al., 2011). Publications on fungal denitrification in temperate or boreal soils have been gathering momentum over the past 15 years, however apparently only one study has compared the contribution of fungi and bacteria to denitrification within tropical forest and agricultural soils (Yanai, et al., 2007). Working in Indonesian Borneo, Yanai et al. (2007) found N_2O emissions from the arable peat soil to be largely from fungal rather than bacterial nitrification and/or denitrification. Furthermore, emissions of N_2O were much higher in agricultural sites relative to the natural forest, though the authors stopped short of relating these land-use differences to changes in microbial community and relative abundance of fungal denitrifiers. Data on fungal denitrification lags behind that of bacterial denitrification, however, there is evidence that fungal denitrification is significant across a range of environments. For example, fungi have been shown to be important denitrifiers in birch forests on drained Swedish peat (Rütting, et al., 2013), coniferous forests in The Netherlands (Laverman, et al., 2000), Irish grasslands (Laughlin & Stevens, 2002) and semi-arid riparian soils in Arizona (McLain & Martens, 2006). The challenge of ongoing research is to employ recent advances in molecular methods to relate community composition and possibly the fungal community in tropical soils to ecosystem functions such as N_2O emission.

2.3.4.2 The flow through the pipe

The inherent difficulty in measuring the large spatial and temporal variability of denitrification complicates assessment of process rates (Groffman, et al., 2006). Hotspots and hot moments account for a significant proportion of total denitrification in soils, which must be taken into account when scaling up rate estimates (McClain, et al., 2003). Methodological difficulties also arise from the high atmospheric concentrations of N_2. As a result, isotopic tracers are generally used to differentiate the small changes in N_2 relative to the large background concentration. The considerable cost involved in isotope analysis has historically

favoured the reporting of denitrification rates measured as the production of N_2O rather than N_2. The two most common methods of measuring N_2O production are through the use of static soil gas chambers or the denitrification enzyme activity (DEA) assay. Measuring N_2O production however, is not without its own problems. Both nitrification and denitrification (in addition to several other processes) emit N_2O and apportioning production between the two is not always obvious. WFPS is commonly used as a process discriminator but whilst the model presented by Davidson et al. (1991) (Figure 2-4) in general holds true, the use of ^{15}N tracers has highlighted the need for caution in using soil moisture as the only mechanism of differentiation (Wolf & Brumme, 2002; Müller, et al., 2004). For example, even at very high WFPS (87%), N_2O emissions in a fertilised Costa Rican coffee plantation still came primarily from autotrophic nitrification and were greater than N_2 emissions from denitrification, (Hergoualc'h, et al., 2007). Furthermore, heterotrophic nitrifiers were primarily responsible for nitrous oxide production across a range of forest and agricultural land uses on acidic soils in central Sumatra (Nakajima, et al., 2005). The possibility of heterotrophic nitrifier and fungal denitrifier contribution to N_2O emissions raises an important methodological point. Acetylene gas (C_2H_2) is commonly employed as a process inhibitor in estimates of nitrification and denitrification (Davidson, et al., 1986). At low (10 Pa) concentrations C_2H_2 blocks ammonium monooxygenase and at high (10 kPa) concentrations it blocks nitrous oxide reductase. Thus, denitrification as the sum of N_2O+N_2 production can be measured solely through the production of N_2O. However, it appears that heterotrophic nitrification is not inhibited by the low concentrations that affect autotrophic nitrification (Hynes & Knowles, 1978), and that fungal denitrifiers are not inhibited by C_2H_2 at high concentrations (Yanai, et al., 2007). Therefore, in soils where fungi dominate, using an acetylene block will result in little differentiation between treatments with and without acetylene and may underestimate

the total denitrification occurring within the soil. Conversely, if nitrification is not blocked by C_2H_2, then estimates of denitrification may be greater in soils where heterotrophic nitrifiers dominate. This is particularly important for soils where nitrification and denitrification are closely coupled, as the latter process is strongly dependent on NO_3^- availability from the former.

Despite difficulties in distinguishing the source of N gas efflux, tropical soils are generally observed to be large emitters of N_2O (Davidson & Kingerlee, 1997; Bouwman, 1998; Zhuang, et al., 2012). The proportion of N_2O to N_2 released from tropical soils also appears to be higher than in temperate soils with some researchers noting very low or absent N_2 emissions (Griffiths, et al., 1993; Silver, et al., 2001). Globally, denitrification rates are reported to span 0-239 kg N ha^{-1} y^{-1}, however, despite the large range, mean rates are somewhat more restrained with estimates of 2kg N ha^{-1} y^{-1} for forest soils and 13kg N ha^{-1} y^{-1} for agricultural soils (Barton, et al., 1999). Several early studies in tropical soils noted that denitrification rates appeared similar to those reported in temperate soils (Parsons, et al., 1993; Griffiths, et al., 1993; Robertson & Tiedje, 1988), although, some very high rates have also been observed in fertilised agricultural soils, (Hall & Matson, 2003; Nakajima, et al., 2005; Templer, et al., 2005; Skiba, et al., 2012). For example, in-situ emission rates from the palm circle[2] of an oil palm plantation in Sabah, Borneo following fertiliser application were 6.5mg N m^{-2} h^{-1}, (Skiba, et al., 2012). Scaled up to the areal extent of the plantation, this would equate to an enormous 575kg N ha^{-1} y^{-1}. However, the difficulty in accounting for hot spots and hot moments applies as much to tropical soils as it does to temperate, and when rates were estimated for the entire plantation, a much more modest 4.4 ± 3.5kg N ha^{-1} y^{-1} was recorded.

[2] The palm circle is an area extending roughly 2 m in radius from the base of the palm trunk where fertiliser is applied.

Thus, the spatial variability of denitrification once again highlights the need to consider agricultural management practices such as heterogeneous fertiliser application when estimating process rates.

As with other microbial processes, forest clearance often elevates rates of denitrification in the immediate period following deforestation and burning, (Luizão, et al., 1989; Keller, et al., 1993; Nakajima, et al., 2005). Subsequently, the conversion to agriculture often requires ongoing N fertilisation due to the high rates of weathering and lack of fresh organic matter inputs. Therefore the input-export balance of inorganic N, together with the response of the microbial community to changes in soil organic matter, are important regulators of denitrification response to forest conversion (Veldkamp & Keller, 1997; Groffmann, et al., 2001). For example, low soil organic matter and microbial biomass in oil palm plantations in the Dominican Republic were associated with low rates of potential denitrification (Templer, et al., 2005). Conversely, forest clearance in Brazil increased soil N availability in newly established Amazonian pastures and elevated rates of N_2O emission above those of the native forest (Neill, et al., 2005). However, as pastures age, N becomes limiting in the absence of fresh organic inputs and rates decline relative to forested sites (Keller, et al., 1993; Verchot, et al., 1999; Veldkamp & Keller, 1997; Neill, et al., 2005). As oil palm plantations mature, removal of understory vegetation reduces organic matter returns to the soil, which primarily come in the form of pruned palm fronds or fresh fruit bunches. It is unclear therefore whether these limited returns will equate to an increasing N deficit and/or reductions in microbial biomass over the life of the plantation. The majority of oil palm plantations require fertilisation and therefore, increased N inputs will likely result in increased losses from denitrification post application. For example, in Papua New Guinea, fertilisation of oil palm soils resulted in a 7-10 fold increase in N_2O flux (Banabas, 2007). On deep peat soils in

Indonesia, the application of a modest 28 kg N ha^{-1} fertiliser has been shown to release 8.8±1.7 kg N_2O-N ha^{-1} (equivalent to 115 kg N ha^{-1} y^{-1}) in the 28 days following application relative to an unfertilised control of 0.3±0.3 kg N_2O-N ha^{-1} (4 kg N ha^{-1} y^{-1}) (Hergoualc'h et al., 2012). N fertilisation may also be one reason for the changes in atmospheric chemistry resulting from oil palm plantation establishment observed by the Oxidant and Particle Photochemical Processes (OP3) project conducted in in Sabah during 2008 (Hewitt, et al., 2010). From airborne measurements aboard an atmospheric research aircraft, researchers discovered that emissions of N_2O above oil palm plantations were 50% higher than those of neighbouring forest (Hewitt et al., 2009). Furthermore, emissions of NO_x were 250% higher from palm plantations relative to the forested areas. NO_x emissions are of particular concern as in combination with the oil palm's naturally high emissions of volatile organic compounds (VOCs), the potential for ground-level ozone creation is greatly increased (Hewitt, et al., 2009). In the presence of sunlight, VOCs combine with NO_x to form ozone, which can cause local breathing problems and damage plant health. Furthermore, the creation of ozone may result in a positive feedback whereby plants under ozone stress increase the biogenic VOC emission, (Llusià, et al., 2002). The extent of the current oil palm crop (11 million ha in Southeast Asia as of 2012 (FAOSTAT, 2013)) coupled to the predicted growth in crop area and anthropogenic N additions has the potential to push regional ozone levels well beyond the World Health Organisation's safe threshold of 50 ppbv (Pyle, et al., 2011). In addition to regional health concerns, trace gas emissions from tropical regions is one of the major uncertainties constraining predictions of global climate change. As such, understanding current emission levels and their response to both changes in proximal and distal controls as a result of land use change is of prime importance.

2.3.5 Dissimilatory nitrate reduction to ammonia (DNRA)

Dissimilatory nitrate reduction to ammonia (DNRA) is, most probably, the second major pathway of nitrate reduction in soils after denitrification (Figure 2-2). Like denitrification, DNRA occurs under conditions of anaerobisis and requires a carbon donor to proceed. It also produces N_2O as an intermediate product (Tiedje, 1988; Philippot & Højberg, 1999). However, rather than depleting soil inorganic N, DNRA conserves nitrogen through transformation of NO_3^- to the less mobile NH_4^+ pool.

DNRA appears to be a relatively important N transformation process in some, although not all, tropical soils (Silver, et al., 2001; Templer, et al., 2008; Pett-Ridge, et al., 2006), whilst its relative importance (calculated as the proportion of NO_3^- consumed) may be greater in temperate soils (Rütting, et al., 2011). Whether DNRA is favoured over denitrification is a function of the soil oxidation status and NO_3^- and C availability (Tiedje, et al., 1982; Fazzolari, et al., 1998; Silver, et al., 2005). Under highly reducing conditions, where electron acceptors (i.e. NO_3^- and NO_2^-) are limited, DNRA can transfer more electrons per mole of NO_3^- (Tiedje, et al., 1982). As such, DNRA derives more free energy for cell synthesis than denitrification where the ratio of electron donor (C) is high relative to electron acceptor (N), (Strohm, et al., 2007).

Rutting et al. (2011) reviewed DNRA rates reported across the temperate (forest and grassland), sub-tropical forest and tropical forest ecosystems (summarised in Table 2-2). They concluded that rates in tropical forests were the highest of any biome ranging from 0.03 – 2.89 μg N g^{-1} soil d^{-1}. Although low-end estimates of DNRA appear to be similar across ecosystems, mean rates in tropical regions were generally an order of magnitude greater than in other biomes.

Table 2-2: Summary of DNRA rates across temperate, sub-tropical and tropical ecosystems (from Rutting et al. (2011))

Ecosystem	DNRA Rates ($\mu g\ N\ g^{-1}$ soil d^{-1})	References (lower estimate; upper estimates)
Temperate forest	0.004 (±0.001) – 1 (±0.20)	Staelans et al (2011); Huygens et al (2008)
Temperate grassland	0.034 (±0.002) – 0.27 (±0.01)	Rutting et al (2010); Muller et al (2009)
Sub-tropical forest	0.015 (±0.008) – 0.053 (±0.009)	Zhang et al (2011a); Zhang et al (2011b)
Tropical forest	0.03 (±n.d.) – 2.89 (±0.57)	Templer et al (2008); Pett-Ridge et al (2006)

Few studies report rates of DNRA in tropical soils and seemingly, only one has measured rates outside the 'natural' forest environment (Silver et al 2005). In this case, DNRA in a Spanish elm *(Cordia alliodora)* plantation (0.23±0.08 $\mu g\ N\ g^{-1}\ d^{-1}$) in Costa Rica was indistinguishable from DNRA in old-growth forests (0.24±0.08 $\mu g\ N\ g^{-1}\ d^{-1}$). In Puerto Rico, Silver et al. (2001) found rates of DNRA to be three times higher than rates of denitrification, which was attributed to the competitive advantage of DNRA under conditions of NO_3^- limitation. Thus, in situations where NO_3^- is limited, such as aquatic sediments or floodplain soils where nitrification is supressed through anoxia, DNRA may be relatively more important than denitrification or anaerobic ammonium oxidation (anammox) (Dong, et al., 2011). For example, working with tropical estuarine sediments in Indonesia, Thailand and Fiji, Dong et al. (2013) recently found that DNRA was the main NO_3^- consuming process above that of denitrification: a fact attributed to the energetic advantage of DNRA and a higher affinity for nitrate of nitrate ammonifiers. Although rates of DNRA reported for Puerto Rico and Costa Rica have been relatively high (Silver, et al., 2001; Pett-Ridge, et al., 2006; Templer, et al., 2008), lower rates have been reported for sandy soils in Brazil (Doff Sotta, et al., 2008), montane sites in Hawaii, (Holtgrieve, et al., 2006) and rice field sediment

in the Philippines (MacRae, et al., 1968). The paucity of estimated DNRA rates in tropical soils highlights the need for further work to assess the importance of this process at the biome level. However, current analysis suggests that rates are likely to be high in tropical soils with high organic content and limited NO_3^- availability, (Rütting, et al., 2011).

2.3.6 Nitrate reduction coupled to iron oxidation

A relatively recent discovery is the process of nitrate reduction (biotic and abiotic) combined with ferrous iron (Fe(II)) oxidation, (Figure 2-5). Under anoxic conditions, nitrate reduction has been observed to be biotically coupled to the oxidation of Fe(II) in aquatic, wetland and flooded rice environments, (Straub, et al., 1996; Ratering & Schnell, 2000; Weber, et al., 2006). However, when biologically mediated, the process occurs at relatively low temperatures with circumneutral pH (~pH 7) and is thus unlikely to be an important nitrate consumer in acidic, humid tropical soils (Postma, et al., 1991; Weber, et al., 2001).

More controversial is the pathway of abiotic reduction of nitrate coupled to iron oxidation (otherwise known as the ferrous wheel hypothesis), whereby nitrate reacts with Fe(II) (or Mn(II)) to form nitrite that subsequently binds to organic matter resulting in DON (Davidson, et al., 2003) (Figure 2-5). In several temperate and tropical studies employing the isotope dilution technique, recovery rates of the applied $^{15}NO_3^-$ label have been too low to permit accurate estimates of gross nitrification (Corre, et al., 2006; Arnold, et al., 2009). Rapid assimilation of NO_3^- into the organic N pool immediately after addition is often observed, or assumed to be the cause (Dail, et al., 2001; Perakis & Hedin, 2002; Corre, et al., 2006; Huygens, et al., 2008; Doff Sotta, et al., 2008). When testing this hypothesis, Coleman et al. (2007) reported that the assumed abiotic retention was more likely the result of underestimation of NO_3^- concentrations due to Fe interference when using the indophenol method of determining extracted NO_3^-.

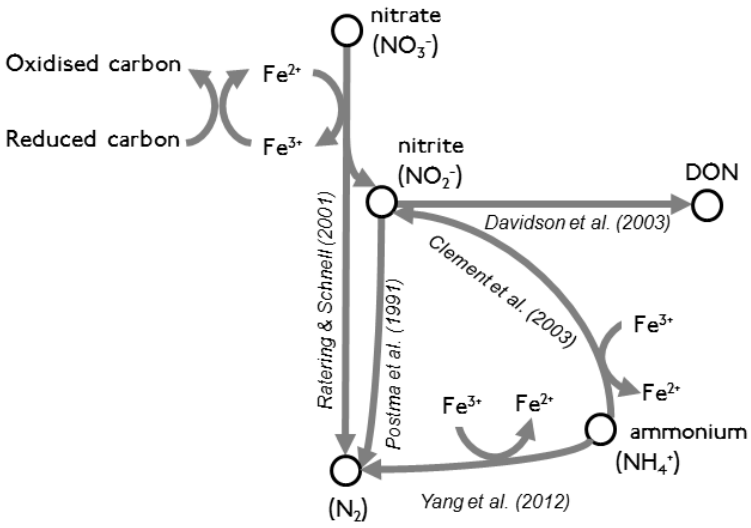


Figure 2-5: Schematic of potential pathways of nitrate reduction and ammonia oxidation coupled to iron cycling.

The ensuing debate has yet to be resolved (Davidson, et al., 2008; Coleman, et al., 2008) and the ferrous wheel hypothesis remains controversial with limited evidence that both supports (Torres-Canabate, et al., 2008; Zhang, et al., 2010; Huygens, et al., 2008; Dail, et al., 2001) and refutes (Schmidt & Matzner, 2009) its existence. However, abiotic NO_3^- reduction has been observed in temperate forests (Dail, et al., 2001), Mediterranean coniferous forests (Torres-Canabate, et al., 2008), temperate soils of Eastern China (Zhang, et al., 2010), and temperate evergreen rainforests (Huygens, et al., 2008). In the absence of any alternative, it remains the only hypothesis currently offered as an explanation for the rapid transformation of NO_3^- to DON, though there is only indirect evidence of its existence and the exact mechanism requires further investigation. Furthermore, the importance of the process has yet to be established for tropical soils (Zhang, et al., 2010).

2.3.7 Anaerobic ammonium oxidation

The discovery of anaerobic ammonium oxidation (anammox) is one of the processes challenging the long-held belief that denitrification is the only microbially mediated process within the nitrogen cycle that converts bioavailable N back to atmospheric N_2. Anammox bacteria use nitrite as an electron acceptor to oxidise NH_4^+ to N_2 via the following pathway:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_20$$

The process was first hypothesised from ammonium oxidation observed in the anoxic deep ocean and seabed sediments and was later confirmed in anaerobic wastewater treatment plants (Richards, 1965; Emerson, et al., 1980; Mulder, et al., 1995; van de Graaf, et al., 1997). There is increasing evidence of its substantial contribution to aquatic nitrogen cycling with estimates ranging between 20-40% of total global N_2 production (Thamdrup, 2012). Within soils, however, the process has been largely ignored and may only contribute minimally to terrestrial nitrogen cycling (Thamdrup, 2012; Humbert, 2011). Ammonia oxidation can utilise other electron acceptors such as iron, sulphate or manganese oxide (Luther, et al., 1997; Hulth, et al., 1999; Shrestha, et al., 2009), but coupled to iron reduction, NH_4^+ has been reported to be oxidised to NO_2^- in wetland soils (Clement, et al., 2005; Shrestha, et al., 2009), and to N_2 in tropical forests (Yang, et al., 2012). In Puerto Rico, ammonia oxidation rates were similar to those of aerobic nitrification and denitrification and represented a significant process in the nitrogen cycle of the upland soils (Yang, et al., 2012). The utilisation of new molecular methods has shown that anammox bacteria are present in soils and their diversity may be greater than is present in the marine environment, however further work is required to determine whether they actively produce N_2 within soils and if so, though what mechanism and in what quantity.

2.4 SUMMARY AND FURTHER RESEARCH

Unlike most temperate climax communities, many tropical forests appear limited by nutrients other than N. High soil N, coupled to warm year-round temperatures and abundant soil moisture, are conducive to an up-scaling of microbial activity during almost every step of the nitrogen cycle (Bai, et al., 2012). For example, there is evidence that fixation (Cleveland, et al., 1999), mineralisation, nitrification (Booth, et al., 2005), nitrous oxide emission (Zhuang, et al., 2012) and DNRA (Silver, et al., 2001) are all higher in the tropics than in other biomes.

Although research has lagged behind that conducted in other parts of the world, the importance of understanding tropical nitrogen cycling is exemplified by the large contribution that the region makes to global N_2O emissions (Bouwman, 1998). The main processes responsible for the release of N_2O from soils are nitrification and denitrification. Even in the absence of fertilisation, losses of N_2O (and possibly N_2) from undisturbed tropical soils appear relatively high. However, deforestation within tropical regions proceeded at such a rate over the past half-Century that approximately half of the world's remaining forests are now of secondary quality (Chazdon, et al., 2009). The factors driving deforestation are intimately linked to population increase, industrialisation and agricultural development, the latter of which is exemplified by the advance of oil palm across much of Indonesia and Malaysia since the 1970s. The removal of natural vegetation and replacement with monoculture plantations that require artificial fertilisation is likely to result in changes to soil biogeochemistry with important consequences for global nitrogen (and carbon) cycling. Southeast Asia in particular has seen a N fertiliser use increase from 5 to 7 million tonnes over the nine year period from 2002 to 2011 (FAOSTAT, 2013). Yet, little work has been done on the impact that forest clearance and agricultural development has on nitrogen cycling

within these soils. Quantifying key process rates in tropical N cycling is the first step to prevention of nitrogen saturation in the future and closes the knowledge gap on one of the major uncertainties in global climate change modelling, namely predicting future N-based trace gas emissions.

Advances in both molecular and isotopic tracer methods over recent years have vastly improved our knowledge of nitrogen cycling and opened the way for investigation of novel N-transformation pathways and new groups of organisms responsible for otherwise well-studied processes such as heterotrophic denitrification and anammox. Many of these new developments are still in their infancy in research in temperate zones. Research in tropical regions has traditionally lagged behind the developed world in terms of nitrogen cycling, yet the opportunities are even greater. Even at a most basic level, the database of rate estimates for well-known processes such as mineralisation, autotrophic nitrification and denitrification is very limited, which constrains the robustness of regional estimates relative to say European or North American equivalents. Yet climatic conditions within the tropics are such that the region plays a decisive role in global biogeochemical cycling with significant contributions to *inter alia* global N_2O, NO, and CO_2 budgets. In addition to improving global rate estimates, tropical nitrogen biogeochemistry is replete with other opportunities for research development: Resolution of the "nitrogen paradox"; the contribution of heterotrophic nitrifiers to NO_3^- production; microbial diversity (particularly fungi) and their role in ecosystem function (e.g. N_2O emissions); the quantitative contribution of novel pathways such as DNRA, anammox and abiotic nitrate reduction to overall N cycling and the response of tropical soils to increasing anthropogenic N, are to name a few. Methodological advances of the past few decades have permitted new discoveries in N cycling research. The challenge for

the tropics will be to employ these new methods in a region where the pace of economic and industrial growth makes future predictions uncertain.

By examining process rates in tropical forests and oil palm plantations in Sabah, Malaysia, this thesis will address some of the uncertainties highlighted by this review. In particular, the aim is to:

1. Establish the nitrogen status of secondary forests along a trajectory of recovery post-disturbance.
2. Examine spatial variability of nitrogen cycling indicatory within plantations.
3. Examine temporal variability in nitrogen cycling across season and through plantation age.
4. Assess the impact of land use change by comparing process rates in plantations with forests.

CHAPTER 3: SITE SELECTION, FIELD AND ANALYTICAL METHODS

3.1 INTRODUCTION

This chapter gives an overview of the sites selected for study and the field and analytical methods used to produce the results set out in Chapters 4 to 7. This Chapter aims to provide general information on the area of study, namely climatological, topographical and geological information rather than an in-depth description of each study site. Full details of the locations sampled are provided within the data chapters that present results on the study sites discussed. Namely, the secondary forest sites are described in detail within Chapter 4, and the plantation sites are described in Chapter 6.

3.2 SITE SELECTION

3.2.1 The Kinabatangan Lowlands, Sabah, Borneo

Nitrogen cycling indices were determined in soils sampled from ten study sites situated in the Kinabatangan Lowlands, North Eastern Sabah, Malaysian Borneo (Figure 3-1). Borneo lies below the Southeast Asian monsoon and typhoon belt and experiences a humid tropical climate with high annual rainfall, high humidity and greater diurnal than monthly variation in temperature. Mean annual temperature is 27.4°C (2008-2013) and annual rainfall is typically between 2500 – 3500 mm with no obvious dry month.

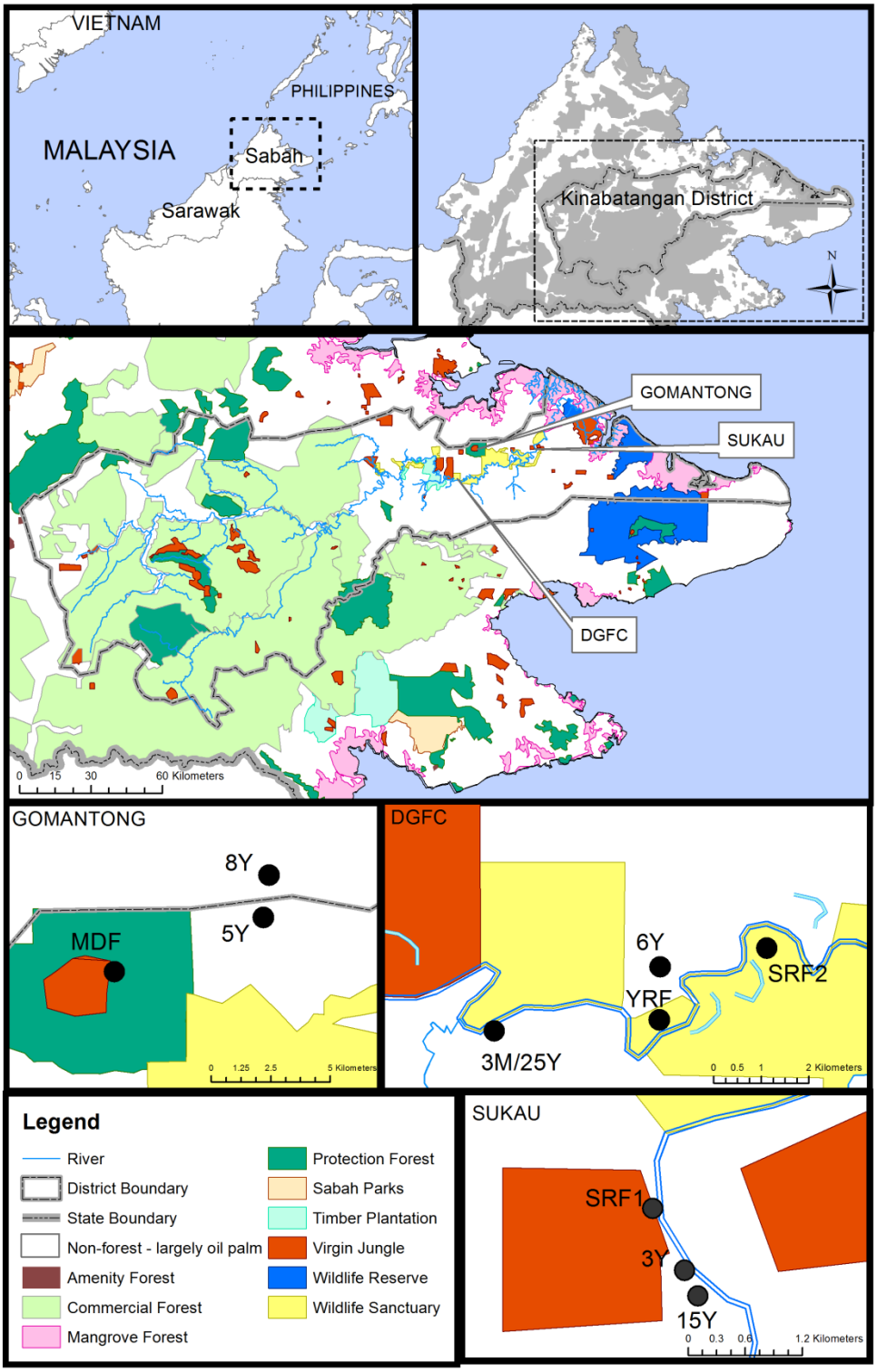


Figure 3-1: Location of the study sites in Sabah, Malaysian North Borneo. Study sites included four secondary forest sites (YRF, SRF1, SRF2, MDF) and six oil palm plantation sites (3M/25Y, 3Y, 5Y, 6Y, 8Y, 15Y). Sites were located on the lower Kinabatangan River floodplain where patches of forest (coloured areas) remain in the larger matrix of primarily oil palm plantations and settlements (white areas).

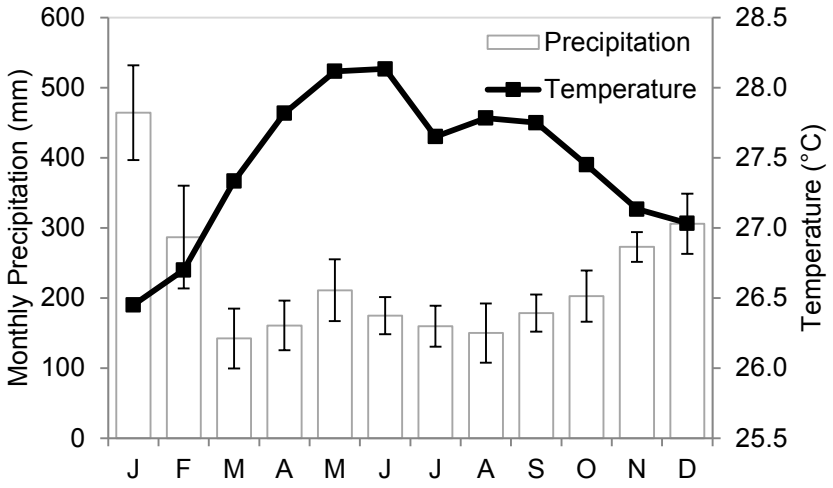


Figure 3-2: Average monthly precipitation (mm) and temperature (°C) for Kota Kinabatangan rain station between 2008 and 2013 (*Malaysian Meteorological Department*). Data excludes 2011 when September and October received 2400mm taking the annual rainfall for that year to >6000mm. Error bars represent standard error of the mean.

The climate of Sabah is influenced by two monsoons: the northeast from November to March; and the southwest between May and September. Inter-monsoonal periods span March to May and September to November (Figure 3-2). The northeast monsoon brings the wettest weather with November to January experiencing monthly rainfall averages typically in excess of 300 mm. The southwest monsoon brings less rain than northeast so that the period from May to September is referred to as the "dry season" throughout Malaysia. However, during this period Eastern Sabah still receives relatively high monthly rainfall (e.g. > 100 mm) from typhoons which hit the Philippines to the north east.

3.2.2 Topography and geology

The Kinabatangan floodplain dominates the eastern lowlands of Sabah where the topography is low-lying with occasional limestone outcrops. The Kinabatangan River is the largest in

Sabah, covering a distance of 560 km and a catchment area of 16,800 km^2. During the wettest months of the year (December – January), much of the floodplain is prone to seasonal flooding. Soils of this region form part of the Kinabatangan series and are typically silty clay loams formed on either alluvium (Tuaran Association) or mudstone and sandstones (Rumidi Association) (Acres & Folland, 1975). For this thesis, the alluvial soils are broadly classified as "riparian" and the mudstone and sandstone soils as "*terra firme*". However, as the periodicity of inundation varies between riparian sites these labels are used primarily to differentiate sites adjacent to the Kinabatangan River (referred to as DGCF and Sukau on Figure 3-1) from those sites further inland (referred to as Gomantong on Figure 3-1).

Along the meander belt of the Kinabatangan River, the Tuaran Association forms the soils found on levees, meander scars and scrolls and ox-bow lakes. The levees are generally courser textured and less frequently inundated by floodwaters than those of the aggrading banks. As a result, not all riparian areas are subject to regular or annual inundation. Dominant soils include Eutric Fluvisols, Cambic Arenosols and Eutric Gleysols with Gelyic Luvisols and Gleysols occupying the poorly drained meander scrolls and backwater swamps (Acres & Folland, 1975). Vegetation on the Tuaran Association is primarily riparian or riverine forest with flood-tolerant species that are lower in stature than the *terra firme* evergreen dipterocarp forest that once dominated this region. The proximity of this association to the navigable Kinabatangan has meant that logging, shifting agriculture and inhabitation are common historic and on-going land uses. Remaining forested areas are therefore of secondary quality.

The Rumidi Association is extensive across the *terra firme* middle and lower reaches of the Kinabatangan occupying low hills (< 30m a.s.l) and narrow alluvial flats where the gradient is typically between 5-15°. The unstratified inter-bedded mudstone and sandstone substrate of

volcanic and sedimentary origin is extremely diverse. Consequently, a large number of soils are formed within the association though Ferric, Orthic and Gleyic Acrisols and Luvisols are most common. The suitability of the Rumidi Association for agriculture has seen most of the lowland dipterocarp forest logged and replaced with oil palm plantations progressively since the mid-1970's.

3.2.3 Vegetation and land use change

The African oil palm (*Elaeis guineensis*) was first planted in Malaysia as a commercial crop in 1917. At the time, the country was financially dependent on exports of rubber and tin and the diversification into oil palm was an important safeguard against economic vulnerability to these two commodities. By the 1960's production on the mainland was increasing rapidly, largely through the replanting of existing rubber estates. In the 1970's, policy makers began looking eastwards to the vast expanse of undeveloped land in the Bornean states of Sabah and Sarawak. At the time of Acres and Folland's comprehensive 1976 land classification series, approximately 75% of Sabah was still forested with less than 20% of that designated as secondary quality (Acres & Folland, 1975; Table 3-1). Within the Sandakan residence (incorporating the districts of Kinabatangan, Sandakan, and Labuk and Sugut), forest was even more widespread with 97% coverage and less than 13% of that disturbed (Acres & Folland, 1975). By 1980, just five years after publication, all of the lowland dipterocarp forests had been logged out or licenced for logging and in less than 20 years primary forest cover had declined by 60-75%, (Vincent & Rozali, 2005).

The decline of primary forest has been succeeded by a commensurate rise in the proportion of land covered by secondary forest. Early successional or disturbed forests are now the dominant forest type within Sabah covering 38% of the total land area (Table 3-1). By contrast, oil palm estates cover ~20% of the state with the majority of development having

taken place in the eastern lowlands where the land is of low relief (Figure 3-1). Although forest cover is extensive in the highland areas of the Kinabatangan catchment, the majority of these forests are maintained for commercial forestry. Within the lowlands, some *terra firme* patches of primary forest remain having been classified as virgin jungle reserves or given designated protection status from activities such as logging and poaching. A large proportion of the riparian forest on the Tuaran Association has also been protected since 2005 through the establishment of the Lower Kinabatangan Wildlife Sanctuary, though this forest has been extensively logged in the past. In the coastal inter-tidal zone, the vegetation is primarily nipa palm (*Nypa fruticans)* and mangrove swamp, parts of which have been granted protection, virgin jungle, or Ramsar wetland status.

Table 3-1: Estimated forest cover (by vegetation type) in Sabah 1975 and 1995, (ICZM, 1998).

Forest Type	1975 Area (ha)	Percentage of total land in Sabah	1995 Area (ha)	Percentage of total land in Sabah
Mangrove	365 500	4.96	317 400	4.30
Transitional, beach and swamp	203 256	2.76	193 000	2.62
Undisturbed late successional	2 800 236	37.99	300 000	4.07
Montane	711 874	10.47	700 000	9.50
Secondary	1 399 024	18.98	2 799 220	37.97
TOTAL	5 539 890	75.16	4 309 620	58.46

3.2.4 Sampling design

Samples were collected from 10 study sites (Figure 3-1) during the inter-monsoonal period from 28 September to 24 October 2010 and again at the end of the wet season between 29 March and 18 April 2012. Monthly temperature and rainfall averages for both sampling seasons are set out in Figure 3-3. The purpose of the study was to examine nitrogen cycling across land use gradients and to compare rates in secondary forests with those of oil palm

plantations. Accordingly, the four secondary forest sites had experienced different levels of disturbance and the six oil palm plantations ranged in age from 3 months to 25 years.

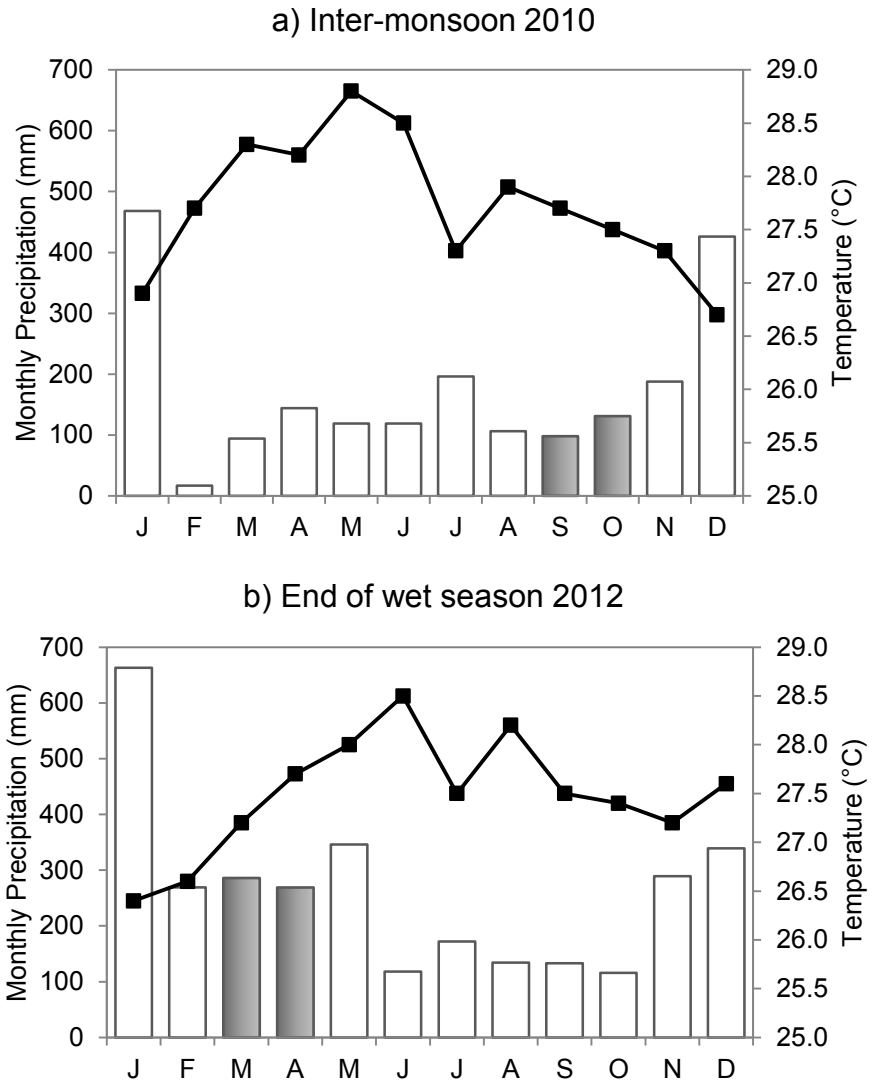


Figure 3-3: Monthly rainfall (bars) and temperature (points) for a) the inter-monsoon and b) end of wet season sampling at Kota Kinabatangan weather station *(Malaysian Meteorological Department)*. Shaded bars represent the months when sampling took place.

Site selection reflected logistical constraints such as ease of access, time availability, and in the case of oil palm plantations, permission from estate owners. Ten locations represented the

maximum number of sites that could be sampled within a two month time frame given the need to obtain permission to access sites, the resolution of sampling (i.e. 12 repetitions per site) and the time taken to handle samples, many of which required processing within hours of collection. Of the four forest locations, three were riparian at different stages of successional development: namely a young riparian forest (YRF), and two mid-successional riparian forests (SRF1 and SRF2). The remaining forest was a *terra firme* old-growth lowland dipterocarp site (MDF) that had been under government protection since 1925. A detailed description of the four forested sites is presented in Chapter 4. The six oil palm plantation sites spanned and age range of 3 months – 25 years with five of them being described by their age at time of sampling in 2012, namely 3Y is a 3-year old plantation at the end of wet season sampling. Site 25Y was a 25-year old mature plantation reaching the end of its economic life during the inter-monsoonal sampling in 2010. However, when the site was revisited at the end of the wet season in 2012, the mature trees had been felled and new seedlings planted out 3 months (3M) prior to sampling. Accordingly, 3M and 25Y are the same plantation but with different aged palms over the two seasons sampled. Two of the plantations (5Y and 8Y) were situated on *terra firme* soils of the Rumidi Association and the remaining four plantations (3Y, 6Y, 15Y and 25Y) were in the riparian zone of the Kinabatangan River on recent alluvium. The sites also incorporated first and second generation plantings within large estates and smallholdings further details of which are set out in Chapter 6 below.

3.3 PHYSICAL METHODS

3.3.1 Soil sampling

Samples were collected from 10 study sites, first during the inter-monsoonal period between September and October 2010 and secondly at the end of the wet season between March and

April 2012. During both sampling periods (i.e. the inter-monsoonal and end of wet season), 12 replicates were chosen within an area of ~150 m^2 at each study site. A 4cm diameter PVC tube was driven into the soil to a depth of 10cm for each replicate plot. The core was weighed in-situ for determination of bulk density before removal of large roots, homogenisation and sub-division for physical and chemical analysis. During the 2012 (end of wet season) sampling, a further four cores were collected from eight of the twelve replicate plots for measurements of gross mineralisation and nitrification. A summary of the samples taken over both seasons is set out in Table 3-2.

Table 3-2: Summary of sample analysis conducted for the inter-monsoon 2010 and end of wet season 2012 sampling.

		n (per sampling site)	
Variable	Method	2010 Season	2012 Season
Soil moisture	Loss on ignition	12	12
Bulk density	4 cm diameter x 10 cm length core	3	12
Soil organic matter	Loss on ignition	12	12
Particle size	Laser diffraction	3	12
pH_w	Glass electrode	3	12
NH_4^+	Salicylate method	12	12
NO_3^-	Reduction to NO_2^- by copperised Cd & Griess-Ilosvay method	12	12
Gross mineralisation	Isotope pool dilution		8
Gross nitrification	Isotope pool dilution		8
NH_4^+ consumption	Isotope pool dilution		8
NO_3^- consumption	Isotope pool dilution		8
N_2O	Soil chambers	12	12
N_2O+N_2	Soil chambers and C_2H_2 inhibition	12	12
Potential denitrification	^{15}N tracer	12	12
Potential DNRA	^{15}N tracer	12	12
Soil ^{15}N	Isotope ratio mass spectrometry		12
Soil ^{13}C	Isotope ratio mass spectrometry		12
Foliar ^{15}N	Isotope ratio mass spectrometry		6
Foliar N	Isotope ratio mass spectrometry		6
Soil total N	Elemental analysis		12
Soil total C	Elemental analysis		12

3.3.2 Moisture, bulk density and soil organic matter (SOM) content

Gravimetric soil moisture was determined by drying a 50g sub-sample at 105°C for 24 hours to enable the calculation of soil bulk density from:

$$\rho_d = \frac{M_s}{V_t} \quad \text{Equation 3-1}$$

where ρ_d is soil bulk density (g cm^{-3}), M_s = mass of dry soil (g) per core volume and V_t = total volume of the soil core (cm^3). Soil water content was then converted to percentage water-filled pore space (% WFPS) using the equation of Linn & Doran., (1984):

$$\%\, WFPS = \frac{\theta_v}{P_t} \cdot 100 \quad \text{Equation 3-2}$$

where θ_v = volumetric water content (g g^{-1}) and total porosity (P_t) is defined as:

$$P_t = 1 - \frac{\rho_d}{\rho_p} \quad \text{Equation 3-3}$$

and ρ_p = particle density (assumed to be 2.65 g cm^{-3}).

Soil organic matter (SOM) content was determined from loss on ignition at 450°C for 3 hours.

3.3.3 Particle size analysis

Particle size was analysed by laser diffraction at Birmingham on a Mastersizer2000 equipped with Hydro MU wet sample dispersion unit (Malvern Instruments, Malvern, UK). Prior sieving of samples through a 2mm mesh confirmed the absence of a coarse fraction. Samples were treated with sodium hydroxide to remove organic matter (Mikutta, et al., 2005; Anderson, 1963) before being prepared in accordance with the "dry" sample preparation method set out in Sperazza et al., (2004). Approximately 0.1g of oven-dry soil was placed in a 30ml bottle containing 20ml of 5.5g l^{-1} sodium hexametaphospate ($(NaPO_3)_6$) and left for

24 hours to aid dispersion. The entire content of the bottle was then introduced to the laser particle sizer and grain size analysed between the range of 0.02 – 2000μm. Machine operational settings are summarised in Table 3-3 below.

Table 3-3: Summary of laser diffractometer operational settings (after Sperazza et al. (2004)).

Operation	Method details
Sample Preparation and introduction	
Pump Speed	2000 rpm
Sonication	60 seconds with 10 μm tip displacement
Reservoir Dispersant	500 ml of 5.5 g l^{-1} sodium hexametaphosphate with refractive index of 1.478
Machine parameters	
Obscuration	15-20%
Particle Absorption Index	0.1
Particle Refractive Index	1.533

Table 3-4: Manufacturer target tolerance levels for glass bead standards and measured result for particle size analysis by laser diffraction.

	D_{10}	D_{50}	D_{90}
Manufacturer target tolerance			
Upper tolerance level	29.28	47.73	79.31
Target tolerance	28.43	46.80	77.00
Lower tolerance level	26.72	45.39	72.38
Measured Parameters			
Result	28.196	46.518	76.02
% difference	-0.82	-0.60	-1.27

Results for particle size analysis are reported as the average of three consecutive measurements, each of 12 seconds duration. Precision, as a coefficient of variation (CV), was better than 1% for the D_{10}, 2.5% for the D_{50} and 5% for the D_{90} percentile limits. Accuracy of the instrument was determined by use of a 15-120 μm glass microsphere standard (Malvern Instruments) which showed that results were within manufacturer tolerance

levels (i.e. <1.3% deviation from the target tolerance for the tenth, fiftieth and ninetieth percentiles; Table 3-4.

3.4 CHEMICAL METHODS

3.4.1 Soil pH

Measurements of soil pH were performed with a bench-top pH meter (Hannah Instruments) equipped with glass electrode following the method described by Thomas (1996). Approximately 10g of air-dried soil was weighed into a 50ml beaker to which 10ml of de-ionised water (DIW) was added. The content of the beaker was mixed well and allowed to stand for 10 minutes before the pH of the slurry was determined three times in close succession. Values are the average of these three readings and are reported as pH_w. Buffer solutions of pH 4 and pH 7 were used to calibrate the electrode to the second decimal place after every 5 measurements.

3.4.2 Inorganic N

2M KCl was used to extract inorganic nitrogen (NH_4^+, NO_3^- and NO_2^-) from 10g of field-moist soil using a soil:extractant ratio of 1:5 (w:v) (Bremner & Keeney, 1966). Sample preparation differed slightly between the two years' sampling and as such caution needs to be employed in comparing soil inorganic nitrogen concentrations between years. Specifically, during the inter-monsoon extraction took place approximately 24 hours after collection and storage at ambient temperature. By contrast at the end of the wet season, samples were weighed in the field immediately following extraction into a pre-prepared bottle containing 50ml 2M KCl then placed in a cool bag for transportation back to the laboratory. Samples were kept on ice to minimise the stimulation of NO_3^- production and the repression of NH_4^+

production that can occur where soils are held in storage prior to extraction, (Arnold, et al., 2008). Once returned to the laboratory, bottles were shaken for 1 hour before being filtered through a Whatman 42 filter. Samples were then frozen pending transportation to the UK and subsequent analysis. During transit from Sabah to Birmingham, frozen samples defrosted despite insulation but were still cold to the touch and were immediately refrozen on arrival.

Table 3-5: Methods of determination for limit of detection for inorganic nitrogen content in 2M KCl extracts.

Inorg. N	Method	λ (nm)	LOD
NH_4^+	Salicylate method	667	0.003 mg NH_4^+ l^{-1}
NO_3^-	Reduction to NO_2^- by copperised Cd & Griess-Ilosvay method	540	0.01 mg NO_3^- l^{-1}
NO_2^-	Griess-Ilosvay method	540	0.001 mg NO_2^- l^{-1}

NH_4^+, NO_3^- and NO_2^- were determined colorimetrically on a double beam spectrophotometer (Jenway 6800, Bibby Scientific). A summary of each method of determination together with the operational wavelength and limit of detection (LOD) is set out in Table 3-5. The LOD was determined at the 95% confidence interval from the standard deviation of 10 replicate blanks using the equation:

$$LOD = SD \cdot t \qquad \text{Equation 3-4}$$

where *LOD* = limit of detection (mg l^{-1}), *SD* = standard deviation of the blanks, and *t* = the student's *t* statistic at the 95% confidence interval for *n-1* degrees of freedom.

3.4.2.1 Ammonia

A modified version of the indophenol blue method of Keeney and Nelson (1982) was used for determination of NH_4^+ (Mulvaney, 1996). An aliquot of 0.5-5 ml of KCl-extracted sample

was placed in a 25 ml volumetric flask to which was added 1ml of 6% Na_2EDTA (disodium salt of ethylenediameinetetraacetic acid) and 4 ml of a sodium salicylate-sodium nitroprusside reagent (78.13g l^{-1} of $NaC_7H_5O_3$ and 1.25g l^{-1} of $Na_2Fe(CN)_5NO \cdot 5H_2O$). The contents were brought to ~20 ml volume by the addition of DIW before 2 ml of a buffered hypochlorite reagent at pH 13 (29.6g l^{-1} of NaOH, 99.6g l^{-1} of $Na_2HPO_4 \cdot 7H_2O$, and 10 ml of NaOCl solution) was added. Flask content was brought to volume and left in a 37°C water bath for 30 minutes for colour to develop. Once removed from the bath, the sample was allowed to cool before being measured colorimetrically with reference to a set of six standards containing between 0-20µg NH_4^+-N.

3.4.2.2 Nitrate

For analysis of NO_3^-, the sample was first passed through a copperised cadmium column in order to reduce to NO_3^- to NO_2^- (Keeney & Nelson, 1982; Dorich & Nelson, 1984). A 100ml volumetric flask was attached to the outlet of the column and 2-5ml of sample flushed through by the addition of 75ml of 13g l^{-1} NH_4Cl solution. The resulting solution was collected in the 100ml flask (containing both the initial NO_2^- present in the soil and the NO_2^- formed by column reduction of NO_3^-) and then the total NO_2^- concentration determined via the Griess-Ilosvay method described below. Initial NO_2^- concentrations were determined separately to and deducted from the total NO_2^- value to give the final concentration of extractable soil nitrate.

Calibration was performed by passing a set of six standards through the column of known NO_3^- concentration. Column efficiency at reducing NO_3^- was tested every fifth sample against a parallel set of NO_2^- standards that had not passed through the column but which

were analysed via the Griess-Ilosvay method. Linear reductions in column efficiency over time were adjusted for and a new column prepared where efficiency fell below 90%.

3.4.2.3 Nitrite (Griess-Ilosvay method)

For analysis of NO_2^-, a 5ml aliquot of sample was placed in a 100ml flask. The flask was brought to 75ml volume by the addition of DIW and 2ml of a diazotizing reagent (5g l^{-1} sulphanilamide in 2.4 M HCl) introduced. The solution was mixed and allowed to stand for 5 minutes before adding 2ml of a coupling reagent (2g l^{-1} N-(1-napthyl)-ethylenediamine dihydrochloride in 0.12 M HCl) and bringing the flask to volume. Flasks were then placed in the dark for 20 minutes to allow colour to develop before being analysed on the spectrophotometer at 540 nm.

3.5 BIOGEOCHEMICAL METHODS

3.5.1 Gross mineralisation, immobilisation and nitrification

Gross rates of mineralisation and nitrification were measured using the isotope pool dilution method of Kirkham & Bartholomew (1954). The technique involves labelling the end product pool and is generally preferred to tracer methods that artificially stimulate microbial activity by providing the substrate necessary for the process of interest to occur (Hart, et al., 1994).

Rates of gross mineralisation were estimated by enriching the soil with $^{15}NH_4^+$ and then observing dilution of the atomic % enrichment over time as ^{14}N is mineralised and added to the total NH_4^+ pool. Gross consumption of NH_4^+ was also estimated by observing the change in the size of the NH_4^+ pool over time. The principle is identical for measurements of gross nitrification and NO_3^- consumption but with the substitution of $^{15}NO_3^-$ for $^{15}NH_4^+$. Whilst

consumption processes remove both ^{14}N and ^{15}N from the total NH_4^+ pool over the incubation period, it is assumed that these processes draw on the heavy and light isotope equally and that that they do not affect ^{15}N abundance. In addition, it is assumed that the measured process rate is constant over the time of the incubation and ^{15}N is not re-mineralised during the incubation period. Although some discrimination in favour of the lighter ^{14}N does take place in microbial N transformations (Delwichs & Steyn, 1970), any error associated with it is considered to be small in incubations of a few days or less where enrichment is greatly above natural abundance (Davidson et al 1991). Where errors do occur due to microbial discrimination, it is often the result of heterogeneous distribution of the applied ^{15}N through the soil profile resulting in incomplete mixing with the ambient ^{14}N pool (Watson, et al., 2000). These errors can be minimised by repeated injections of the label through the soil core as set out in Davidson et al,. (1991). The second assumption (that rates remain constant throughout the incubation period) may lead to large errors in some circumstances, for example, where application of ^{15}N wets the soil after a prolonged drought, or where soil has been fumigated prior to addition of the label. Soils sampled here were not prone to water stress, nor were they fumigated and in all probability the error occurring from the assumption of linearity is likely to be less than the experimental error (Bjarnason, 1987). The final assumption, that remineralisation of ^{15}N does not occur over the incubation period, is probably not an issue for incubations of less than a few days such as the 1-day incubation used in this study (Bjarnason, 1987; Davidson, et al., 1991).

To measure gross rates, eight replicate plots were established in each site and four intact cores (4cm diameter x 10cm length), at each of those replicate locations, driven into the soil. Once removed, cores were capped at the surface end and inverted to allow injection of ^{15}N solution through the bottom. The two cores used to determine mineralisation each received 6ml of

$^{15}(NH_4)_2SO_4$ solution (Sigma Aldrich 98 atomic % ^{15}N). The remaining two cores were used to estimate nitrification, and accordingly 6ml of $K^{15}NO_3^-$ (Sigma Aldrich 98 atomic % ^{15}N) was substituted for ammonium. Both the $^{15}NH_4^+$ and the $^{15}NO_3^-$ solutions contained 30 mg N l^{-1} thereby enriching the soil by approximately 2 μg N g^{-1}. Injections were made 1ml at a time with an 18 gauge spinal needle that had been sunk through the bottom of the core to a depth of 1cm from the capped end. The ^{15}N solution was injected by slowly depressing the syringe and drawing the needle upwards through the core to ensure even distribution of the heavy isotope through the soil profile and homogenous mixing with ambient ^{14}N. Any solution that had collected in the capped end was distributed back through the soil by turning the core upright.

Following injection, one core from each pair (the T_0 core) was removed from the PVC tube, immediately placed in a plastic bag and homogenised. A sub-sample of 10g from the homogenised soil was then placed in a 120ml Nalgene bottle containing 50ml of 2M KCl that was placed on ice pending transportation back to the laboratory. The time between injection and introduction into the KCl was approximately 15 minutes. The remaining core from each pair (the T_1 core), was returned to the soil to be incubated at ambient temperature. After 24 hour of incubation, the T_1 cores were extracted in the same manner as the T_0 cores. Mean recovery rates of the added ^{15}N label from the T_0 cores 10 minutes after addition were 63±7% for NH_4^+ and 26±5% for NO_3^-. Once back in the laboratory, inorganic N was extracted as per Section 3.4.2 with the modification that slurries were filtered through pre-leached Whatman No.1 filters. Filtrates were frozen until transportation back to the UK for analysis. NH_4^+ and NO_3^- was determined as per the modified Griess-Ilosvay and cadmium reduction methods respectively described in Sections 3.4.2.1 and 3.4.2.2 above.

Samples were prepared for isotopic analysis using the diffusion procedure set out in Brooks, et al., (1989). 20-40ml of KCl filtrate was placed in a 150ml plastic urine cup together with 20ml of a carrier solution containing 40μg of ^{14}N in the form of either $(NH_4)_2SO_4$ (for $^{15}NH_4^+$ amended cores) or KNO_3^- (for $^{15}NO_3^-$ amended cores). The purpose of the carrier solution was to ensure a sufficient N signal during ^{15}N analysis by isotope ratio mass spectrometry (IRMS). Where the solution held $^{15}NH_4^+$, a 7 mm filter disc (Whatman No.3) loaded with 10μl of 2.5M $KHSO_4$ was suspended on wire in the lid of the urine cup. 0.2g of MgO was then added before the lid was quickly attached so that the filter disc was suspended above the sample. The MgO functioned to make the solution basic thereby releasing NH_3 vapour into the headspace to be trapped on the acidified filter disc. Filtrates containing KNO_3^- also had 0.2g of MgO added to them but the lids were not replaced immediately. Instead, the cups were left open for six days to allow any NH_4^+ present in the sample to escape from the solution. After the six days had passed, an acidified filter disc and 0.4g of Devarda's alloy (which reduces NO_3^- to NH_4^+) was introduced to the cups before the tops were closed and the solution gently swirled. The filter discs were removed from the urine cups after 6 days and were then placed in a desiccator for 48 hours to dry. Once dry, discs were wrapped in tin caps and analysed for ^{15}N enrichment by IRMS on a continuous-flow Isoprime™ IRMS connected to an Elementar PYRO cube© in the University of Birmingham stable isotope facility.

The equations of Kirkham and Bartholomew (1954) were used to calculate rates of mineralisation and consumption as follows:

$$m = \frac{M_0 - M_1}{t} \cdot \frac{\log\left(\frac{H_0 M_1}{H_1 M_0}\right)}{\log\left(\frac{M_0}{M_1}\right)} \qquad \text{Equation 3-5}$$

$$c = \frac{M_0 - M_1}{t} \cdot \frac{\log\left(\frac{H_0}{H_1}\right)}{\log\left(\frac{M_0}{M_1}\right)}$$ Equation 3-6

Where $m > c$ *and* m = the rate of mineralisation (μg N g^{-1} dry soil d^{-1}); c = the rate of consumption (μg N g^{-1} dry soil d^{-1}); t = the time period of the incubation (days); M_0 = the $^{14+15}$N pool at T_0 (μg N g^{-1} dry soil); M_1= the $^{14+15}$N pool at T_1 (μg N g^{-1} dry soil); H_0 = the ^{15}N pool at T_0 (μg N g^{-1} dry soil); H_1 = the ^{15}N pool at T_1 (μg N g^{-1} dry soil). Where $c > m$ or $m = c$ then the alternative equations set out in Kirkham and Bartholomew (1954) are substituted for equations 3-5 and 3-6. Nitrification rates were calculated from the same equations with the substitution of n for m.

To account for the effect that the carrier solution and background N had on the atom% ^{15}N enrichment of the samples, calibration was performed using the following equation of Kelley et al., (1991):

$$A = \frac{A_s M_{s+c} - M_c A_c}{M_{s+c} - M_c}$$ Equation 3-7

where A is the corrected atom% ^{15}N enrichment; A_s is the atom % ^{15}N enrichment of the sample and the carrier solution; M_{s+c} is the mass of N in the sample and the carrier solution recovered on the filter (μg); M_c is the mass of N recovered on the filter from the carrier; and A_c is the atom % ^{15}N enrichment of the carrier solution (assumed to be 0.366%).

A comparison of diffusion standards containing 3.5 atom% ^{15}N and ranging from 10 to 80μg N with the laboratory working standard (IAEA N1 $(NH_4)_2SO_4$ $\delta^{15}N$ = 0.4‰) showed that N recovery averaged 94% (SD 11) and 94% (SD 10) for NH_4^+ and NO_3^- respectively. Mean atom% ^{15}N of the standards was 3.4546 (SD 0.16). Where recovery of N on the filter is

incomplete, fractionation during the diffusion process can result in atom% enrichment errors (Lory & Russelle, 1994). In their evaluation of the diffusion method, Khan, et al., (1998) found that for samples containing NH_4^+-N a 75% recovery rate resulted in an error of <1% but this decreased to 0.2% when recovery was 99%. For NO_3^--N errors were 4.2% and 0.8% where recovery was 73% and 99% respectively. On this basis all samples with <80% recovery were excluded from the analysis to keep methodological error for this step <5%. The multiple steps involved in calculating gross rates though isotope pool dilution can result in the accumulation of analytical error throughout the experiment. The total propagated methodological error for rates of gross mineralisation and nitrification were 4% and 7% respectively. However, the greatest source of error in the in-situ isotope pool dilution experiment results from the estimate of the initial pool size at t_0. Although not employed for this study, Davidson et al. (1991) propose the extraction of concentric soil cores with the outer core being used to estimate the initial pool size. Meanwhile, the inner core is injected with ^{15}N and then used to estimate the extraction efficiency at t_0. However, in heterogeneous soils, errors of >20% have still been observed with this revised procedure (Stark, 2000). Given, in this case, the size of the t_0 pool is estimated only from the t_0 core pool size prior to addition of the $^{15}NO_3^-$, there is likely to be a large degree of uncertainty in the reported rates of gross mineralisation and nitrification reported for this thesis and errors in excess of 20% cannot be ruled out.

3.5.2 Denitrification and DNRA

3.5.2.1 N_2O emission and in-situ denitrification

In situ denitrification was estimated from the production of N_2O in static chambers using an acetylene block. Static chambers consisted of capped 110mm diameter PVC soil bossed pipes (Osma 4S583G) with side port for sample extraction and venting. At each of the 12 replicate

locations, two chambers were sunk into the soil to a depth of 5cm. One chamber in each pair received no amendment from which samples were drawn to estimate ambient N_2O production. The other chamber was treated with acetylene gas (C_2H_2) applied in the form of 8g of granular CaC_2 (Sigma Aldrich) prior to closing (Aulakh, et al., 1991). The purpose of the C_2H_2 was to prevent complete reduction of NO_3^- by blocking operation of the nitrous oxide reductase thereby releasing N_2O rather than N_2 as the end product of denitrification. Sampling with and without the acetylene block yielded an estimate of total denitrification ($N_2O + N_2$) and allowed the ratio of N_2O:($N_2O + N_2$) emission to be determined. Samples were extracted immediately after closing the chamber (t_0), and after one (t_1) and two hours (t_2) of incubation which permitted collection of soil cores for analysis of soil variables in the interim period. However, results reported here are only for the two hour (t_2) incubation. Each 4ml sample was transferred to a 3ml Exetainer vial (Labco Ltd., High Wycombe, UK) for transport to the UK for analysis. Vials were over-pressurised to minimise inward gas diffusion during transport. N_2O concentrations were determined within one month of sample collection by gas chromatography (Perkin Elmer Clarus 500 fitted with a ^{63}Ni electron capture device) in Birmingham using a pure N_2 carrier gas. Chromatograph signal (area (mv)) was converted to parts per million (ppm) using three standards of 20, 50 and 100ppm (Standard and Technical Gases Ltd). Concentration (ppm) were then expressed as time averaged emissions in μg m^{-2} h^{-1} using the following equation:

$$\mu g\ N2O\ m^{-2}h^{-1} = \frac{ppm \cdot 44(g\ mole^{-1}) \cdot 10^6(\mu g g^{-1})}{24.45 \cdot 10^{-3}(m^3 mole^{-1})} \cdot \frac{V(m^3)}{A\ (m^2) \cdot t(h)} \qquad \text{Equation 3-8}$$

Where *ppm* = the calibrated value from the GCMS expressed as a decimal; *44* = the molar mass of N_2O (g $mole^{-1}$); *V* = static chamber volume (m^3); *24.45* = litres of air occupied by 1

mole of gas at 25°C and 1 atm; A = chamber area (m^2); t = time of incubation. t_1 results were calculated from the mean of three replicate samples. Precision as a coefficient of variation was better than 5% and N_2O emission is reported as the difference between the t_1 and t_0 incubation results where a linear rate of gas accumulation was assumed.

Although chambers were placed immediately adjacent to the area where soil cores were extracted, the use of disparate soil samples for in-situ denitrification and determination of general soil parameters may lead to some incomparability when conducting the subsequent correlation analysis. To circumvent this, rates of potential denitrification were also measured from the production of ^{15}N-N_2 following application of $K^{15}NO_3$ to incubated slurries that incorporated a sub-sample of the soil from which general soil characteristics were derived. Measuring potential denitrification rates also provided an additional denitrification estimate that was free of the rate under-estimation problems inherent in the C_2H_2 approach: Namely, inhibition of nitrification, incomplete diffusion in fine textures or saturated soils, decomposition of C_2H_2 and suppression of microbial respiration (Groffman, et al., 2006).

3.5.2.2 Potential denitrification and DNRA

Potential rates of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) were estimated by addition of ^{15}N to anaerobic soil slurries using a modified method of Trimmer et al. (2003) described in Lansdown et al. (2012). Approximately 1g of field-moist soil was placed in a 3ml Exetainer, filled to the top with DIW and incubated at ~28^0C for 24-36 hours. This pre-incubation was to deplete ambient $^{14}NO_3^-$ and $^{14}NO_2^-$ to below detection limits for NO_3^- analysis and has been shown to be 96.3±0.7% successful for slurries containing ~500μM $^{14}NO_3^-$, (Sgouridis, 2010). An injection of 125 μl of 9.8 mM $K^{15}NO_3$ (98^+ atom% ^{15}N Sigma Aldrich) through the rubber septa enriched the slurries by 250 μM $^{15}NO_3^-$ which

was marginally higher than the average ambient slurry $^{14}NO_3^-$ concentration of 212 μM $^{15}NO_3^-$ across all sites. Vials were shaken and incubated for 0.5-2.5 hours before denitrification activity was stopped by injection of 100 μl 50% w/v $ZnCl_2$. Production of ^{15}N-N_2 gas was determined by continuous flow IRMS (Thermo-Finnegan, Delta Matt Plus) following creation of a 1ml helium headspace above the slurry in Queen Mary University, London (Trimmer & Nicholls, 2009; Lansdown, et al., 2012). NO and N_2O were reduced to N_2 by passing through reduction and oxidation columns at 980°C. Separation of N_2 was conducted on a GC Porapak column (PoraPlot Q). Precision as a coefficient of variation was better than 1%. Increase in ^{30}N abundance was determined by reference to control vials that did not receive any ^{15}N and whose microbial activity was stopped at the beginning of the experiment with ZnCl. Pre-incubation of the vials should have removed the ambient nitrate within the slurry resulting in primarily the production of $^{30}N_2$ gas from denitrification after the addition of $^{15}NO_3^-$. As the added NO_3^- was 98% ^{15}N, the predicted proportion of $^{29}N_2$ from denitrification should therefore be less than 2% (i.e. $P^{29}N = 0.02 + (2*0.98)$). However, many of the samples showed $^{29}N_2$ in concentrations substantially above this predicted amount indicating that anammox was likely occurring within these slurries. Accordingly, denitrification of the $^{15}NO_3^-$ spike is reported only as the production of $^{30}N_2$ in mg N kg^{-1} d^{-1} from:

$$p^{30}N_2 = \left[\left(\frac{^{30}N_2}{\Sigma N_2}\right)_s - \left(\frac{^{30}N_2}{\Sigma N_2}\right)_r\right] \cdot \Sigma N_{2_{sample}} \cdot \alpha^{-1} \cdot V \cdot 30 \cdot \frac{1}{m} \cdot 10^{-6} \cdot t^{-1} \qquad \text{Equation 3-9}$$

where $p^{30}N_2$ is the excess $^{30}N_2$ in the headspace of the Exetainer; $\frac{^{30}N_2}{\Sigma N_2}$ is the ratio of heavy isotope to total N_2 signal for either sample (s) or reference (r); α is a calibration factor determined by reference to atmospheric air; V is the volume in the headspace (ml); *30* is the

molecular weight of N_2; *m* is the dry mass of soil in the vial (g); and *t* is the time of the incubation (h). An estimate of the aerial rate of denitrification potential in g N m^{-2} d^{-1} was obtained from:

$$Denitrification_{potential} = p^{30}N_2 \cdot \rho_d \cdot 10 \cdot 0.1m \qquad \text{Equation 3-10}$$

where $p^{30}N_2$ is the denitrification of the added ^{15}N label derived from Equation 3-9; ρ_d is the bulk density of the soil derived from Equation 3-1; 10 is the unit conversion factor; and 0.1 is the depth sampled (m).

The same slurries used to estimate denitrification potential were also used to assess DNRA potential by quantifying the increase in $^{15}NH_4^+$ abundance over the course of the incubation. Following determination of ^{15}N-N_2 production from denitrification, the slurries were transferred to a centrifuge tube containing 15ml of 2M KCl and shaken for 1 hour. The slurry was centrifuged for 5 minutes (2000 rpm) and the supernatant filtered through a 0.45µm ptfe syringe filter. 1ml was transferred to a new Exetainer through which helium was bubbled for 20 minutes to remove trace ^{15}N-N_2 from denitrification. An aliquot of the supernatant was also used for determination of NH_4^+concentration as described per Section 3.4.2.1. $^{15}NH_4^+$contained in the vial was converted to ^{15}N-N_2 gas by hypobromite oxidation as per Risgaard-Petersen, et al. (1997), and subsequently quantified by IRMS. Due to the low (<1 atomic %) ^{15}N enrichment of the samples, the equation of Plazner (1997) was used to calculate atomic % enrichment from:

$$A^{15}N = \frac{100}{(2 \cdot \frac{^{28}N_2}{^{29}N_2} + 1)} \qquad \text{Equation 3-11}$$

The ^{15}N of the headspace atmospheric air mix was calculated from the following equation adapted from Fry (2006):

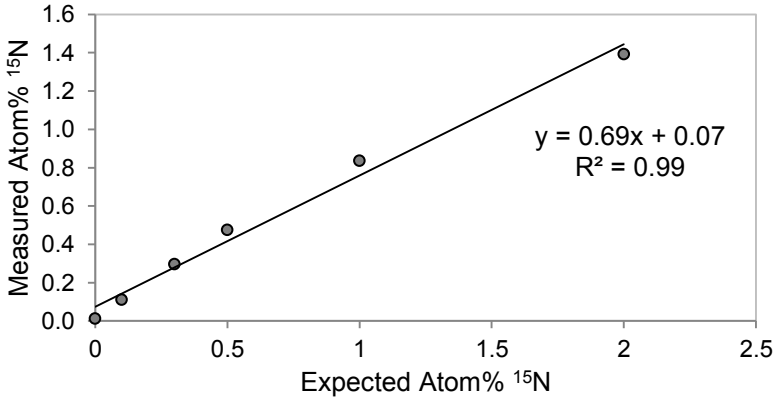
$$^{15}N\%_{mix} = \frac{(M_{air} \cdot {}^{15}N_{air}) + (M_{sample} \cdot {}^{15}N_{sample})}{(M_{air} + M_{sample})} \qquad \text{Equation 3-12}$$

Where M_{air} and M_{sample} are the amount of N_2 deriving from the atmospheric background and the sample respectively (μmol); $^{15}N_{air}$ is the atomic % of the background N_2 and $^{15}N_{sample}$ is the atomic % of the sample N_2. Combining Equations 3-12 and 3-13 gives an estimate of the true enrichment of the sample by:

$$^{15}N_{sample}\text{at.}\% = \frac{[(\text{A}^{15}\text{N}) \cdot (M_{air} + M_{sample})] - (M_{air} \cdot {}^{15}N_{air})}{M_{sample}} \qquad \text{Equation 3-13}$$

The efficacy of the hypobromite oxidation and validity of Equation 3-14 were examined through testing of five standards containing 0.1 to 2% ^{15}N with a final NH_4^+ concentration of 1mM. Measured and expected values agreed well and did not differ statistically (Figure 3-4).

Figure 3-4: Measured ^{15}N atom % versus expected values in 1mM NH_4^+ following hypobromite oxidation.



Finally, the production of DNRA (mg kg^{-1} d^{-1}) was calculated as excess over the reference from the equation:

$$pDNRA\ (mg\ N_2\ kg^{-1}d^{-1}) = \frac{[^{15}N_{sample} - {}^{15}N_{ref}]}{100} \cdot NH_4^+ \cdot 5 \cdot V \cdot 15 \cdot \frac{1}{m} \cdot 10^{-6} \cdot t^{-1}$$

Equation 3-14

Where $^{15}N_{sample}$ and $^{15}N_{ref}$ are the atomic percent enrichment of the sample and reference calculated from Equation 3-14; NH_4^+ is the ammonium concentration (μM); *5* is the dilution factor applied when extracting the solution (15ml KCl:3ml slurry); *V* is the volume of the solution; *15* is the atomic mass of N; *m* is the dry weight of soil (g); and *t* is the incubation time. Areal rates were calculated in accordance with Equation 3-11.

3.5.3 Soil and plant ^{15}N and ^{13}C

Subsamples of the homogenised soil cores (*n* = 12 per site) were separated after collection and immediately air dried before return to the laboratory. A composite sample of vegetation was also taken within the vicinity of soil core collection from (primarily) herbaceous species. In the secondary forest sites, vegetation was naturally abundant but, in one oil palm site (plantation 5Y), understory vegetation was scarce and consisted almost exclusively of young oil palm seedlings that had not yet been removed through intensive plantation management. Accordingly, vegetation samples from 5Y consisted only of young palm leaves. Six composite vegetation samples (minimum 3 species) were taken from the remaining nine locations and then air dried before transportation to the UK.

Soil samples were analysed in Paris at BioEMCo's (Continental Ecosystems Biogeochemistry and Ecology) stable isotope facility in the University Pierre and Marie Curie. Soils were ground to a powder and 10-40 mg placed in a tin capsule before elemental analysis (Vario PYRO cube, Elementar, Hanau, Germany) coupled to IRMS (Isoprime, micromass, Manchester, UK). Capsules were dropped into the combustion furnace at 1120°C, passed through the reduction furnace (850°C) and reduced to N_2 prior to separation by molecular

sieve and analysis of ^{15}N and ^{13}C content. Results were adjusted by use of a tyrosine working standard (^{15}N = 9.98‰ and ^{13}C = -23.2‰). Precision was 0.2‰ for ^{13}C and 0.3‰ for ^{15}N. Vegetation samples were ground and ~2 mg placed in a tin capsule for N elemental isotopic analysis at the University of Birmingham stable isotope facility. Results were adjusted by use of IAEA-N1 ammonium sulfate ($(NH_4)_2SO_4$; $\delta^{15}N$ = +0.4) working standard.

The difference in ^{15}N natural abundance of soil and plants is expressed as $\delta^{15}N$(‰) relative to the ^{15}N of atmospheric N_2 (0.3663%), and is calculated from:

$$\delta^{15}N‰ = 1000 \cdot \left[\frac{\left(\frac{^{15}N}{^{14}N}_{sample} \right)}{\left(\frac{^{15}N}{^{14}N}_{standard} \right)} - 1 \right] \qquad \text{Equation 3-15}$$

The ^{13}C natural abundance of soil is expressed as $\delta^{13}C$(‰) relative to the ^{13}C of Pee Dee Belemnite (PDB) where the $^{13}C/^{12}C$ ratio is 0.0112 and is calculated from:

$$\delta^{13}C‰ = 1000 \cdot \left[\frac{\left(\frac{^{13}C}{^{12}C}_{sample} \right)}{\left(\frac{^{13}C}{^{12}C}_{standard} \right)} - 1 \right] \qquad \text{Equation 3-16}$$

The natural abundance ^{15}N of leaves and soil was used to calculate an average per mil enrichment factor (ε) of the product (the soil) relative to the substrate (the leaves) from the Rayleigh equation as per the approximation given in Mariotti et al (1981) where:

$$\varepsilon = \frac{\ln \delta_s - \delta_{so}}{\ln f}$$ Equation 3-17

Such that δ_s is the δ^{15}N‰ of the soil, δ_{so} is the δ^{15}N‰ of the leaves and f is the fraction of total N remaining in the soil (i.e. (soil total N%)/(leaf total N%)).

3.5.4 Statistical methods

Prior to analysis, data were tested for normality and homogeneity of variance using the Kolmogorov-Smirnov test and Levene's test. For normally distributed data, parametric tests were used. If data did not meet the requirements of homogeneity of variance, results are reported using robust estimates. For non-normal data, non-parametric equivalent tests were employed and where non-parametric tests were not available, data were transformed prior to analysis. If following transformation, the requirements of normality were still not met, then robust measures were employed (i.e. bootstrapping or trimmed means). Effect sizes for post-hoc contrasts are calculated from:

$$r = \sqrt{\frac{t^2}{t^2 + df}}$$

Where t^2 is the *t*-statistic for the comparison and df is the number of degrees of freedom.

Analysis was conducted using SPSS v.20 (IBM Corp.) or R v.2.15.3(Development Core Team 2013).

CHAPTER 4:CHARACTERISATION OF SECONDARY FOREST SITES IN THE LOWER KINABATANGAN, SABAH

4.1 INTRODUCTION

It is a common generalisation that tropical forests are nitrogen rich and phosphorus poor. However, this often-recited abstraction is a gross over-simplification of the vast biodiversity and complexity contributing to biogeochemical heterogeneity in tropical soils. Factors such as soil and vegetation type, climate and altitude are as diverse in the tropics as they are in any other biome. In many tropical forests, nitrogen has been observed to accumulate and be recycled at faster rates than equivalent temperate forests (Jenny, 1950; Vitousek, 1984; Vitousek & Sanford, 1986; Matson, et al., 1999; Houlton, et al., 2006; Davidson, et al., 2007; Brookshire, et al., 2012). It does not mean that an accelerated internal nitrogen cycle equates to an absence of nitrogen limitation. Rather, rapid decomposition and turnover could equally be a prelude to N loss, particularly where rates of nitrification are high (Robertson, 1984; Templer, et al., 2008; Corre, et al., 2010). This propensity for N loss, coupled to intensive competition for nutrients, may therefore be the reason for the conservative N strategies observed in some tropical soils (Harte & Kinzig, 1993; Silver, et al., 2001; Templer, et al., 2008). These two conflicting paradigms, i.e. a leaky, up-regulated N cycle versus a conservative, but N-rich one, underpin the ambiguities present in tropical nitrogen biogeochemistry.

For old-growth forests at low elevations, large inorganic N losses might be indicative of nitrogen saturation rather than an up-regulated inefficient system (Hall & Matson, 1999; Brookshire, et al., 2012). However, the majority of tropical forests do not fall into this

category of intact or near-pristine environments. Specifically, disturbed and regenerating forests cover almost 60% of forested land worldwide (FAO, 2010): a figure that is mirrored in Sabah, Malaysia, where most undeveloped land falls within the definition of secondary forest as proposed by Chokkalingam & De Jong, (2001), namely that they:

> *"…are forests regenerating largely through natural processes after significant human and/or natural disturbance of the original forest vegetation at a single point in time or over an extended period, and displaying a major difference in forest structure and/or canopy species composition with respect to nearby primary forests on similar sites."*

The dominance of secondary forests within the tropical landscape affirms their increasing importance both economically and environmentally. For example, secondary forests provide a local food and timber resource (Kanel & Shrestha, 2001; Gavin, 2009), help maintain species biodiversity (Brown & Lugo, 1990a; Turner & Corlett, 1996; Chazdon, et al., 2009), act as carbon sink in offsetting regional and global CO_2 emissions (Silver, et al., 2000a; Feldpausch, et al., 2004) and mitigate alterations to hydrological function in deeply weathered soils (Bruijnzeel, 2004; De Graff, et al., 2012; Yang, et al., 2010). Additionally, with more than half of the world's forests classified as secondary, land use change is more likely to involve the conversion of secondary, rather than primary, forests (FAO, 2010). As such, within regions like Kinabatangan, where no primary forest remains outside of protected nature reserves, secondary forests provide an appropriate baseline by which to assess the impact of land use change.

The growing prominence of secondary forests is reflected in the large body of research directed towards understanding their recovery post-disturbance. Following activities such as logging and burning, substantial reductions in soil C and N can result through biomass

removal, losses to the atmosphere, erosion and leaching, (Matson & Vitousek, 1987; Kauffman, et al., 1995; Malmer, 1996; Ellingson, et al., 2000; Herbert, et al., 2003; Davidson, et al., 2004). Long-term C and N recovery is dependent on, inter alia, the nature and intensity of previous land use (Vitousek, 1984; Erickson, et al., 2001; Silver, et al., 2000a), climate (Marín-Spiotta & Sharma, 2013; Raich, et al., 2006), soil type (Nardoto, et al., 2008), initial soil nutrient status (Erickson, et al., 2001; Knops & Tilman, 2000) and presence or absence of N fixers (Batterman, et al., 2013). The multitude of factors contributing to soil recovery, therefore, means that patterns of nitrogen accumulation through successional development remain poorly understood. Nevertheless, if secondary forests are to be used as the baseline by which changes in soil processes are compared, the developmental stage of the forest may be an important consideration for the choice of representative site. For example, young successional forests may have low C and N storage (relative to mature forests) resulting in little change in soil carbon and nitrogen following land conversion. Perhaps then, it is no surprise that with regard to C and N status, contradictory trends have been reported in secondary forest chronosequence (space-for-time) studies. There is evidence that both supports (Vitousek, et al., 1989; Davidson, et al., 2007; Amazonas, et al., 2011; Don, 2011; Knops & Tilman, 2000; Feldpausch, et al., 2004) and refutes (Ewel, et al., 1991; Neumann-Cosel, et al., 2011; Martin, et al., 2013; Marín-Spiotta & Sharma, 2013; Sierra, et al., 2012) the incremental accrual of C and N with time since disturbance. Patterns of nitrogen cycling during forest recovery are also difficult to categorise through successional stage. The same controls of land use history, climate, soil and vegetation type apply equally to process rates as they do to nutrient status. Though in some cases, rates of N mineralization and/or nitrification are seen to be following a trajectory of recovery towards a state more akin to that of primary forests (Robertson, 1984; Vitousek, et al., 1989; Davidson, et al., 2007; Amazonas, et al.,

2011; Pérez, et al., 2004; Yan, et al., 2009). In others, patterns of increased (Groffmann, et al., 2001; Erickson, et al., 2001) or decreased (Yan, et al., 2009; Marin-Spiotta, et al., 2009) mineralisation and nitrification in mid-successional forests have been reported. Whether successional trends are observed or not, increased nitrification is accompanied by a greater potential for nitrogen loss (Neill, et al., 1995; Davidson, et al., 2000; Davidson, et al., 2007), although actual losses will depend on nitrogen conservation mechanisms. For example, N loss may be mitigated where retention through dissimilatory nitrate reduction to ammonia (DNRA) is substantial or where plant and microbial competition for N is strong (Keller & Reiners, 1994; Davidson, et al., 2000; Erickson, et al., 2001; Robertson & Tiedje, 1988; Silver, et al., 2001; Ewel, et al., 1981).

The uncertainties outlined above raise a number of questions about the secondary forests sampled for this study. The objective of this chapter is therefore to examine indicators of N status and cycling capability within each of the forests undergoing secondary succession spanning an age range from 16 years post-disturbance to near-primary state. By examining these indicators, it is expected that the higher aboveground biomass observed in the mid-successional and near-primary forests will be reflected by higher belowground N and C storage. It is also expected that increasing N with forest recovery will result in greater NO_3^- (relative to NH_4^+) availability and greater gaseous losses of N through processes such as nitrification and denitrification. Accordingly, it is hypothesised that forests will follow a trajectory of increasing N availability and loss with time since disturbance as they transition from N conservation towards a more "open" N cycle akin to that of primary forests. If nitrogen cycling does follow predictable patterns through forest development, then the choice of representative site and their successional stage may be further dictated by the historical development of land use change; i.e. a form of path dependence where the future recovery of

the site is dependent on past land use histories. Where, as in this case, the purpose is to determine the effect of land use change from the initial forested state (i.e. primary forest) to agricultural use , late successional forests should be more suitable representatives of the initial soil nutrient status (i.e. the baseline condition) than young secondary forests. However, it is acknowledged that in many cases it may be degraded forests and those that are in the early stages of secondary regrowth that are the primary source of new agricultural land in future, in which case mature forests may not be the most suitable baseline to assess the impact of ongoing land use change.

4.2 MATERIALS AND METHODS

4.2.1 Site description

Four sites were sampled across a gradient of secondary forest succession during April 2012 (Figure 4-1; Figure 3-1, p.47). Of the four sites, three were riparian in nature and were situated on alluvial soils of the Tuaran Association that are periodically inundated by the Kinabatangan River (Table 4-1). The riparian sites are represented by a young riparian forest (YRF), and two mid-successional riparian forests subjected to different

Figure 4-1: The four secondary forest sites at different stages of succession. Clockwise from top left: YRF, SRF1, MDF and SRF2.

Table 4-1: Location, protection status, soil and vegetation details for the four secondary forest sites sampled in the Lower Kinabatangan.

	Young riparian forest (YRF)	Secondary riparian forest (SRF1)	Secondary riparian forest (SRF2)	Mixed dipterocarp forest (MDF)
Location	5°24′11.N 118°01′09.E	5°31′35.N 118°17′26.E	5°24′50.N 118°02′16.E	5°31′52.N 118°04′35.E
Year of protection and classification status[2]	1997-2001 – Bird sanctuary 2005 – Wildlife Sanctuary	1930 – Gazetted 1935 – Amenity FR 1948 – Domestic FR 1956 – Protection FR 1984 – Virgin JR	1997-2001 – Bird sanctuary 2005 – Wildlife Sanctuary	1925 – Gazetted 1971 – Extended 1984 – Virgin FR – central reserve & Protection FR – wider reserve
Soil association [α]	Tuaran	Tuaran	Tuaran	Rumidi
Substrate [α]	Alluvium	Alluvium	Alluvium	Mudstone and Sandstone
Soil classification	Silty loam	Silty loam	Silty loam	Silty loam
Vegetation type [α]	Riparian	Riverine	Riparian	Mixed Dipterocarp
Dominant species	*Colona serratifolia* (Tiliaceae) (58%), *Kleinhovia hospita* (Sterculiaceae) (30%), *Ficus obpyramidata* (Moraceae) (6.6%). Species making up < 3% not reported.[γ]	*Colona serratifolia* (Tiliaceae), *Dillenia excelsa* (Dilleniaceae), *Ficus nota* (Moraceae), *Pterospermum elongatum* (Sterculiaceae) and *Dracontomelon dao* (Anacardiaceae).[β]	*Dillenia excelsa* (Dilleniaceae) (45%), *Vatica rassak* (Dipterocarpaceae) (7.8%), *Xyloma sp.* (Flacourtiaceae) (7.8%), *Mallotus muticus* (Euphorbiaceae) (7.2%) and *Pterospermum macrocarpum* (Sterculiaceae).[γ]	*Polyalthia insignis* (Annonaceae), *Diospyros sp.* (Ebenaceae), *Magnolia sp.* (Magnoliaceae) and *H. beccariana* (Dipterocarpaceae), *Hopea beccariana, Hopea sp., Shorea atrinervosa, S. hypoleuca, Shorea sp*.[β]
Tree recruitment[iii]	~2 ha^{-1} y^{-1}	n.d.	~20 ha^{-1} y^{-1}	n.d.
Mean canopy height	5-10[γ] m	15[β] m	10-15[γ] m	20-25[β] m
Bulk Density (g cm^{-3})	1.11 (0.04)	0.91 (0.05)	1.06 (0.08)	0.87 (0.04)
Sand (%)	20.43 (1.03) a A	27.27 (4.17) b B	14.88 (1.30) a A	25.27 (2.21) A
Silt (%)	54.59 (0.63) a B	51.57 (3.79) b B	59.67 (1.45) abA	50.61 (1.38) A
Clay (%)	24.97 (0.77) a	20.61 (1.15) a	25.45 (0.79) b	24.12 (1.05)
pH_w	4.94 (0.08)	5.16 (0.13)	5.09 (0.11)	5.10 (0.10)
WFPS (%)	67.52 (3.44) a A	48.52 (1.49) ab B	53.77 (4.05) b B	67.74 (3.82) A

Notes: Values reported are means with standard error in parenthesis. Different lowercase letters indicate significant differences between the three riparian sites (one-way ANOVA). Different uppercase letters indicate significant differences between MDF and each of the riparian sites (Student's *t*-test for pairwise comparison). Differences reported as $p < 0.05$. [α] (Acres & Folland, 1975); [β] Conservation Areas Information and Monitoring System (CAIMS), Sabah Forestry Department; [γ] (Penpoul, 2012).

intensities of disturbance (SRF1 and SRF2). It was not possible to sample a primary riparian forest due to extensive logging activity in the latter half of the 20th Century and historical shifting agricultural practices along the meander belt of the Kinabatangan River. However, a *terra firme* mature dipterocarp forest (MDF) was sampled in one of the least disturbed forested areas in the Kinabatangan lowlands. This near-primary site was located ~9 km away from the meander belt adjacent to a shallow 5m-wide tributary flowing over mudstone and sandstone forming part of the Rumidi Association (Table 4-1). Due to differences in vegetation and substrate, it was not possible to include the MDF in statistical analysis of successional status. However, inclusion of the site within the broader analysis of site characterisation provides a useful comparison with what was once the principal land cover in this region.

Site YRF is located on the ridge and swale formation of a meander bend on land that had been clear-cut in September 1997 for oil palm cultivation, though the area is likely to have been logged prior to this. Subsequently, the extent of the plantation fell short of the meander bend and the forest has been allowed to regenerate naturally. In 2005, YRF, along with a further 26 $x10^3$ ha of predominantly riparian vegetation, was designated part of the Lower Kinabatangan Wildlife Sanctuary affording it State government protection status from timber exploitation and hunting (Table 4-1). Tree recruitment has been slow (~2 trees ha^{-1} y^{-1}) resulting in large areas of open canopy and a mean canopy height between 5-10 m (Penpoul, 2012). Pioneering vines and grasses dominate in the open areas of the shrub layer where light penetration is high. Species richness is low with only 8 species recorded throughout the plot as of 2012. *Colona serratifolia* (Tiliaceae) comprised 58% of the total number of trees >10cm diameter at breast height (DBH). There was also a significant cover of *Kleinhovia hospita* (Sterculiaceae) and *Ficus obpyramidata* (Moraceae) (30% and 6.6% coverage respectively; Penpoul, 2012). The prevalence of *Colona serratifolia* in this area may be indicative of past fire damage as it

is a pioneering species and effective coloniser during post-burn forest recovery. *K. hospita* is a light-demanding low-stature (< 20m) species which, being flood-tolerant, is common to riparian vegetation on the alluvial floodplain.

SRF1 lies within the protected Keruak Forest Reserve on a meander levee of the river at the edge of Sukau village. Although the forest has been under State government protection since 1930, the protection status has changed from Amenity forest reserve (FR) in 1935 to Domestic FR by 1948, Protection FR in 1956 and finally Virgin jungle reserve (JR) in 1984. The amenity status established the forest as an area of recreational opportunity for the general public and was partly to protect the edible birds' nest caves within the small limestone outcrop located towards the centre of the reserve. Domestic status allowed hunting and harvesting of minor forest produce (subject to relevant permits) by the local community: an activity that, due to the reserve's proximity to the village of Sukau, has continued illegally through to the present. Protection status prohibits logging and land conversion for the purpose of environmental protection and biodiversity conservation, whereas the Virgin JR status is designed to protect the forest from timber harvest and hunting and aims to conserve the area for scientific research and biodiversity conservation. Tree recruitment data were unavailable for this sampling location although species diversity was relatively poor being dominated by *Colona serratifolia* which in 2001 comprised 48% of total trees (CAIMS, 2001).

SRF2 was also located on an internal meander bend where logging activity (most likely prior to 1995) had cleared almost all large or commercially important tree species from the forest structure. It is not possible to know the full extent and intensity of historical logging activity, however, the forest appears to have recovered well with mean canopy height of 10-15 m and tree recruitment of ~20 trees ha^{-1} y^{-1} (Penpoul, 2012). Species richness was greater than the

YRF and SRF1 although *Dillenia excelsa* (Dilleniaceae) dominated with 45% coverage. Other important species included *Vatica rassak* (Dipterocarpaceae) (7.8%), *Xyloma sp.* (Flacourtiaceae) (7.8%), *Mallotus muticus* (Euphorbiaceae) (7.2%) and *Pterospermum macrocarpum* (Sterculiaceae) (Penpoul, 2012). As for YRF, SRF2 was protected in 2005 under the gazettement of the Lower Kinabatangan Wildlife Sanctuary.

The above-ground vegetation density at the two mid-successional sites was similar in appearance (Figure 4-1). Both sites had a mean canopy height in the region of 15 m, however the level of disturbance at each site (both historically and ongoing) is likely to be very different. For example, SRF1 has been under government protection 75 years longer than SRF2 and some very large (i.e. >30m) trees are extant. Although, it is likely that SRF1's proximity to, and accessibility from, the adjacent village has had a significant impact on the forest quality when compared to the more isolated SRF2. In particular, during the 2001 survey, tyre tracks and spent bullet cartridges were visible in SRF1 suggesting that illegal logging and poaching activity was still occurring. Additionally, there are well-defined paths through SRF1 that are used by both villagers and tourists. The quality of the aboveground vegetation also provides some indication of disturbance level. In SRF1, the presence and persistence of *C. serratafolia* is suggestive of past fire damage and it is likely that at least part of this site was inhabited at some point (CAIMS, 2001). There are also no dipterocarps extant amongst the most numerous species within SRF1 indicating intensive logging in the past. By contrast, SRF2 is accessible only by boat and has minimal disturbance from local fishermen, and staff and researchers at the scientific field centre located nearby. The understory vegetation was better developed in SRF2 than in SRF1 and the presence of *Vatica rassak* (a dipterocarp) may be further indicative of a less disturbed environment.

The *terra firme* MDF falls within the Gomantong Forest Reserve on soils of the Rumidi Association (Table 4-1). The central forest reserve of the MDF was initially gazetted in 1925 and was later extended to the surrounding forest in 1971. Currently, the MDF has the protection status of Virgin JR on account of its importance for tourism and harvest of edible birds' nests. Large areas of both the central and surrounding reserve have been selectively logged in the past, but the forest has regenerated well with a mean canopy height of 20-25 m recorded in 2001. Samples were taken on the border of the central and surrounding reserve where predominantly low stature species were recorded including *Polyalthia insignis* (Annonaceae), *Diospyros* sp. (Ebenaceae), *Magnolia* sp. (Magnoliaceae) and *H. beccariana* (Dipterocarpaceae). Although the MDF is located on a different substrate to that of the riparian forests, soils from all four locations fell into the same soil textural class: that of silty clay loams (Table 4-1).

4.2.2 Soil analysis

Samples were collected and processed in accordance with the methods set out in Chapter 2. Results are reported from the end of wet (2012) sampling season to allow comparison of a full suite of nitrogen cycling variables.

4.2.3 Statistical analysis

Variables were tested for normality using the Kologorov-Smirnov test and for homogeneity using Levene's statistic. Robust measures were employed and are reported where variance did not meet the requirements of homogeneity. Differences between sites were tested with one-way analysis of variance (ANOVA) and post-hoc Tukey multiple comparisons (between riparian sites) or Student's *t*-test (MDF versus each riparian site). Variables violating the assumption of normality were tested using non-parametric Kruskal-Wallace *H*-test with post-

hoc pairwise comparisons (Dunn 1964) or Mann-Whitney *U*-test. Statistical tests were carried out with SPSS v.20 statistical software (IMB SPSS, Inc. Chicago, Il, USA).

4.3 RESULTS

4.3.1 Patterns of C and N storage

Soil carbon and nitrogen storage in both leaves and soil increased from SRF1 to the MDF following a decline from YRF to SRF1 (Table 4-2; Figure 4-2). Mean upper soil (0-10 cm) carbon storage in SRF2 was approximately double that of SRF1 when soil bulk density was accounted for with a one-way analysis of variance between the three riparian forests confirming carbon stocks in SRF1 to be significantly below that of SRF2 ($r = 0.80$, $p = 0.001$) and YRF ($r = 0.61$, $p = 0.018$) (Table 4-2). In the MDF, soil C was significantly higher than that of SRF1 ($r = 0.75$, $p = 0.009$) but not the YRF or SRF2.

Soil carbon and nitrogen were highly correlated ($r = 0.96$, $p < 0.001$) and as such soil N, foliar N and soil $\delta^{15}N$ followed the same dynamic as soil C through the riparian sites (Figure 4-2). Specifically, N storage and soil $\delta^{15}N$ declined from YRF to SRF1 before increasing through SRF1 to the MDF. Differences between riparian sites were significant for both soil N ($F(2, 33) = 32.81$, $p < 0.001$) and foliar N ($F(2,15) = 5.826$, $p = 0.013$) (Table 4-2). Soil N and foliar N were also higher in the MDF than in YRF and SRF1 but not SRF2 (Table 4-2). Soil texture affected mean C and N storage and a decline in both was apparent through the riparian sites as the percentage sand content of soils, however, there were no significant correlations of sand with other variables (Figure 4-3). The C:N ratio did not differ statistically but was marginally higher in SRF2 than the other sites and declined through YRF, SRF1 and MDF did however, it did not differ between riparian and *terra firme* sites (Figure 4-2).

Table 4-2: Mean values for indicators of nitrogen cycling (SE in parenthesis) across the four secondary forest sites together with result for analysis of variance (between the three riparian sites YRF, SRF1 and SRF2) and Student's t-test statistic (between the *terra firme* MDF and each of the riparian sites).

	C (Mg ha^{-1})	N (Mg ha^{-1})	Soil $\delta^{15}N$ (‰)	C:N	Foliar N (%)	Foliar $\delta^{15}N$ (‰)	NO_3^- (g m^{-2})	NH_4^+ (g m^{-2})	Gross min (g m^{-2} d^{-1})	Net min (g m^{-2} d^{-1})	Gross nit (g m^{-2} d^{-1})	Net nit (g m^{-2} d^{-1})	DNRA (g m^{-2} d^{-1})	N_2O-N (g m^{-2} d^{-1})	(N_2O+N_2) -N (g m^{-2} d^{-1})	$Denit_{pot}$ (g m^{-2} d^{-1})
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
YRF	22.60 (2.16)	2.10 (0.10)	7.16 (0.58)	10.66 (0.64)	1.79 (0.10)	4.85 (0.69)	2.00 (0.32)	20.18 (4.69)	11.08 (1.83)	1.16 (0.84)	9.59 (2.54)	-2.01 (0.56)	$2.22_{x10^{-3}}$ (0.38)	$0.13_{x10^{-3}}$ (0.01)	$4.45_{x10^{-3}}$ (1.06)	0.67 (0.11)
SRF1	15.11 (1.07)	1.52 (0.08)	6.36 (0.34)	10.11 (0.31)	1.40 (1.10)	4.05 (1.08)	1.08 (0.22)	6.65 (0.80)	9.30 (1.52)	1.18 (0.81)	-	-	$2.56_{x10^{-3}}$ (0.21)	$0.14_{x10^{-3}}$ (0.03)	$2.44_{x10^{-3}}$ (1.10)	1.73 (0.24)
SRF2	32.74 (3.51)	2.90 (0.17)	7.11 (0.69)	11.06 (0.68)	2.35 (0.30)	5.52 (0.90)	1.75 (0.24)	10.91 (1.06)	4.85 (1.22)	1.27 (0.51)	9.73 (1.14)	-0.08 (0.23)	$3.41_{x10^{-3}}$ (1.72)	Below detection	$5.81_{x10^{-3}}$ (1.09)	2.53 (0.45)
MDF	35.23 (5.44)	3.12 (0.44)	9.25 (0.34)	9.78 (0.40)	2.84 (0.33)	4.92 (1.07)	2.90 (0.62)	6.62 (0.98)	5.78 (2.14)	-0.12 (0.88)	5.27 (1.62)	-1.03 (0.37)	$2.29_{x10^{-3}}$ (0.47)	$0.88_{x10^{-3}}$ (0.27)	$8.52_{x10^{-3}}$ (1.79)	10.35 (1.85)
Riparian	ANOVA/ Kruskal-Wallace *H*-test *p*-value															
YRF-SRF1	0.018	0.005	ns	ns	ns	ns	ns	0.004	ns	ns	-	-	ns	ns	ns	0.003
YRF-SRF2	ns	<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.021	ns	-	ns	0.005
SRF1-SRF2	0.001	<0.001	ns	ns	0.010	ns	ns	ns	ns	ns	-	-	ns	-	ns	ns
Terra firme	Student's *t*-test/Mann-Whitney *U*-test *p*-value															
MDF-YRF	ns	0.043	0.006	ns	0.01	ns	ns	0.016	ns	ns	ns	ns	ns	<0.001	ns	<0.001
MDF-SRF1	0.009	0.004	<0.001	ns	0.01	ns	0.015	ns	ns	ns	-	-	ns	<0.001	0.001	0.001
MDF-SRF2	ns	ns	ns	ns	ns	ns	ns	0.007	ns	ns	0.039	ns	ns	-	ns	0.002

Notes: Gross min = gross mineralisation, gross nit = gross nitrification, net min = gross min – gross NH_4^+ consumption, net nit = gross nit – NO_3^- consumption, DNRA = potential DNRA, $Denit_{pot}$ = potential denitrification, and N_2O+N_2 = in-situ denitrification.

Table 4-3: Values of the correlation coefficient (*r*) for selected soil variables and process rates. Significant correlations are marked by an asterisk; * indicates correlation is significant at the level $p < 0.05$ and ** indicates a significant correlation at the level $p < 0.01$.

	Total C (%)	Total N (%)	C:N ratio	Soil $\delta^{15}N$ (‰)	Sand (%)	Bulk density (g cm^{-3})	WFPS (%)	NO_3^- (g m^{-2})	NH_4^+ (g m^{-2})	NO_3^- :NH_4^+	Net min (g m^{-2})	Net nit (g m^{-2})	N_2O (mg m^{-2} d^{-1})	N_2O+N_2 (mg m^{-2} d^{-1})	$Denit_{pot}$ (g m^{-2} d^{-1})	DNRA (mg m^{-2} d^{-1})
Total C (%)	1.000	**.957****	**.437****	-0.096	-0.218	**-.423****	**.472****	0.268	-0.026	0.259	-0.017	0.142	**.555****	**.491****	0.186	**-.401****
Total N (%)		1.000	**.403****	-0.027	-0.229	**-.376****	**.434****	**.290***	-0.039	**.316***	-0.024	0.196	**.540****	**.416****	**.415****	**-.362***
C:N ratio			1.000	**-.377****	-0.081	0.066	0.139	-0.010	0.097	-0.074	0.151	-0.145	0.054	0.218	-0.042	-0.251
Soil $\delta^{15}N$ (‰)				1.000	0.189	**.377****	-0.067	0.231	-0.026	0.211	-0.145	-0.057	0.296	-0.072	**.474****	**.326***
Sand (%)					1.000	-0.072	0.039	0.040	-0.255	0.203	-0.188	-0.223	0.160	-0.036	0.168	0.130
Bulk density (g cm^{-3})						1.000	-0.098	0.095	**.474****	-0.272	0.168	0.071	-0.262	-0.268	-0.030	**.323***
WFPS (%)							1.000	**.380****	0.156	0.127	0.041	-0.108	0.328	**.310***	0.097	**-.337***
NO_3^- (g m^{-2})								1.000	0.074	**.610****	**-.425***	-0.324	**.368***	0.148	0.092	0.009
NH_4^+ (g m^{-2})									1.000	**-.659****	-0.048	**.426***	-0.201	-0.043	-0.103	-0.145
NO_3^-:NH_4^+										1.000	-0.193	**-.434***	**.447****	0.159	0.243	0.077
Net min (g m^{-2})											1.000	0.158	-0.125	0.200	-0.244	-0.021
Net nit (g m^{-2})												1.000	-0.355	0.028	0.239	0.018
N_2O (mg m^{-2} d^{-1})													1.000	**.584****	**.597****	**.336***
N_2O+N_2 (mg m^{-2} d^{-1})														1.000	0.225	**-.319***
$Denit_{pot}$ (g m^{-2} d^{-1})															1.000	-0.099
DNRA (mg m^{-2} d^{-1})																1.000

Notes: Net min = gross min – gross NH_4^+ consumption, net nit = gross nit – NO_3^- consumption, DNRA = potential DNRA, $Denit_{pot}$ = potential denitrification, and N_2O+N_2 = in-situ denitrification.

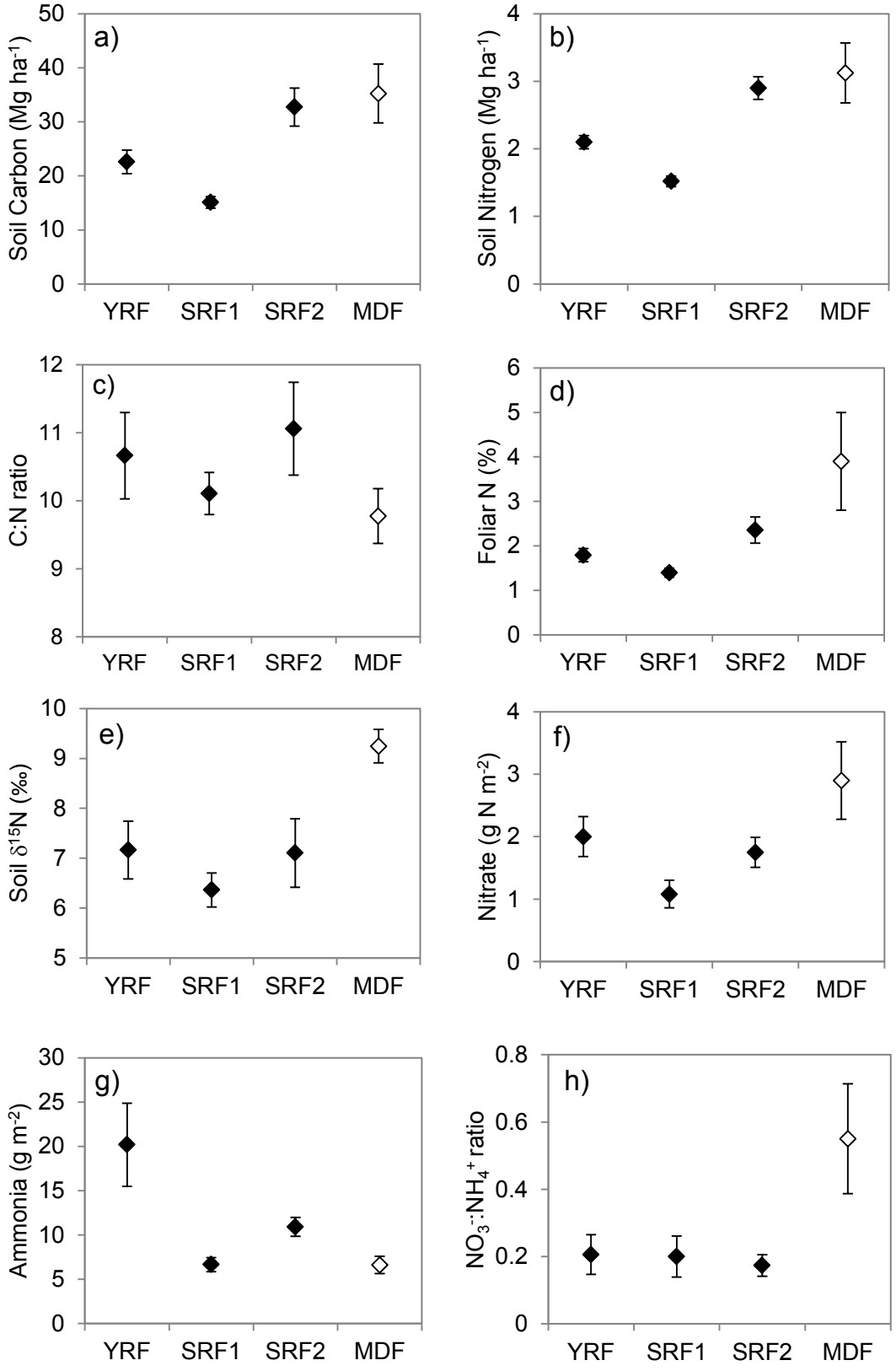


Figure 4-2: Indicators of soil N and C status through the three riparian sites (black diamonds) and the *terra-firme* MDF (white diamond). Error bars are standard error of the mean.

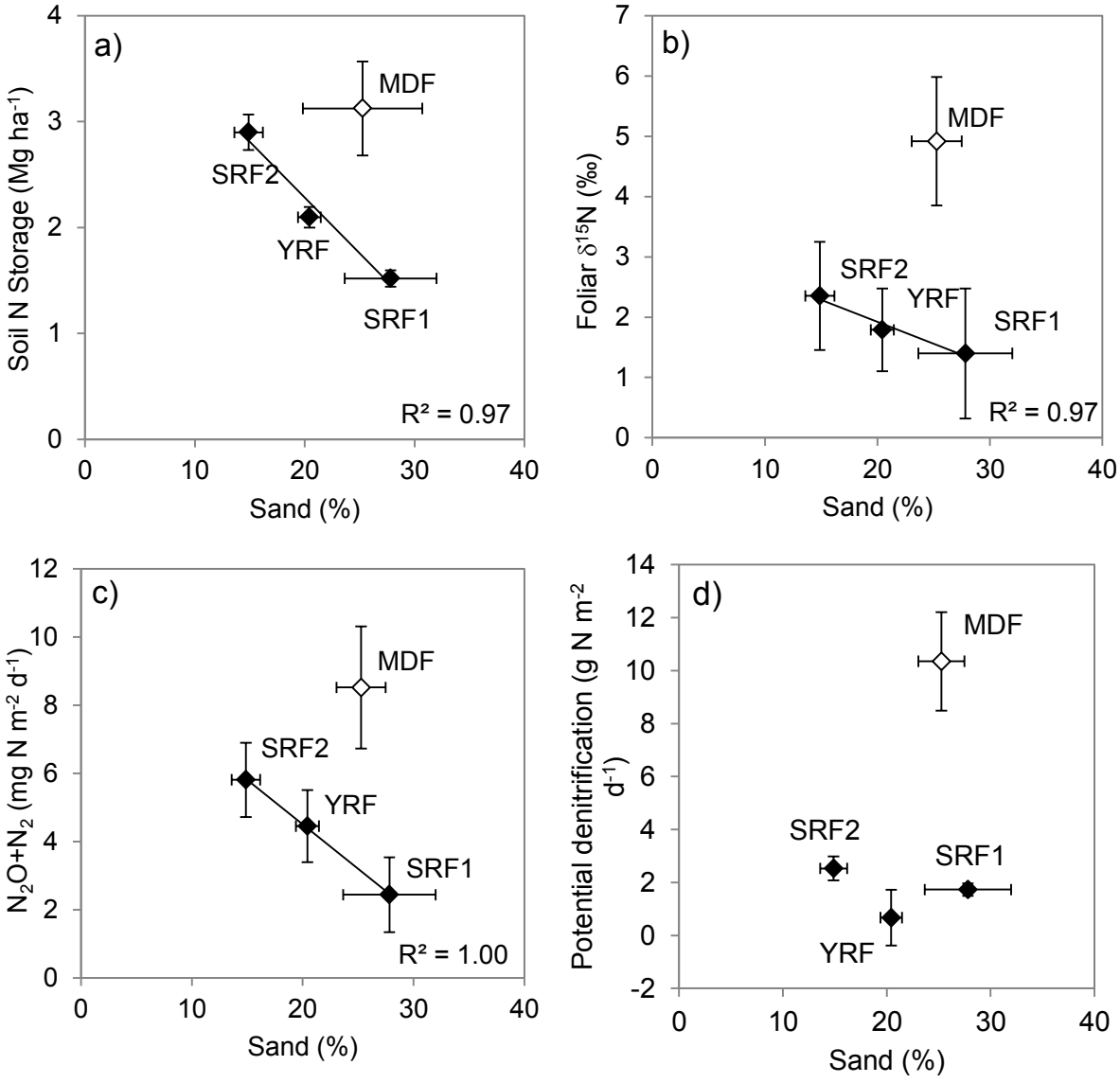


Figure 4-3: The effect of soil texture (percent sand content) on a) soil N storage; b) foliar $\delta^{15}N$ (‰); c) in-situ denitrification as the production of N_2O+N_2; and d) potential denitrification. Error bars are standard error of the mean.

Soil $\delta^{15}N$ in the riparian sites ranged from 4.06 – 11.62‰ compared to a range of 6.58 – 10.86‰ in the MDF. Whilst there was no statistical difference between the three successional forests the MDF soil was more enriched in the heavier isotope than the YRF (r = 0.60, p = 0.006) and SRF1 (r = 0.79, p < 0.001) though not SRF2. Foliar $\delta^{15}N$ did not differ between riparian forests, nor was there a difference between the *terra firme* and riparian sites.

However, mean foliar $\delta^{15}N$ (like mean C and N) was negatively correlated with % sand content through the three riparian sites (Figure 4-3).

4.3.2 Inorganic nitrogen

For the riparian forests, the concentration of soil NO_3^- was uniformly low, spanning 0.14-3.82 g N m^{-2}. Meanwhile extractable NH_4^+ was more variable ranging from 3.35-50.22 g N m^{-2} with both minimum and maximum values recorded in the YRF. Soil nitrate decreased from YRF to SRF1, then increased from SRF1 to the MDF (Figure 4-2), and was positively correlated with total N ($r = 0.29$, $p = 0.045$) and WFPS ($r = 0.38$, $p = 0.008$). Soil ammonia was correlated with bulk density ($r = 0.47$, $p < 0.001$) and showed a decreasing trend through YRF, SRF2 and MDF, but was again lowest in SRF1 (Figure 4-2). The YRF held significantly more soil ammonia than SRF1 ($r = 0.47$, $p = 0.004$) but not SRF2. Ratios of NO_3^-:NH_4^+ were similar in riparian sites where ammonia dominated the inorganic nitrogen pool, making up 79-83% of total inorganic nitrogen (Figure 4-2). Extractable ammonia in the *terra firme* MDF was similar to that of SRF1 but was significantly lower than in SRF2 ($r = 0.60$, $p = 0.007$) and YRF ($r = 0.57$, $p = 0.016$). Meanwhile, extractable nitrate in the MDF was similar to YRF and SRF2 but was significantly higher than SRF1 ($r = 0.60$, $p = 0.015$). The ratio of nitrate to ammonia was highly variable in the MDF but averaged 55 ±16% compared with ratios <21% in the riparian sites. This large variability meant that, when compared to the riparian sites, the ratio of NO_3^-:NH_4^+ in the MDF was only significantly higher than that of SRF2 ($r = 0.55$, $p = 0.044$).

4.3.3 Mineralisation, nitrification and DNRA

Gross mineralisation rates decreased through the three riparian sites though differences between them were not significant (Figure 4-4; Table 4-2). Throughout all sites (riparian and

terra firme), NH_4^+ consumption was highly correlated with ($r = 0.84$, $p < 0.001$), and in the case of MDF exceeded, NH_4^+ production resulting in negative net mineralisation. SRF1 was excluded from the results for gross nitrification and NO_3^- consumption as nitrogen diffused onto the filter discs was below the concentration necessary to measure ^{15}N atom% reliably by IRMS. However, in the remaining sites, mean gross nitrification was high, ranging from 4.85 g N m^{-2} d^{-1} in SRF2 to 11.08 g N m^{-2} d^{-1} in the YRF. Nitrate consumption was also highly correlated with ($r = 0.99$, $p < 0.001$) and exceeded and NO_3^- production resulting in negative net nitrification across all sites for which data was available. Nitrate consumption was stronger in YRF than SRF2 resulting in a more negative net nitrification rate. However, in SRF2, gross nitrification surpassed the rate of gross mineralisation by 100% (Table 4-4).

Table 4-4: Ratios of gross nitrification to gross mineralisation (Nit:Min); in-situ denitrification to gross nitrification ((N_2O+N_2):Nit); N_2O:in-situ denitrification (N_2:(N_2O+N_2)); in-situ denitrification to potential denitrification ((N_2O+N_2):$Denit_{pot}$); and potential DNRA to in-situ denitrification ($DNRA_{pot}$:(N_2O+N_2)).

	Nit:Min		(N_2O+N_2): Nit		N_2O: (N_2O+N_2)		(N_2O+N_2): $Denit_{pot}$		$DNRA_{pot}$: (N_2O+N_2)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
YRF	0.87	0.27	.001	1_x10^{-4}	0.03	0.007	6.6_x10^{-3}	1.9_x10^{-4}	0.50	0.10
SRF1	-	-	-	-	0.06	0.029	1.4_x10^{-3}	6.6_x10^{-4}	1.05	0.34
SRF2	2.01	0.46	.001	1_x10^{-4}	-	-	2.3_x10^{-3}	5.8_x10^{-4}	0.59	0.22
MDF	0.91	0.41	.002	4_x10^{-4}	0.10	0.038	8.2_x10^{-4}	2.2_x10^{-4}	0.27	0.06

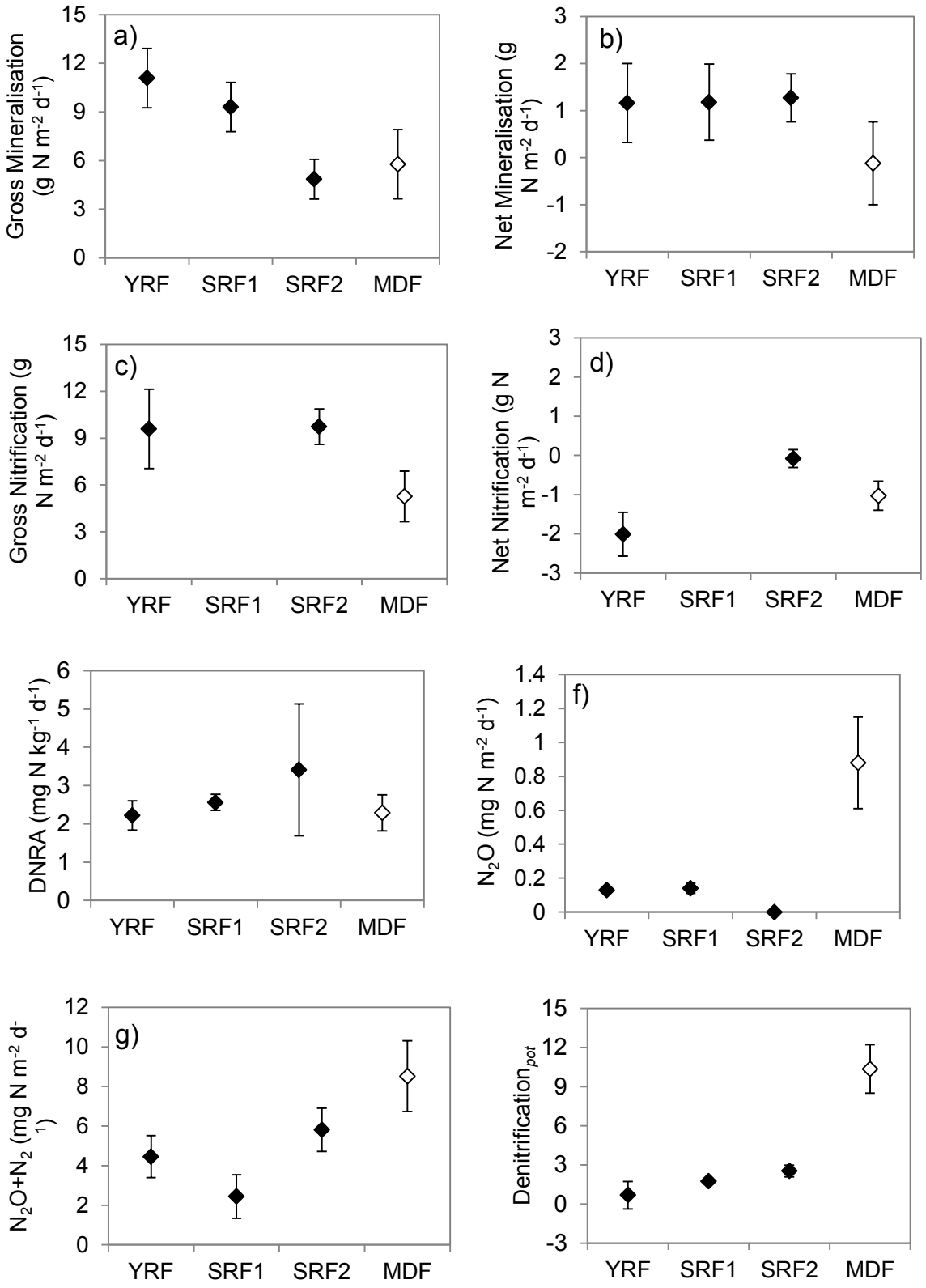


Figure 4-4: Nitrogen cycling process rates through the four secondary forests: a) gross mineralisation; b) net mineralisation; c) gross nitrification; d) net nitrification; e) potential DNRA; f) N_2O emission; g) in-situ denitrification (as the production of N_2O+N_2); and h) potential denitrification ($Denit_{pot}$). Error bars are standard error of the mean.

Rates of gross mineralisation, net mineralisation and net nitrification did not differ between riparian and *terra firme* sites, through gross nitrification in the MDF was significantly lower than that of SRF2 ($r = 0.54$, $p = 0.039$). Potential DNRA was three orders of magnitude lower than potential denitrification, indicating that that denitrification is the primary dissimilatory nitrate consumption process in soils of these tropical forests. Potential DNRA rates were, however, similar to in-situ denitrification rates (Table 4-4). An increasing trend for DNRA through YRF to SRF2 was observed, although rates were highly variable in SRF2 and did not differ significantly between riparian sites, nor was there any difference in DNRA between the MDF and the riparian sites. However, there were positive correlations of DNRA with $\delta^{15}N$ ($r = 0.33$, $p = 0.02$) and bulk density ($r = 0.32$, $p = 0.025$), and negative correlations with total C ($r = -0.40$, $p = 0.006$), total N ($r = -0.36$, $p = 0.012$), WFPS ($r = -0.34$, $p = 0.02$) and in-situ denitrification ($r = -0.32$, $p = 0.03$).

4.3.4 Losses of N through denitrification and N_2O emission

In-situ denitrification increased through SRF1 to the MDF following the same pattern as C and N storage and foliar N (%) with rates declining from YRF to SRF1 before increasing through SRF1 to the MDF (Figure 4-4). As such, the production of N_2O+N_2 was positively correlated with total N (%) ($r = 0.42$, $p = 0.003$) and total C (%) ($r = 0.49$, $p = 0.001$) across all sites. In-situ rates also increased with WFPS ($r = 0.31$, $p = 0.03$).

N_2O emissions in the riparian sites ranged from below detection to 0.46 mg N_2O -N m^{-2} d^{-1}. Despite the relatively high rate of in-situ denitrification in SRF2 (5.81 mg N m^{-2} d^{-1}), N_2O emissions for this site were below detection in all samples taken from each of 12 soil gas chambers. Accordingly, SRF2 was excluded from correlation analysis of N_2O with other variables resulting in positive relationships of N_2O with total C (%) ($r = 0.56$, $p = 0.001$), total

N (%) ($r = 0.54$, $p = 0.001$), in-situ denitrification ($r = 0.58$, $p < 0.001$), nitrate ($r = 0.37$, $p = 0.027$), the ratio of nitrate to ammonia ($r = 0.45$, $p = 0.006$) and potential denitrification ($r = 0.58$, $p < 0.001$) and DNRA ($r = 0.34$, $p < 0.05$). Rates of potential denitrification were significantly lower in YRF than in SRF1 and SRF2 and there was an increasing trend of potential rates through secondary forest recovery (Figure 4-4). Soil texture affected in-situ denitrification with rates in the riparian sites decreasing with increasing sand content, however the same trend was not apparent for potential denitrification. (Figure 4-3). Potential denitrification was also positively correlated with total N ($r = 0.42$, $p = 0.005$) and $\delta^{15}N$ ($r = 0.48$, $p = 0.001$).

Rates of in-situ denitrification in the MDF were similar to those observed in the YRF and SRF2 but were three and a half times higher than in SRF1 ($r = 0.73$, $p < 0.001$). However, emissions of N_2O from the *terra firme* site (0.14 – 3.10 mg N_2O-N m^{-2} d^{-1}) were over six times greater than in the riparian sites (below detection– 0.46 mg N_2O-N m^{-2} d^{-1}) and spanned a much greater range. Similarly, rates of potential denitrification were higher in the MDF than in all three riparian sites. The proportion of N_2O being released through denitrification in MDF was also greater, being 10% vis-à-vis 3% in YRF and 6% in SRF1 (Table 4-4).

4.4 DISCUSSION

4.4.1 Trends of increasing carbon storage and nitrogen availability through forest succession

The prediction that soil carbon storage and nitrogen availability would increase with time since disturbance was broadly, although not universally, true for these secondary forests. Whilst all secondary forest sites conformed to the same soil textural class and had similar pH, any analysis of trend through forest recovery remains statistically constrained by the small

sample size and possible differences in nutrient availability (resulting from differing substrate) in the riparian and *terra firme* sites. Soil C, and N in both leaves and soil, increased from SRF1 to the MDF, although methodological difficulties in controlling for edaphic variables such as soil texture and initial soil nutrient status, or prior land use history are possible reasons for the pattern of lower C and N storage in SRF1 relative to YRF.

Soil texture, and consequently soil fertility, are strong determinants of forest recovery from disturbance, and therefore can affect the rate of soil N availability and C accumulation (Moran, et al., 2000; Johnson, et al., 2000). Soil organic matter tends to increase linearly with the clay content of soils (Schimel, et al., 1994), whereas sandier soils are associated with higher fine root density, greater leaching losses, greater nutrient limitation, and higher soil aeration (Cuevas & Medina, 1988; Gaines & Gaines, 1994; Austin, et al., 2004). Along a texture gradient within the riparian sites, sandier soils had lower N storage (and consequently C storage and foliar N) than soils with proportionally greater silt and clay content. C and N storage through the riparian sites therefore, likely reflected differences in soil texture with sandier soils having lower organic matter and consequently storing less C and N than more clay-rich soils.

In addition to soil texture, the legacy of prior land use may also affect C and N accumulation. Contradictory trends reported in studies of soil carbon and nitrogen accumulation along forest recovery chronosequences have highlighted the effect of prior land use history on belowground nutrient status (Hughes, et al., 1999; Erickson, et al., 2001; Marin-Spiotta, et al., 2009; Orihuela-Belmonte, et al., 2013). Whilst almost all investigations of tropical succession report an increase in aboveground biomass with time since disturbance, studies of belowground C and N accumulation are more equivocal. For example, in Puerto Rico, mid-successional forests have been found to have the highest (Erickson, et al., 2001) and lowest

(Marin-Spiotta, et al., 2009) C and N storage following pasture abandonment. Others have found no relationship between forest age and soil C accrual (Orihuela-Belmonte, et al., 2013; Hughes, et al., 1999). However, forests that have been subjected to intense, repeated burning are likely to take longer to recover than those that have had only one period of moderate burning (Uhl & Jordan, 1984; Uhl & Kauffman, 1990). Similarly, the intensity of logging activity will affect soil erosion, compaction and nutrient losses. In Sipitang, Sabah, manual log extraction and burning resulted in a 50% reduction in losses of N, P, K and Mg from soils compared with those subjected to mechanised logging and burning (Malmer, 1996). It is possible, therefore, that where land has been clear cut and logs removed mechanically, losses of soil N and C will be greater than from soils where low-impact selective logging has occurred. Land use legacies for these forests are largely unknown, however as mentioned above, the presence of *C. serratifolia* in YRF and SRF1 is suggestive of previous fire at both of these sites. Furthermore, the fact that *C. serratifolia* has not yet been crowded out by later-successional species in SRF1 may be indicative of disturbance that is either ongoing, or of greater magnitude, than at other sites. Site accessibility, evidence of previous inhabitation and, more recently, tyre tracks lends additional weight to the argument that the intensity of previous land use was greater in SRF1 than in the other riparian sites and may be one explanation for the low C and N storage in this site. Higher C and N in YRF relative to SRF1 may also reflect the legacy of the earlier forest on this site. In the case of conversion to agriculture, losses appear to be exponential during the first few years of disturbance but have been shown to continue to occur for up to ~23 years before reaching a new equilibrium level (Fearnside & Barbosa, 1998; de Blecourt, et al., 2013). YRF was cleared 16 years prior to sampling and it is possible, therefore, that given the slow rate of tree recruitment, losses of soil carbon are still occurring. Without knowledge of prior land use and disturbance history,

the assumption that these forests accurately represent a successional continuum rather than independent trajectories highlight the difficulties inherent in space-for-time substitutions, (Chazdon, et al., 2007).

Soil nitrate followed the same pattern of C and N storage increasing through SRF1 to MDF, although uniformly low (rarely exceeding 4 g N m^{-2}) concentrations did not result in statistically different results through the three riparian sites. Once again, SRF1 had statistically lower soil ammonia than YRF, which may be due to the same constraints on C and N accumulation for that site described above. The ratio of NO_3^-:NH_4^+ is often observed to increase as secondary forests recover indicating overall greater N availability (Keller & Reiners, 1994; Verchot, et al., 1999; Erickson, et al., 2001). Davidson et al. (2007) suggest ratios of NO_3^-:NH_4^+ > 1 to be a characteristic of soils prone to large N losses through N gas emission or leaching. However, in the riparian sites, the ratio of NO_3^-:NH_4^+ was always below 0.8 and in only two of twelve samples in the MDF did the ratio exceed 1.

4.4.2 Does increasing N availability equate to increasing N loss?

Within the framework of the nitrogen saturation model, greater N availability results in elevated N losses as a result of increased N mineralisation and nitrification following satisfaction of plant and microbial demand. In these forests, there was no difference in rates of gross or net mineralisation and no increasing trend of either gross or net nitrification despite significantly greater soil N in the later successional sites. In fact, increasing N availability resulted in a non-significant decline in mineralisation as is often observed following increasing N availability (Booth, et al., 2005). Rates of both mineralisation and nitrification were higher than reported for most tropical sites (Table 2-1), and gross nitrification consumed a very high (60-190%) proportion of mineralised NH_4^+. High gross nitrification rates, however, were accompanied by negative rates of net nitrification indicating

strong NO_3^- demand. As net production rates were calculated through the subtraction of NH_4^+ or NO_3^- consumption from NH_4^+ or NO_3^- production via the isotope pool dilution method, the addition of ^{15}N may have had the effect of priming microbial immobilisation (Watson, et al., 2000; Accoe, et al., 2004). Whilst ^{15}N additions (2 μg N g^{-1} dry soil) were equivalent to, or lower than, ambient soil N concentrations (2.5 μg NO_3^--N g^{-1} and 11.2 μg NH_4^+-N g^{-1}), the addition of similar concentrations to European grassland soils have been found to stimulate microbial consumption (Watson, et al., 2000; Accoe, et al., 2004). Therefore, gross consumption rates may be overestimated and thus responsible for the negative net rates observed. Nevertheless, NO_3^- demand was tightly coupled to supply as indicated by the high correlation coefficient between gross nitrification and gross nitrate consumption ($r = 0.99$, $p < 0.001$). Consumption processes that maintain low soil NO_3^- either retain nitrate in an alternative N pool (e.g. DNRA, microbial and plant assimilation or abiotic assimilation in the DON pool), or remove NO_3^- from soils through denitrification. The extent to which the N cycle is open or closed therefore depends on the relative importance of these loss and conservation processes.

4.4.2.1 N loss processes: denitrification and emissions of N_2O

Although rates of N_2O emission and in-situ did not differ statistically between riparian sites, in-situ denitrification mirrored C and N storage by following a trajectory of higher emissions (as N_2O+N_2) through SRF1 to the MDF. Significant correlations of in-situ denitrification with both carbon and nitrogen storage, also resulted in a similar relationship between in-situ rates and percentage sand content through the riparian sites (Figure 4-3). A reduction in the rate of denitrification with increasing sand content is often observed in both tropical and temperate forests, and is largely attributed to increased aeration due to better drainage and

lower WFPS in sandier soils (Matson & Vitousek, 1987; Groffman & Tiedje, 1989). Here, in-situ denitrification was lower in soils that had a greater sand content, and was positively correlated with WFPS indicating that soil aeration was a factor controlling N_2O+N_2 production rates. The $\delta^{15}N$ signature of the vegetation also provides additional insights into N cycling within these secondary forests. The $\delta^{15}N$ of plants reflects *inter alia*: i) the ^{15}N signature of the plant N source (e.g. N_2-fixation, soil N, atmospheric deposition); ii) the form of N taken up (organic N, NH_4^+ or NO_3^-); iii) the depth at which the plant N pool is within the soil profile; and iv) fractionation processes within the plant (Högberg, 1997). The competing factors contributing to plant ^{15}N signatures highlight the difficulty in attributing foliar $\delta^{15}N$ to soil N cycling processes. However, taking composite samples of only understory herbaceous vegetation was designed to minimise ^{15}N variability that could be attributable to plant phenotypical characteristics (Section 3.5.3). The reduction in foliar ^{15}N with increased sand content is therefore likely a result of reduced fractionating losses (e.g. through gaseous N emissions) which is consistent with the lower in-situ denitrification observed in the sandier riparian soils.

In-situ rates of denitrification during this end of wet season sampling ranged from 9 kg N ha y^{-1} in SRF1 to 31 kg N ha y^{-1} in the MDF, which is above the mean rate of 2 kg N ha y^{-1} estimated for non-agricultural land. However, denitrification consumed only a small proportion (i.e. <1%) of the total N cycled within these forests at the time of measurement and was much lower than estimated potential rates. In tropical forests, a substantial proportion of N gas released through denitrification is often observed to be emitted as N_2O, however emissions in these forests were only a fraction (3-10%) of in-situ rates. N_2O emissions (below detection-0.31 mg N m^{-2} d^{-1}) were also lower than the rates reported for selectively logged forests on Ultisols in Rondonia (1.6-1.8 mg m^{-2} d^{-1}; Garcia-Montiel, et al., 2001) and

coastal lowland forests in Queensland, Australia (1.9-5.8 mg m^{-2} d^{-1}; Kiese, et al., 2002). Rates were however higher than those reported for riparian and floodplain emissions from the Luquillo Experimental Forest (<0.048 mg m^{-2} d^{-1}) in Puerto Rico, (Bowden, et al., 1992). In addition to relatively low emissions from the YRF and SRF1 (<0.14 mg N m^{-2} d^{-1}), SRF2 appeared to act as a sink for N_2O. Given the fact that in samples taken from this site N_2O was below detection limits, the probability of experimental error during sample extraction is low. In addition, as chambers were deployed over two days for each site, temporal variability of emission rates were not captured. The lack of temporal resolution to emissions is highlighted through comparison of the end of wet season results reported here with samples taken during the inter-monsoon in 2010 (Table 4-5). During the inter-monsoon, rates in SRF1 and the MDF were 3-4 times greater, rates in YRF were almost 20 times greater, and in SRF2, dry season emissions were 2.68 mg N_2O-N m^{-2} d^{-1} compared to a probable negative flux at the end of the wet season.

Table 4-5: Difference between mean N_2O and in-situ denitrification (N_2O+N_2) emission rates and the ratio of N_2O :(N_2O+N_2) at the end of the wet season and during the inter-monsoon.

	End of wet season			Inter-monsoon		
	N_2O (mg m^{-2} d^{-1})	(N_2O+N_2) (mg m^{-2} d^{-1})	N_2O: (N_2O+N_2)	N_2O (mg m^{-2} d^{-1})	(N_2O+N_2) (mg m^{-2} d^{-1})	N_2O: (N_2O+N_2)
YRF	0.13	4.45	0.03	2.35	6.08	0.39
SRF1	0.14	2.44	0.06	0.57	6.32	0.09
SRF2	0	5.81	-	2.68	1.46	1.84
MDF	0.88	8.52	0.10	2.80	1.30	2.15

There was also a notable difference in the proportion of N_2O emitted relative to denitrification, which during the inter-monsoon ranged from 9-215% compared to 3-10% at the end of the wet season. Furthermore, during the inter-monsoon, for the two more mature forests (SRF2 and the MDF), nitrous oxide emissions exceeded in-situ denitrification rates by ~100%. Although rates of gross and net nitrification were not measured during the inter-

monsoon, lower WFPS and higher soil nitrate suggest that nitrification was a significant contributor to N_2O emissions during the drier season, particularly for SRF2 and MDF.

Potential denitrification rates through the riparian sites increased along the trajectory of forest recovery. Similarly, the highest rates of potential denitrification were found in the most mature MDF. Potential denitrification assays reflect the amount of denitrifying enzymes in the soil rather than the actual amount of denitrifying activity taking place in-situ. However, the correlation of both soil and foliar $\delta^{15}N$ and N_2O emissions with rates of potential denitrification is suggestive of greater nitrogen losses through N-isotope fractionating processes and may be indicative of increasing losses through the chronosequence of forest recovery.

4.4.2.2 N conservation processes: DNRA and abiotic retention

For these soils, rates of potential DNRA were much lower than potential denitrification suggesting that this process has little effect on N consumption and conservation. Several studies employing the isotope pool dilution technique have found rates of DNRA to be similar to, or exceed rates of denitrification (Silver, et al., 2001; Pett-Ridge, et al., 2006; Templer, et al., 2008; Doff Sotta, et al., 2008). Comparison of rates is complicated by differences in analytical methods: This thesis employs a ^{15}N tracing technique coupled to hypobromite oxidation rather than the more common isotope pool dilution technique reported in other studies. However rates in forests sampled for this thesis are approximately three orders of magnitude lower than rates in the Luquillo Forest in Puerto Rico (Silver, et al., 2001; Pett-Ridge, et al., 2006; Templer, et al., 2008) and two orders of magnitude lower than those in *terra firme* lowland forests in Brazil (Doff Sotta, et al., 2008). Although, not all studies report high rates of DNRA. For example, in one of the earliest tropical studies of DNRA, MacRae,

et al. (1968) found negligible rates in six samples taken from soils in the Philippines. The reason for the higher rates reported in Puerto Rico may lie in the $C:NO_3^-$ ratio. DNRA is likely to be more competitive for nitrate under highly reducing conditions where the ratio of electron donor (i.e. C) is high relative to electron acceptor (nitrate). In Luquillo, the $C:NO_3^-$ ratio ranged from 8.5–17.38 and was substantially greater than the range of 0.82–1.97 found in these soils (Silver, et al., 2001). In Brazil, $C:NO_3^-$ ratios were lower (i.e. $0.21 < C:NO_3^- < 0.31$) than those reported here, however the authors acknowledge that the 48 hour incubation period may have resulted in an over-estimation of DNRA through NO_3^- immobilisation and remineralisation (Doff Sotta, et al., 2008). Remineralisation was not a concern in the 0.5–2 hour incubations employed for this study.

There is some evidence to suggest that abiotic NO_3^- immobilisation may be an important mechanism in these soils, as recovery rates of the added $^{15}NO_3^-$ label averaged 26±5% fifteen minutes after addition (Section 3.5.1). Plant and microbial demand is also likely to be high, particularly in the mid- and late- successional forests where vegetation growth is vigorous (Robertson & Tiedje, 1988; Groffman, 1995). However, in the absence of direct measurements of either process, no conclusions can be drawn as to the importance of abiotic immobilisation and plant uptake on nitrate retention.

4.4.3 Are mid- and late-successional forests more suitable representatives of the baseline condition for soil N than early-successional forests?

Within the three riparian forests, soil C and N and foliar N varied significantly between sites, indicating that choice of location will have an effect on the baseline condition for soil C and N storage. However, the percentage sand content within the riparian soils also had a significant effect on mean C and N storage, foliar $\delta^{15}N$ and denitrification, highlighting the importance that edaphic variables such as soil texture have on nitrogen status. For the most part,

differences in nitrogen process rates between riparian sites were not significant. However, despite this lack of significance, gross and net mineralisation, net nitrification, potential DNRA and in-situ and potential denitrification were higher in the most mature riparian site (SRF2) when compared to the earlier successional forests. Furthermore, there were many similarities in N storage and process rates between the two later-successional forests, MDF and SRF2. In general, both carbon and nitrogen status increased as soils recovered from disturbance indicating that later successional forests are likely to be a more appropriate proxy for the baseline condition where the purpose is to determine the effect of land use change from the natural or undisturbed state to agricultural use and no primary forest site is available. However, as primary forests have declined rapidly over the past half-century, it is increasingly likely that future land use change involves the conversion of secondary, rather than primary forests. In this case, younger secondary forests may be the more appropriate baseline when determining land use change. As such, it is suggested that where younger secondary forests are used as the baseline condition, edaphic factors may be more important for recovery of nitrogen status and rates of nitrogen loss than successional status when comparing soils under forest cover with those of agriculture.

4.5 CONCLUSION

Indicators of N status and cycling capability in these secondary forests appeared to follow a trajectory of recovery with time since disturbance, although not uniformly so through the chronosequence. Specifically, several indicators of N cycling, including soil C, and soil and foliar N, were higher in the YRF relative to the more mature SRF1, highlighting the importance of prior land use history or site-specific edaphic variables such as soil texture on recovery following disturbance. The importance of the latter is shown by the strong effect that

sand content has on rates of in-situ denitrification and soil and foliar N which decline through the sandier riparian soil. Land use history meanwhile may affect factors such as the time lag of soil carbon response to decreased net primary productivity and organic matter returns emphasising the importance of path dependency through historical land uses.

For the most part, the secondary forests sampled for this study showed a tendency for an increasingly open nitrogen cycling with time since disturbance. Whilst ratios of nitrate to ammonia < 1 placed these sites outside the category of those suggested by Davidson et al. (2007) to be prone to large N losses, they nevertheless had high rates of mineralisation and nitrification, and hence a high potential for nitrogen loss. Furthermore, an increasing trend of potential denitrification through the chronosequence coupled to a positive correlation of potential denitrification with soil and foliar $\delta^{15}N$ is also suggestive of greater nitrogen loses as forests recovery from disturbance. However, a high production rate of nitrate was also coupled to high negative rates of net nitrification indicating strong nitrate consumption that could not be assigned to nitrate retention through DNRA. Furthermore, rates of in-situ denitrification, although high, were only a fraction of the total N turnover in these forests. Although small losses relative to total N turnover is indicative of nitrogen conservation, the large increase in N_2O and in-situ denitrification rates between the wet and dry seasons highlight the temporal variability in N loss. Furthermore, the $\delta^{15}N‰$ of soils provides additional evidence of the long-term N cycling and loss within these soils. Both soil and foliar $\delta^{15}N$ and enrichment values were similar to those predicted for the tropics as a whole (Martinelli, et al., 1999; Amundson, et al., 2003), reflecting high nitrogen turnover rates and potentially large nitrogen losses from these soils that increased with time since disturbance.

CHAPTER 5:THE IMPACT OF MANAGEMENT PRACTICES ON THE SPATIAL VARIABILITY OF SOIL PROPERTIES WITHIN A MATURE OIL PALM PLANTATION

5.1 INTRODUCTION

Oil palm plantations are characteristically uniform in nature. The crop is planted as a monoculture in triangular formation usually at 9m x 9m x 9m distance. However, often within the context what is commonly an industrial-scale homogenous environment, fertilisation regime and organic matter inputs differ at the micro scale. For example, fertiliser is typically applied within 2m radius of the trunk (the "palm circle") where root density is greatest (Schroth, et al., 2000). Additionally, once the palms have reached cropping age, fronds are pruned from the lower branches and placed in the plantation inter-rows to allow nutrients to be recycled within the soil (Figure 5-1). At an average density of 136 palms ha^{-1}, typical areal coverage for the palm circle and frond piles are 15 and 20% respectively (Haron et al., 1998). The remainder of the plantation is kept clear through fertilisation or weeding to allow collection of fresh fruit bunches along harvest paths.

This zonation is likely to lead to disparate nutrient availability between frond piles, palm circle and pathways. Studies looking at oil palm plantation heterogeneity generally report higher soil organic C and N under frond piles when compared to the palm circle and harvest paths (Haron et al., 1998; Lamade & Bouillet, 2005; Nelson et al., 2011; Moradi et al., 2012). However, rapid decomposition of fronds at the soil surface limits the amount of labile carbon penetrating the mineral soil (Lamade, et al., 1996; Haron, et al., 1998). On mainland

Malaysia, Haron et al., (1998) found that biomass C, as a percentage of organic C in mature plantations (10 and 20 years old), did not differ between palm circle and frond piles. Conversely high root density within the palm circle might actually result in higher labile carbon availability than under frond piles (Haron et al., 1998). It follows that microbial processing of nitrogen within plantations does not necessarily follow the pattern of high carbon and nitrogen availability.

Figure 5-1: Plantation 15Y sampled for this study showing areas of frond piles and the palm circle where fertiliser is applied. Palms are planted in triangular formation approximately 9m apart.

In this chapter, geostatistical analyses are undertaken to quantify the magnitude of spatial variability for a number of variables indicative of nitrogen cycling (i.e. total C, total N, C:N, ammonia, nitrate, $\delta^{15}N$ and pH). It is hypothesised that the practice of pruning and stacking palm fronds and the application of inorganic fertiliser to the palm circle will affect the spatial

distribution of soil carbon and nitrogen. In particular, C, N and C:N are expected to be higher under frond piles, and inorganic N to be higher in areas of fertilisation. It is also expected that ammonia and nitrate, which depend on microbial processing, will have greater spatial variability with less obvious zonation commensurate with palm frond inputs. Soil $\delta^{15}N$ is commonly employed as a proxy for soil nitrogen cycling with more enriched values indicative of fractionation during processes such as nitrification and denitrification. By mapping soil $\delta^{15}N$ we aimed to determine whether differences in N isotopic enrichment are impacted by management practices within the plantation. Accordingly, where palm frond inputs overlap with potentially high N concentrations from fertilisation, high rates of N processing are expected to result in enriched $\delta^{15}N$ values. Conversely, additions of artificial fertiliser (with a $\delta^{15}N \approx 0‰$) are hypothesised to lower $\delta^{15}N$ values and increase inorganic nitrogen concentrations. Finally, the range of spatial autocorrelation for each variable is examined to assess the assumption of statistical independence.

5.2 METHODS

5.2.1 Soil sampling

Samples were taken from a 28m x 13m plot in site 15Y (see p.136) at the end of the wet season in April 2012. This intensive, within-plot sampling was conducted approximately one week after sampling of the wider plantation had taken place, the results of which are reported in Chapter 6. The samples collected for this Chapter did not form part of the original field design, but rather, were extracted opportunistically at the end of the field trip when sample collection for the main project had been completed. As a result, the statistical

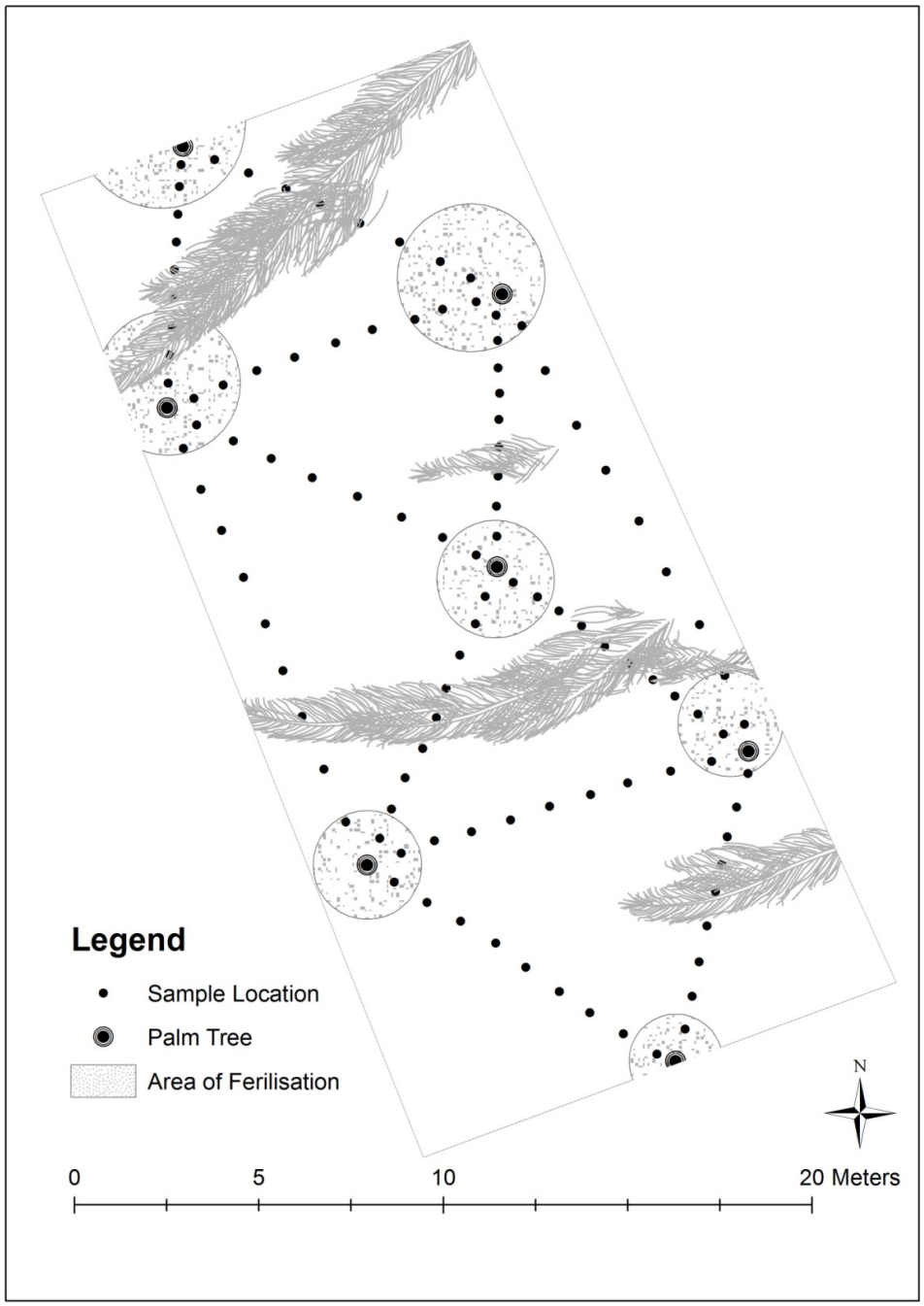


Figure 5-2: Location of the 107 sampling points within the 28 x 13 m plot. Cut palm fronds are placed in the inter-rows and an area of fertilisation extends approximately 2m from the base of the palm trunk ("the palm circle").

methodology for processing results of this chapter was not determined prior to sample collection and design. Accordingly, 107 samples were extracted along 12 transects drawn between 7 palms (Figure 5-2) to determine the influence of frond piles and fertilisation across the plot. Of the 12 transects, decomposing palm fronds were present at the surface of 7 transects; there were no palm fronds through the remaining five transects.

Palm fronds constitute the major addition of organic matter to plantations and most research has reported soil C to be higher in soils beneath them, (Khalid, et al., 1999; Frazao, et al., 2012). It has been estimated that ~24 fronds are typically pruned annually from each tree, equating to the addition of 9-10 Mg ha^{-1} y^{-1} of dry matter (Chan et al., 1980; Haron et al., 1998; Corley & Tinker, 2003). Fronds have a carbon content of roughly 48% and an N percentage of 1% when N content of rachis and leaflet and their relative proportions are accounted for (Haron, et al., 1998; Khalid, et al., 1999). Accordingly, the return from fronds potentially equates to ~4.8 Mg of C and 100 kg^{-1} of N ha^{-1} y^{-1}. Although the exact form and quantity of fertiliser applied in this plantation is unknown, typical applications for this region are 0.5-2.5 kg N $palm^{-1}$ y^{-1} (equivalent to 70-350 kg N ha^{-1} y^{-1}) applied to the palm circle, usually in the form of ammonium nitrate.

In contrast to the method employed for soil variables reported in the remainder of this thesis, sampling depth for this chapter was 5cm using a 5cm diameter core. Accordingly, results are not directly comparable with soil variables reported in other chapters for this site. Samples were homogenised and a subsample immediately placed in 2M KCl with an approximate 5:1 solution:wet soil ratio. Samples were extracted for inorganic N in accordance with the method outlined in Section 3.4.2 above. The remaining sample was air dried before determination of pH, total C, total N, C:N ratio and isotopic analysis as set out in Sections 3.4.1and 3.5.3.

5.2.2 Statistical analysis

Kriging interpolation was used to examine the spatial dependence of soil properties within the plantation plot. The underlying principle of kriging is the assumption of autocorrelation, namely that measurement observations which are relatively close together will be more similar than those further apart. By examining the difference between two measurements *z(x)* and *z(x+h)* at some specified distance the spatial dependence between them can be plotted as a function of the separation distance or lag *(h)*. The resulting plot is the experimental semivariogram (Figure 5-3) calculated from Equation 5-1 where semivariance at distance *(γ (h))* is half of the mean square difference between the two measurements, and *N* is the number of observation pairs in the lag distance class *(h)*.

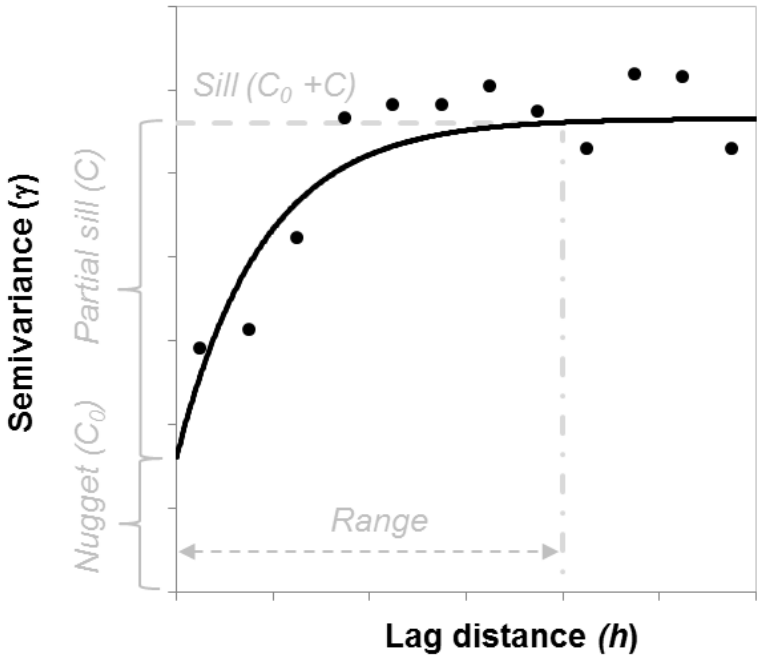


Figure 5-3: Experimental model showing the proportion of semivariance (γ) attributable to spatial dependence over a lag distance (*h*).

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(xi) - z(xi + h)]^2$$

Equation 5-1: Semivariance at distance *(γ (h))* is equal to half of the mean square difference between two measurements and *N* = the number of observation pairs in the lag distance class *(h)*.

The experimental semivariogram is then fitted to an idealised model which can take one of several forms (i.e. linear, spherical, circular, exponential, Gaussian). Typically, semivariance will increase as distance between observations increases until it reaches the point of spatial independence referred to as the sill *(C)*. The distance at which the sill is reached is known as the range and indicates the minimum separation distance between samples to ensure statistical independence. Samples taken in the same location (i.e. $h = 0$) should theoretically be identical and have zero semivariance. However, in practice almost all semivariogram models cross the *y*-axis at a point > 0 indicating: i. variability at distances smaller than the minimum sampling distance; ii. an absence of spatial structure at the scale sampled and/or iii. errors in sampling and measurement. The semivariance at $h = 0$ is referred to as the nugget (C_0). The nugget:sill ratio permits qualitative definition of spatial dependence with ratios < 25%, 25-75% and > 75% expressing strong, moderate and weak effects respectively.

Semivariograms were modelled in ArcMap©, version 10.0 (ESRI 2010) using weighted least-squares analysis. Models were cross-validated and a "best fit" model chosen based on the smallest average error, and average standard error closest to the root means square prediction error. Variables displaying non-normality were transformed before ordinary kriging. Where the presence of outliers impeded semivariogram fitting, they were removed for the initial stage of modelling. Prediction maps were then created from the resulting model (outliers excluded) using the whole dataset (outliers included). Average distance between points was

0.95 m and we therefore allocated a lag size of 1m for each of the models equating to approximately half of the maximum distance between points over the whole plot.

Due to spatial autocorrelation at the scale sampled, the assumption of independence is violated for standard statistical methods. It is not possible therefore to employ the student's *t*-test or Mann-Whitney *U*-test for differences between treatments without obscuring the probability of committing a type I error in the resulting analysis. One way to circumvent this problem is to employ randomisation or bootstrapped *t*-tests (Nash, et al., 1999). This approach obviates the need for assumptions of normality and independence whilst still providing a robust statistical result. Significance is tested by repeated permutations of the test data through random number generation. For each permutation, values were assigned to either the organic matter (i.e. samples under the palm frond pile) or no organic matter treatment (treatment 1) and inside or outside the palm circle where inorganic fertiliser was applied (treatment 2). For each test of difference between treatments, 1000 permutations were carried out to determine probability levels using the bootstrapping facility within SPSS v20 (IBM Inc.).

5.3 RESULTS

On average, soils within the plantation plot were abundant in inorganic nitrogen, had low C:N ratios and were highly acidic (Table 5-1). Samples receiving inputs of fresh organic matter from the palm fronds (treatment 1) had a significantly higher C:N ratio, less nitrate and were less enriched in ^{15}N than samples not under palm frond inputs (Table 5-1). Total C and N were significantly higher within the palm circle (treatment 2), but there was no statistical difference in soil C or N under the palm frond treatment (treatment 1).

From calculations of the C and N content of fresh palm fronds, the difference in soil carbon accumulation between areas that do not receive organic matter inputs from fronds and those that do can be estimated. Pruning occurs 3-4 years after planting out which at an average return of 4.8 Mg C ha^{-1} y^{-1} equates to a total return of 52.8 Mg C ha^{-1} for the 11 years that pruning has occurred (Table 5-3). Cut fronds have also potentially supplied 1.1 Mg N ha^{-1} over the same period equating to 100 kg N ha^{-1} y^{-1}. Observed increases were approximately 90% less than potential inputs averaging 0.53 Mg C ha^{-1} y^{-1} and 10 kg N ha^{-1} y^{-1}. Ammonia and pH did not differ under the palm frond organic matter treatment, but both were significantly higher within the palm circle. Nitrate was also higher within the palm circle but the difference fell just short of significance.

There are many publications on nutrient accumulation within the oil palm as a function of age, which would permit an estimate of N uptake by the palms relative to nitrogen losses. However, calculation of inorganic nitrogen accumulation within the palm circle as a result of fertilisation requires information on fertiliser application rates, which unfortunately were not available at the time of writing. Nevertheless, contrary to expectations, there was no statistical difference in soil $\delta^{15}N$ inside the palm circle where inorganic fertiliser is applied relative to other areas of the plantation. Furthermore, the $\delta^{15}N$ of soil was significantly lower under the frond piles (4.01‰) than in the remainder of the plantation (4.63‰). When soil N was plotted with $\delta^{15}N$, fertilised samples consistently had a greater ^{15}N enrichment relative to samples outside the palm circle (i.e. organic matter, fertiliser and organic matter, or none) (Figure 5-4).

Table 5-1: Properties of soil samples taken at 0-5 cm depth on untransformed data. n = 107 sample locations with as many as 8 missing values.

Variable	Mean	Median	Minimum	Maximum	CV (%)	SD	Variance
Total C (%)	4.69	4.42	1.74	13.18	39	1.83	3.34
Total N (%)	0.38	0.36	0.17	0.87	34	0.13	0.17
C:N	12.30	12.17	7.63	15.85	12	1.50	2.24
Nitrate (mg kg^{-1})	5.20	3.22	0.39	48.39	145	7.50	56.20
Ammonia (mg kg^{-1})	9.47	6.10	1.96	54.29	96	9.05	81.96
$\delta^{15}N$	4.51	4.51	1.82	7.81	27	1.20	1.44
pH	4.74	4.48	3.83	6.54	14	0.68	0.46

Table 5-2: Mean values and randomisation significance tests for soil samples with and without additional organic matter (OM) input from palm fronds (treatment 1) and with and without inorganic nitrogen (IN) inputs to the palm circle (treatment 2). Significant differences are indicated by an asterisk.

		Mean		Sample size			Mean		Sample Size	
	Treatment 1					Treatment 2				
Attribute	p value	OM	No OM	OM	No OM	p value	IN	No IN	IN	No IN
Total C (%)	0.140	5.54	4.49	19	80	**0.013***	5.34	4.41	30	69
Total N (%)	0.548	0.40	0.38	20	87	**0.014***	0.42	0.36	34	73
C:N	**0.001***	13.44	12.03	19	82	0.995	12.30	12.30	32	69
Nitrate (mg kg^{-1})	**0.046***	3.26	5.64	20	87	0.059	8.78	3.53	34	73
Ammonia (mg kg^{-1})	0.913	9.65	9.42	20	86	**0.011***	12.12	8.27	34	73
Soil $\delta^{15}N$ (‰)	**0.033***	4.01	4.63	20	86	0.994	4.83	4.37	33	73
pH	0.480	4.85	4.72	20	87	**0.047***	4.94	4.65	34	73

Table 5-3: Estimated difference between the rate of potential C and N inputs and observed rates of C and N accumulation in soil under pruned palm fronds. Accumulation is calculated by subtracting the C and N in soils not under palm fronds from those that have palm fronds.

	Observed concentration (Mg ha^{-1})		Observed accumulation (Mg ha^{-1})		Potential Inputs from palm fronds (Mg ha^{-1})		Difference between potential inputs and observed accumulation (Mg ha^{-1})	
	OM	No OM	Over 11 years	Per year	Over 11 years	Per year	Over 11 years	Per year
Soil Carbon	30.47	24.70	5.78	0.53	52.8	4.8	-47.03	-4.28
Soil Nitrogen	2.20	2.09	0.11	0.01	1.1	0.1	-0.99	-0.09

Lower median (relative to the mean) values for both nitrate and ammonia reflected the presence of a small number of samples with very high (circa. 50 mg N kg^{-1}) inorganic N (Table 5-1). As a result, both parameters displayed a strong positive skew (Figure 5-5) and were highly variable with coefficients of variation of 145% and 96% respectively (Table 5-1). For ammonia and nitrate, outliers (>20 mg N kg^{-1}) were removed and the remaining data transformed using a Box-Cox conversion where $\lambda = 0.25$. This successfully reduced skew and resulted in a near-normal distributions for both variables (Figure 5-5).

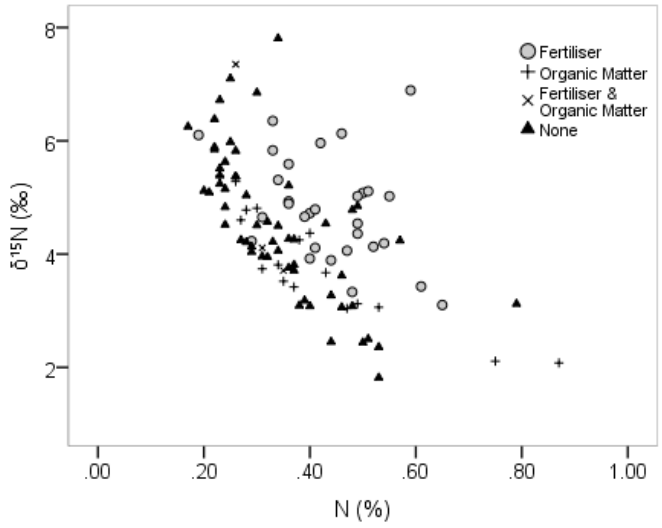
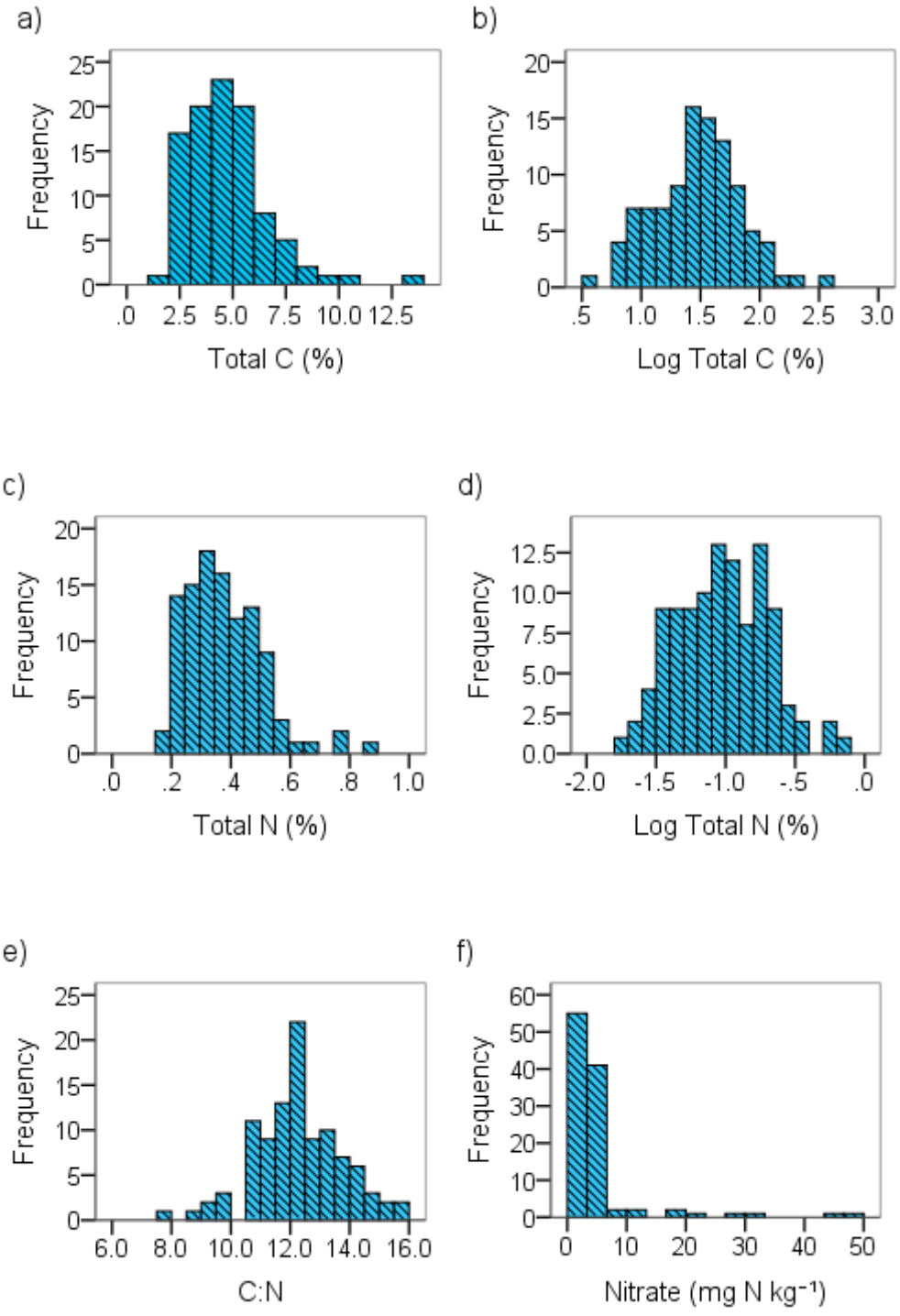


Figure 5-4: Relationship between N% and $\delta^{15}N$ (‰) in soils samples receiving different treatment of fertiliser and organic matter in plantation 15Y.


a)
Frequency
Total C (%)
b)
Frequency
Log Total C (%)
c)
Frequency
Total N (%)
d)
Frequency
Log Total N (%)
e)
Frequency
C:N
f)
Frequency
Nitrate (mg N kg⁻¹)

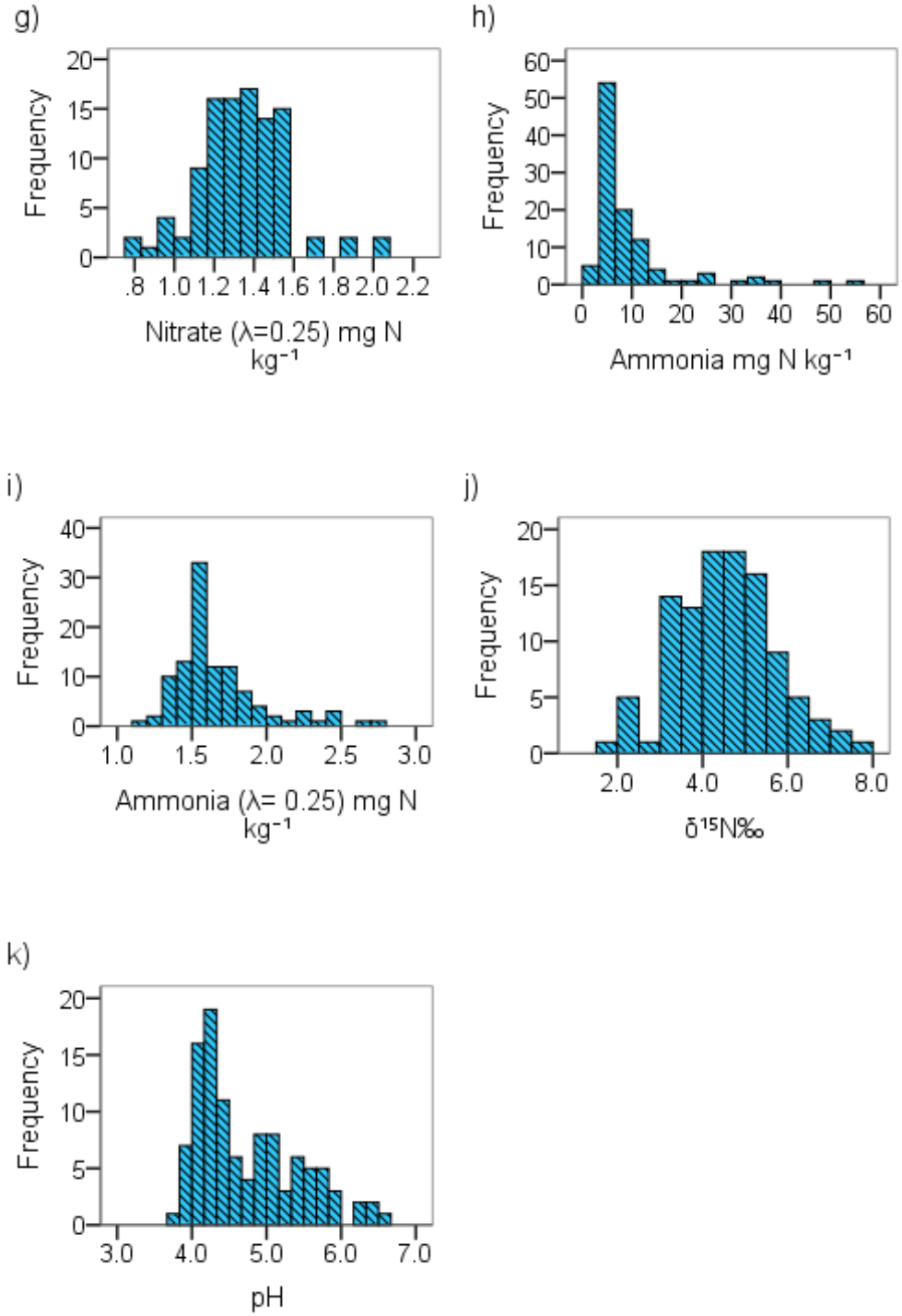
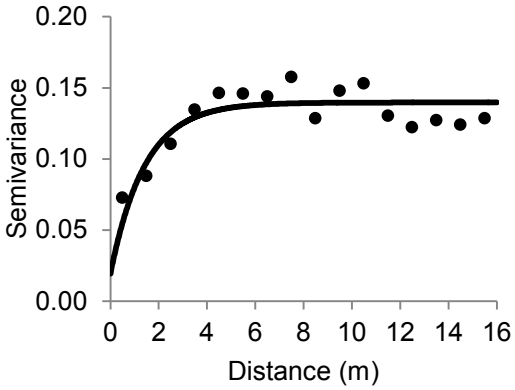
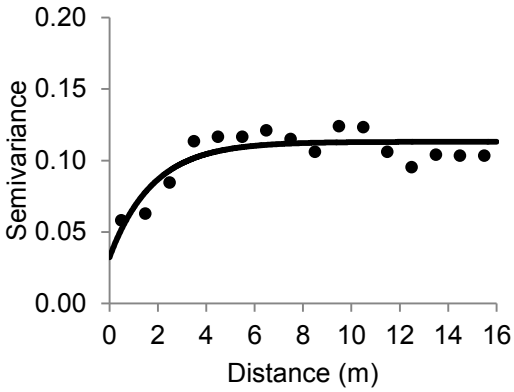


Figure 5-5: Frequency distributions for the variables described in Table 5-1: a) total carbon; b) total carbon (log transformed); c) total nitrogen; d) total nitrogen (log transformed); e) C:N ratio; f) nitrate; g) nitrate (box-cox transformed where λ = 0.25); h) ammonia; i) ammonia (box-cox transformed where λ = 0.25); j) $\delta^{15}N$; k) pH.

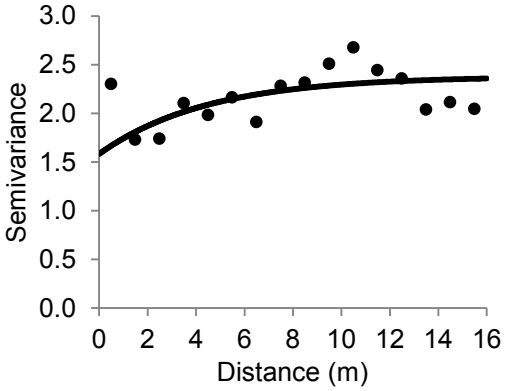
a) Total C



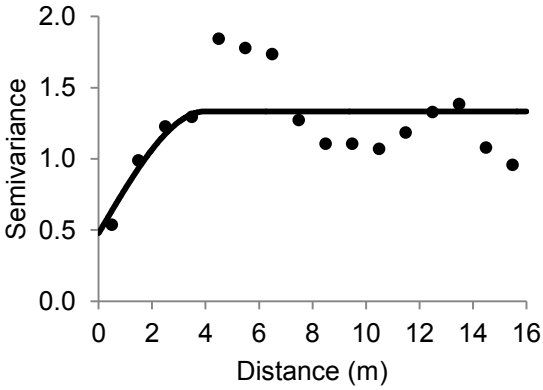
b) Total N



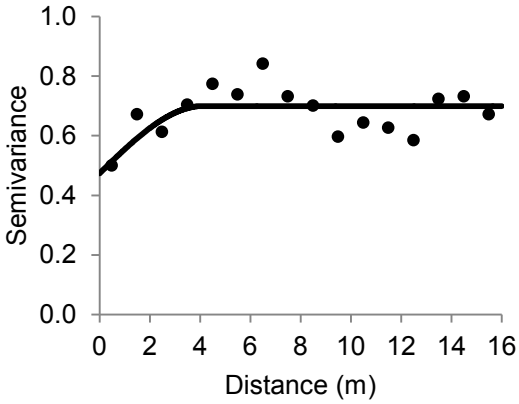
c) C:N



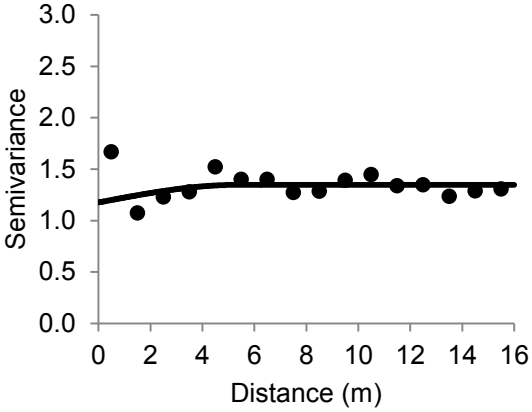
d) Nitrate



e) Ammonia



f) $\delta^{15}N$



g) pH

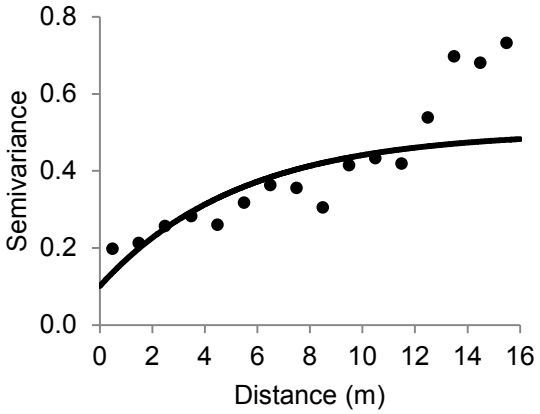


Figure 5-6: Semivariograms for the edaphic variables described in Table 5-1: a) total carbon; b) total nitrogen; c) the C:N ratio; d) nitrate; e) ammonia; f) $\delta^{15}N$; g) pH.

Table 5-4: Model parameters for semivariograms displayed in Figure 5-6.

Variable	Model	*r*	Nugget *(C_0)*	Sill Variance *(C_0+C)*	Nugget:Sill	Range (m)
Total C	Exponential[1]	0.953	0.020	0.140	0.140	4.276
Total N	Exponential[1]	0.969	0.032	0.113	0.286	5.361
C:N	Exponential[1]	0.694	1.583	2.379	0.665	13.376
Nitrate	Spherical[2]	0.899	0.541	1.335	0.405	3.783
Ammonia	Spherical[2]	0.718	0.474	0.700	0.677	4.115
$\delta^{15}N$	Spherical[2]	0.566	1.176	1.348	0.872	5.361
pH	Exponential[1]	0.951	0.101	0.503	0.201	>16

[1] $For\ h > 0, \gamma(h) = C_0 + C\left(1 - exp\left(\frac{-h}{r}\right)\right);\ \gamma(0) = 0$

[2] $For\ 0 < h \leq \alpha, \gamma(h) = C_0 + C\left(\frac{3h}{2\alpha} - \frac{1}{2}\left(\frac{h}{\alpha}\right)^3\right); for\ h > \alpha, \gamma(h) = C_0 + C, \gamma(0) = 0$

Total C and total N varied at similar rates to $\delta^{15}N$, with coefficients of variation between 27 and 39%. C and N also showed slight positive skews to their frequency distributions. Non-normality was corrected by log transformation, which then permitted prediction maps to be created using ordinary kriging (Figure 5-7). The C:N ratio and pH were the least variable having coefficients of variation of 12% and 14% respectively. Frequency distributions for

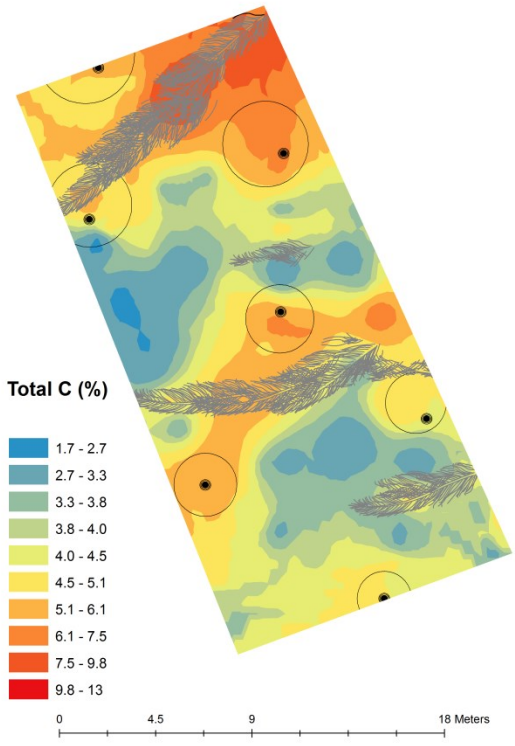
$\delta^{15}N$ and C:N showed normal distributions and accordingly data transformation was unnecessary prior to ordinary kriging. Log transformation of pH did not improve the non-normality of the distribution although, due to the low variation between samples, skewedness of the untransformed data was between the values of -1 and 1. Accordingly, in the absence of extreme outliers the ordinary kriging model performed well on untransformed data and was more accurate than prediction maps created using cokriging or disjunctive kriging methods (results not shown).

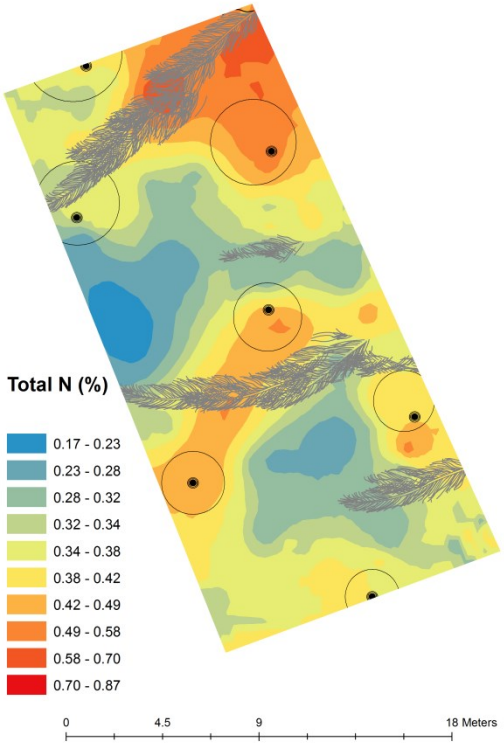
Total C, total N, C:N, and pH were best fitted to the exponential model, whereas the spherical model gave more accurate predictions for nitrate, ammonia and pH (Figure 5-6). The nugget:sill ratio describes the proportion of the population variance which does not contribute to autocorrelation at the lowest scale examined (in this case 1m). For C and pH, a ratio < 0.25 indicates that spatial dependence was particularly strong (Figure 5-6). Total N, nitrate, C:N and ammonia had a moderate proportion of the population variance as a function of spatial autocorrelation (i.e. 0.25 < nugget:sill <0.75). However, the semivariogram for $\delta^{15}N$ displayed an almost pure nugget effect with population variance making only a minimal (13%) contribution to autocorrelation at this scale. The remaining 87% of the variance was due either to autocorrelation at distances < 1m or experimental error. For the majority of variables, autocorrelation was strongest between 3.8 and 5.4 m. However, spatial independence for C:N was found beyond distances of 13.4 m; almost double that of the other variables for which a range was calculable. In the case of pH, a range was not calculated as the semivariogram failed to reach a sill over the distance modelled (i.e. 16 m).

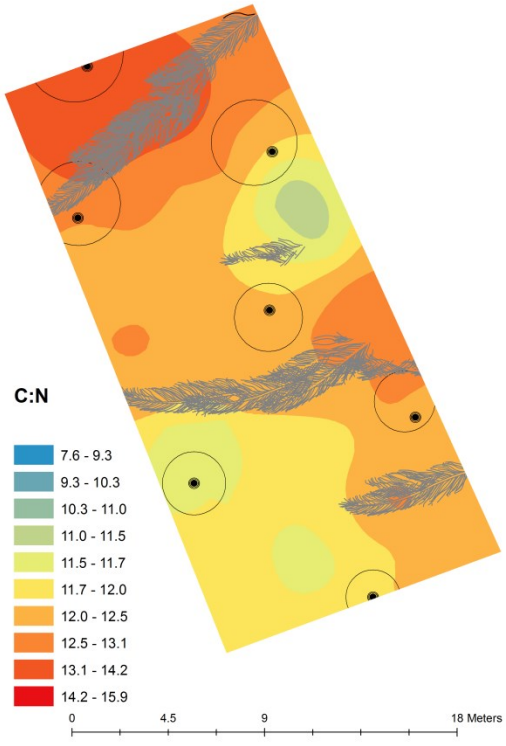
Ordinary kriging at 1m intervals produced the maps set out in Figure 5-7. Total N and C were strongly patterned across the plot and followed similar zonal demarcation as indicated by the

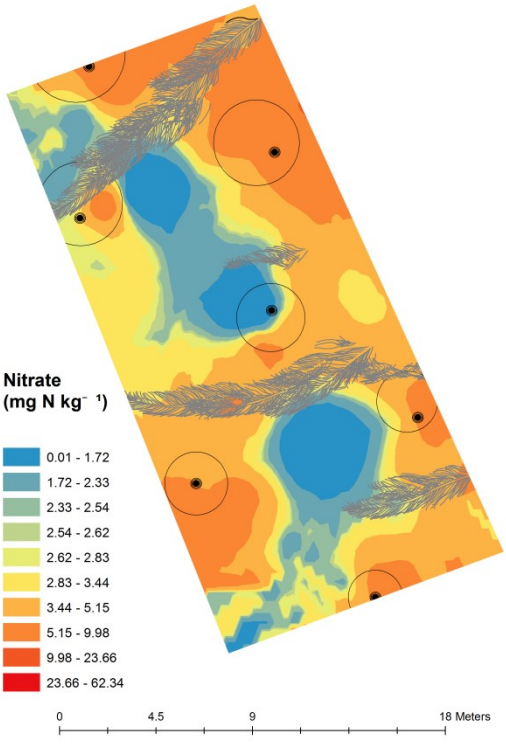
strong positive correlation between the two variables ($r = 0.95$, $p < 0.001$). Areas of high N and C approximately followed the same pattern of palm frond inputs with high values in the northwest corner and again through the middle of the plot. The C:N ratio was fairly uniform across the site as confirmed by the high spatial dependence range, although as with total C and total N, a higher value was expressed under palm fronds relative to the rest of the plantation. There were sharp gradients between high and low nitrate and ammonia concentrations through the plot. In the case of nitrate, areas with higher NO_3^- concentrations tended to be in proximity to the palm trunks where fertilisation takes place rather than in the inter-rows. However, there was a degree of similarity to nitrate and ammonia patterning. For example, soils from the inter-row in the southern half of the plot displayed concentrations of inorganic N that were lower than the plot average. In the northern inter-row, nitrate concentrations were also lower than average although the influence of palm tree location on soil ammonia was smaller than it was for soil nitrate.

Low $\delta^{15}N$ values generally corresponded to areas with higher organic matter inputs. This was confirmed by correlation analysis which revealed an inverse relationship between $\delta^{15}N$ and both total C ($r = - 0.53$, $p < 0.001$) and total N ($r = -0.59$, $p < 0.001$). The spatial patterning of pH was clearly divided between higher values in the north and lower values in the south and was weakly correlated with total C ($r = 41$, $p < 0.001$) and total N ($r = 0.38$, $p < 0.001$).


Total C (%)
1.7 - 2.7
2.7 - 3.3
3.3 - 3.8
3.8 - 4.0
4.0 - 4.5
4.5 - 5.1
5.1 - 6.1
6.1 - 7.5
7.5 - 9.8
9.8 - 13
0
4.5
9
18 Meters


Total N (%)
0.17 - 0.23
0.23 - 0.28
0.28 - 0.32
0.32 - 0.34
0.34 - 0.38
0.38 - 0.42
0.42 - 0.49
0.49 - 0.58
0.58 - 0.70
0.70 - 0.87
0
4.5
9
18 Meters


C:N
7.6 - 9.3
9.3 - 10.3
10.3 - 11.0
11.0 - 11.5
11.5 - 11.7
11.7 - 12.0
12.0 - 12.5
12.5 - 13.1
13.1 - 14.2
14.2 - 15.9
0
4.5
9
18 Meters


Nitrate
(mg N kg⁻ ¹)
0.01 - 1.72
1.72 - 2.33
2.33 - 2.54
2.54 - 2.62
2.62 - 2.83
2.83 - 3.44
3.44 - 5.15
5.15 - 9.98
9.98 - 23.66
23.66 - 62.34
0
4.5
9
18 Meters

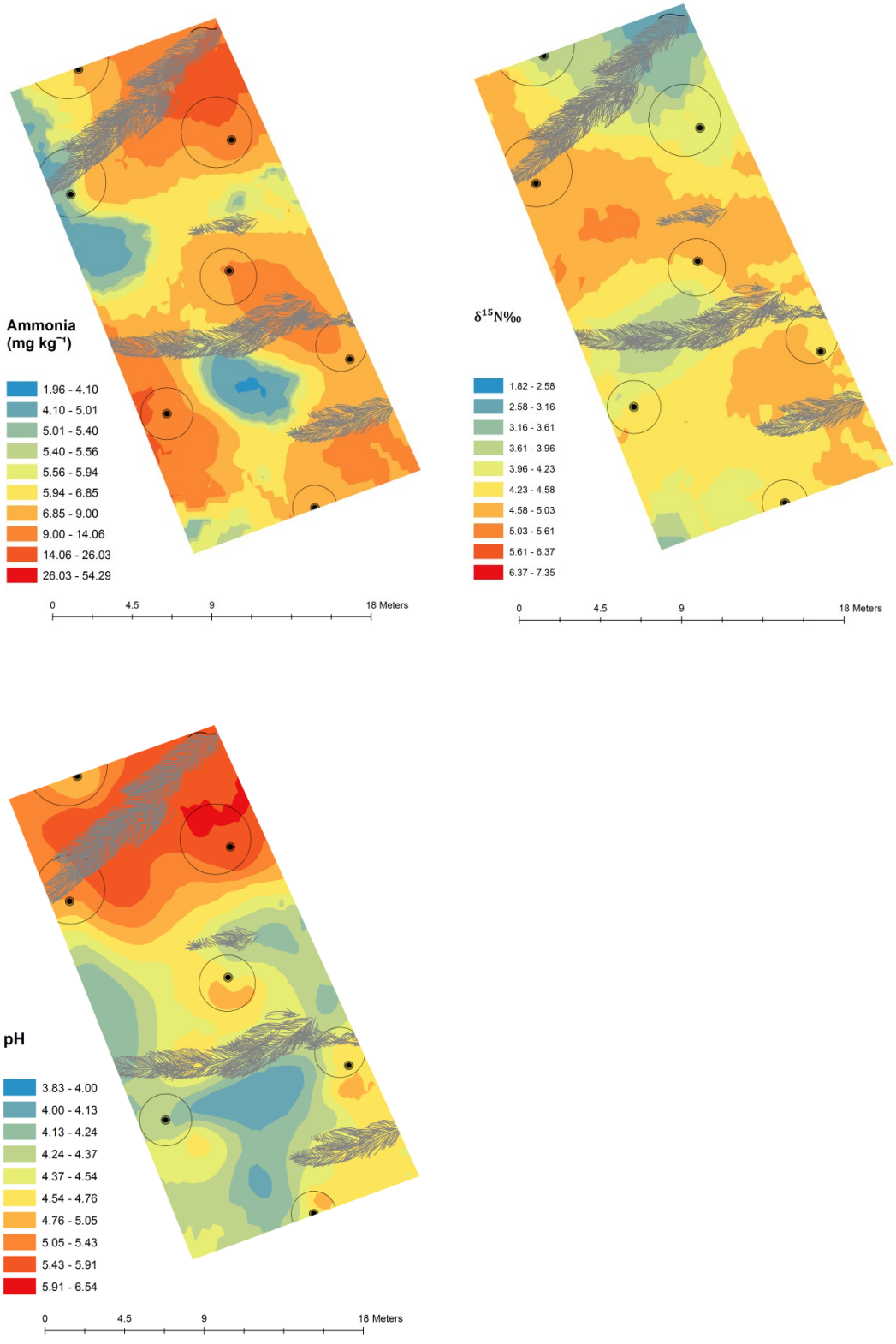


Figure 5-7: Maps of soil variables produced through ordinary kriging.

5.4 DISCUSSION

5.4.1 The effect of management practices on the distribution of C and N

Total C was 24% higher in soils that received inputs of organic matter from the frond piles than those that did not. Total N was 5% higher under frond piles although this increase was not statistically significant. The more modest increase in N relative to C did however result in higher C:N under palm fronds. Soil C increase observed under fronds in this 15 year-old plantation is similar to that reported for a 20-year old plantation and roughly half of that in a 10-year old plantation in mainland Malaysia, (Haron et al., 1998). As in Haron et al., (1998) large inputs of organic matter were not mirrored by the more modest increases in soil C (5.78 Mg C ha^{-1}) and N (110 kg N ha^{-1}) over the 11 years since palm maturity where 89% of frond C and 90% of frond N did not accumulate in the mineral soil. Haron et al., (1998) concluded that limited coupling between above ground inputs and C within the mineral soil was indicative of rapid decomposition at the soil surface. However, estimates of annual carbon accumulation under palm fronds in this plantation (0.53 Mg C ha^{-1} y^{-1}) were higher than the 0.3 Mg C ha^{-1} y^{-1} reported for five-year-old palms on peat soil in the neighbouring state of Sarawak (Melling, et al., 2007). Both the age of the plantation and the sampling depth used for that study (i.e. 20 cm versus 5 cm in this study) may explain some of the disparity in estimated C accumulation rates. As soils were sampled only to a depth of 5 cm, the percentage of carbon that may have accrued in the deeper soil horizons over this period is unknown. C and N that does migrate below the surface also may be rapidly processed by the soil microbial biomass. Biomass C has been observed to be greater under the palm fronds (Haron, et al., 1998; Khalid, et al., 1999), and a larger microbial population will liberate more C through respiration whilst simultaneously requiring N for assimilation. Nitrogen may also be taken up by plant roots or leached from the soil under conditions of sufficient soil moisture

(Schroth, et al., 2000). In addition to assimilation, microbial processes that remove nitrogen (e.g. nitrification and denitrification) from the soil may be greater under palm fronds where a supply of substrate is readily available.

Total C in samples from within the palm circle was roughly equivalent to that under the palm fronds. Despite the lack of frond inputs, increased soil C within the palm circle is likely to be due to the high root density within this zone and the influence of root exudates on carbon availability (Haron et al., 1998). The flat, low-lying topography of this site (Figure 5-1), is likely to minimises excessive hydrological losses of carbon and nitrate from the soil, although plantation inter-rows may be more susceptible to leaching losses than palm circles as a result of lower root density (Schroth, et al., 2000). The structure and development of oil palm roots has been well reported in the literature (Purvis, 1956; Jourdan & Rey, 1997; Khalid, et al., 1999; Corley & Tinker, 2003; Yahya, et al., 2010). In more mature plantations, soil C has been shown to correlate highly with fine root biomass (Syahrinudin, 2005). Root density is highest within a 1m radius of the trunk but drops rapidly with distances beyond 1m before levelling off at about 3m (Syahrinudin, 2005). Root depth depends on soil type and water table depth, although most fine roots are found in the 0-30cm top layer. Average rooting depth appears to be 4-5m (Sommer, et al., 2000; Syahrinudin, 2005), although depths of <1m have been reported for palms where the water table impedes root penetration (Lambourne, 1935). Most of the samples within the fertilisation treatment were at a distance <2 m from the base of the palm and all samples were taken from the shallow top layer where root C would be greatest. However, total C is reported as a percentage without adjustment for bulk density. As a result, any conclusion as to the significance of the difference in soil C inside and outside the area of fertilisation would need to be revised if bulk density within the palm circle is notably lower.

5.4.2 Spatial variability of ammonia and nitrate

Ammonia and nitrate were highly variable throughout the plot, although autocorrelation was stronger for nitrate than for ammonia as indicated by the higher nugget:sill ratio. Nitrate is often observed to be more spatially variable than ammonia: a fact usually attributed to the greater mobility of the NO_3^- ion in soils (Ettema, et al., 1998; Gallardo, 2003; Anuar, et al., 2008). Spatial patterning of inorganic N did not correlate well with frond inputs, however mean nitrate within the palm circle was more than double that of areas outside the circle. This notable, but non-significant, increase was due to several samples from within the circle showing evidence of fertilisation. Specifically, each of the 7 palms in this plot had at least one sample within a 2m radius of the palm trunk where $NO_3^- > 12$ mg N kg^{-1}. The interpolated maps confirmed this tendency for higher nitrate around the base of the palm trees and lower concentrations within the inter-rows. Although outliers were removed from the semivariogram model, this patterning was still apparent in the remaining covariance pairs and resulted in a degree of oscillation to the semivariogram (Figure 5-6). An attempt to model this pseudoperiodicity with the use of a hole effect model indicated that the spherical model remained the most accurate. Like a similar study within fertilised plantations in Sabah, ammonia was significantly higher within the palm circle, (Anuar, et al., 2008). However, the authors in that study also found ammonia to be higher under palm fronds: an observation not mirrored in this plantation.

Soil nitrate was significantly lower under the palm fronds *vis-á-vis* the remainder of the plantation. Assimilation, microbial processing or leaching are the possible candidates for decreased nitrate under fronds. Under the palm fronds, where a fresh supply of organic matter is available, the microbial biomass is likely to be higher than in the plantations inter-rows (Haron, et al., 1998). Therefore, assimilation of N by a larger microbial population during

decomposition may result in lower net mineralisation with less NH_4^+ available for nitrification relative other areas of the plantation. Another explanation is that, where nitrification occurs and soils subsequently become depleted in oxygen, high NO_3^- turnover in combination with abundant organic carbon provides conditions favourable for denitrification and dissimilatory nitrate reduction to ammonia (DNRA). Potential DNRA in the top 0-10cm of this plantation soil was found to be negligible when compared with denitrification (see Chapter 6). As such, the importance of DNRA to NO_3^- and NH_4^+ concentrations is insignificant when compared to the much larger rates of denitrification. Although we did not measure denitrification within this sub plot, in-situ denitrification for this site averaged 19 kg^{-1} N ha^{-1} y^{-1}. Over the course of 11 years, this equates to 210 kg N ha^{-1} and is almost 20% of the deficit of 1.1 Mg N ha^{-1}, which is unaccounted for from the frond inputs (Table 5-3). Therefore, low extractable NO_3^- and greater available organic matter, coupled to the generally high rates of in-situ denitrification observed for this site, suggest that denitrification may keep nitrate concentrations lower under palm fronds relative to other parts of the plantation.

5.4.3 The impact of management practices on $\delta^{15}N$

Interpretation of $\delta^{15}N$ signatures in soils is not without difficulty on account of the complexity in differentiating between the amount and natural abundance of N inputs, together with isotopic fractionation by simultaneously occurring processes such as mineralisation, nitrification, denitrification and volatilisation. Nevertheless, the ratio of ^{15}N to ^{14}N of soil N is commonly used as a proxy for the degree of nitrogen cycling within the soil. Higher $\delta^{15}N$ values are usually indicative of greater N processing rates as microbes discriminate against heavy ^{15}N in favour of lighter ^{14}N. If N processing is greater under the fronds, we might expect to observe soils enriched in $\delta^{15}N$ relative to other areas of the plantation. However, $\delta^{15}N$‰ was lower below palm fronds relative to the remainder of the plantation. Lower

$\delta^{15}N$‰ below palm fronds results from either the influence of depleted ^{15}N inputs without a concurrent increase in fractionating N process rates, or greater N losses (relative to inputs) in the remainder of the plantation (i.e. in the palm circle and harvest paths). There is some evidence for the first hypothesis as, although the $\delta^{15}N$ of palm leaves in this plantation were not sampled, herbaceous vegetation between the palms had a $\delta^{15}N = 3.73$‰; a value lower than the mean delta value for soil samples taken below frond piles. Caution must be exercised when interpreting lower ^{15}N in soil as a result of palm frond inputs because of differences in the form and depth of N uptake and fractionation processes within the plant sampled. But, assuming a similar $\delta^{15}N$ per mil value for palm fronds (which by no means is certain), lighter soil ^{15}N relative to the remainder of the plantation may be a result of high returns of organic matter depleted in ^{15}N relative to the soil. However, the accrual of soil N under palm fronds was <90% of estimated N returns from frond inputs and is suggestive, therefore, of high N losses. The alternative hypothesis proposes that N losses are greater in the remainder of the plantation (relative to inputs) than under the palm fronds. The remainder of the plantation incorporates both the palm circle, where inorganic N fertiliser is applied, and the harvest paths, which receive neither inorganic fertiliser nor organic matter inputs directly. The application of N fertiliser with a $\delta^{15}N$ value of approximately zero would be expected to lower ^{15}N within the palm circle, however, contrary to expectation, $\delta^{15}N$ was higher in the vicinity of the palm trunks than the rest of the plantation. Fractionation losses during mineralisation tend to be small relative to nitrification, denitrification and volatilisation, all of which may be increased by inorganic fertiliser application. Therefore, the addition of nitrogen fertiliser is likely to have increased fractionating processes in soils that are closer to the palm trunks. The fact that fertilised samples tended to have higher $\delta^{15}N$ relative to samples receiving no fertiliser and those receiving organic matter inputs at similar soil N concentrations partly

corroborate this interpretation (Figure 5-4). It is likely, therefore, that isotopic enrichment in areas without palm frond input is a result of increased N losses following fertiliser application.

5.4.4 The range of spatial independence

The majority of variables displayed spatial independence at distances <5.5 m. Sampling design within the wider plantation matrix employed a minimum distance of ~30m thereby confirming that the requirements of spatial independence were met. For total C and N, returns of organic matter to the soil are likely to have enhanced variability within the plantation and reduced the range over which spatial dependence occurred. Ammonia and nitrate were similarly variable, although the range of spatial dependence for ammonia was half that reported for a similar plantation in Sabah (Anuar, et al., 2008). The low C:N ratio and the high nugget value reflects the lack of variability across the plot for C:N. This is consistent with low C:N in tropical soils subject to intensive weathering. The range for $\delta^{15}N$ is similar to the range modelled for C, N, NO_3^- and NH_4^+, although the strength of autocorrelation over this distance was minimal. An almost pure nugget effect for $\delta^{15}N$ suggests that variability occurs at scales other than that modelled here and demands further investigation. Reported ranges for pH vary considerably in tropical landscapes. For example, modelled nuggets range from 25.2m, for pH in Sumatran rubber plantations (Rodenburg, et al., 2003), to 350m in lowland Panamanian forests (Yavitt et al., 2009). In this plot, the distance at which spatial independence occurred was not determinable for pH due to the failure to reach a sill at the distance sampled.

5.5 CONCLUSION

Spatial distributions of carbon and nitrogen within the plantation were influenced by the management practice of frond pruning and stacking. However, the magnitude of nutrient return from fronds was not reflected by significant nor commensurate increases in soil C or N, most likely as a result of rapid decomposition at the soil surface and high rates of microbial respiration within the soil. C and N were significantly higher in samples that received inputs of inorganic N fertiliser, though for C this is possibly due to the high density of oil palm roots within the palm circle where fertiliser is applied. The addition of organic matter in palm inter-rows did significantly increase the C:N ratio and likely contributed to increased spatial variability at the scale sampled.

Both nitrate and ammonia were highly variable within the plot, though the effect was stronger for nitrate than ammonia as evidenced by a higher nugget:sill ratio. As predicted, extractable ammonia was higher within the palm circle, but despite mean soil nitrogen within the area of fertilisation being more than twice that of the remainder of the plantation, results were not significant. Soil nitrate was also significantly lower under the palm fronds when compared to the rest of the plantation, reflecting either lower net nitrification as a result of NH_4^+ assimilation by a large microbial population, or losses of NO_3- through denitrification and leaching.

At the scale sampled, there was no autocorrelation for $\delta^{15}N$. However, contrary to expectations, soil ^{15}N was lower under palm fronds which received fresh organic matter inputs and higher within the palm circle where inorganic fertiliser with a $\delta^{15}N$ of ~0‰ is applied. This spatial distribution of soil ^{15}N most likely reflected increased fractionation

through microbial N processes such as nitrification and denitrification following the addition of inorganic fertiliser to the palm circle.

Finally, the majority of variables were spatially independent over a range much shorter than our minimum sampling distance (~30 m) within the wider plot matrices. We can therefore assume spatial independence within plantation sites for all variables except pH.

CHAPTER 6: DENITRIFICATION AND NITROUS OXIDE EMISSIONS FROM TROPICAL RIPARIAN AND *TERRA FIRME* SOILS PLANTED WITH *ELAEIS GUINEENSIS* (OIL PALM)

6.1 INTRODUCTION

N_2O emissions from tropical forests, have been estimated at 1.3-3.5 Tg N y^{-1}, and represent a major contributor to the global atmospheric budget (Matson & Vitousek, 1990; Bouwman, 1998; Werner, et al., 2007). However, agricultural development within this region has proceeded rapidly over the last 30 years introducing much uncertainty into estimates of trace gas emissions. As emissions of N_2O from agricultural soils are generally higher than those from natural soils, the replacement of natural forest with fertilised tree crops is most likely to require a upwards revision of global estimates.

Where soil N_2O flux has been measured in oil palm plantations, rates of emission appear to be greater than those from adjacent forests, at least on organic soils (Histosols) (Germer & Sauerborn, 2008; Melling, et al., 2007; Hergoualc'h, et al., 2012). However, peat decomposition may contribute to the emission rate irrespective of management practices such as fertilisation (Germer & Sauerborn, 2008; Melling, et al., 2007). On mineral soils, the emission rate is likely to be lower than from Histosols, and rates reported are in the region of -0.08 to 4.4 kg N_2O-N ha^{-1} y^{-1} (Yashiro et al., 2007; Skiba et al., 2012). The Oxidant and Particle Photochemical Processes (OP3) Project conducted in Sabah, Malaysia, during 2008 found that oil palm plantations emitted 50% more N_2O and 250% more NO_x ($NO + NO_2$) than neighbouring rainforests, highlighting the potentially global consequences of land use change in this region (Hewitt et al., 2009). Furthermore, measurements on the ground recorded rates

as high as 5.8 – 6.5 mg N_2O-N m^{-2} h^{-1} within the palm circle where fertiliser is applied (Skiba, et al., 2012; Fowler, et al., 2011).

Only a limited number of studies have reported N_2O emissions from plantations of different ages. In Indonesia, young (<10 year) oil palm plantations emitted three times more N_2O than mature (>10 year) plantations and 30% more N_2O than forests (Ishizuka, et al., 2005). Conversely, N_2O emissions from a 9-year-old plantation in Sarawak were not statistically different to emissions from 1-year-old plantation (Kimura, et al., 2012). The limited number of available estimates of nitrous oxide emissions from plantation soils, together with a lack of information on how emissions might change over the life span of the crop (typically 25 years) is a major contributor to uncertainty. Given that oil palm was the fifth most rapidly expanding crop in harvestable area during the years 1999-2008 (Phalan et al., 2013), and that this growth is predicted to continue through the coming decades (FAO, 2011a), further work is needed to assess the impact of plantations on nitrogen transformations and particularly N_2O emissions.

The objective of the present study was to employ chronosequence analysis to determine the effect of plantation age (time following establishment) on soil nitrogen cycling and N_2O emissions in Sabah, Malaysia. The problems with chronosequence studies are well-documented (Pickett, 1989; Walker, et al., 2010). Thus, to mitigate issues associated with temporal variability, samples were taken from each location during October 2010 and again in April 2012. This also allowed the comparison of emission rates over both seasons with the expectation that N_2O flux will be greater during the wet season than during the inter-monsoon, and that, based on the findings in Ishizuka et al. (2005), emissions would decrease as plantations matured. Rather than provide an estimate of annual N_2O emissions, the purpose of this study is to examine the difference in N_2O flux and N availability (during the wet and dry seasons) though plantation age. Furthermore, as plantations were located on two

different substrates (i.e. riparian plantations on alluvium and *terra firme* plantations on mudstone and sandstone), chronosequence analysis was only conducted on variables that showed no significant effect of location (i.e. riparian versus *terra firme*).

6.2 MATERIALS AND METHODS

6.2.1 Characteristics of oil palm cultivation and the plantations selected for study

Oil palm is primarily cultivated as a monoculture under a continuous cropping system in which the palms have an economic life span of 20-30 years. Fresh fruit bunches (FFB) are harvested by hand with a sickle and pole and this becomes increasingly difficult once the palms grow above 12 m tall. Productivity also declines with age so that beyond 20 years, palms are felled and chipped before being incorporated back into the soil (Figure 6-1). Bare soil is sometimes protected the first few years after planting by sowing a leguminous cover crop, which reduces erosion and fixes atmospheric N in soils that are N poor due to volatilisation (burning), leaching and erosional losses following site clearance. Seedlings are planted out from the nursery at 12-15 months and become harvestable between three and five years later. Canopy closure occurs at about 5 years whereupon any ground cover crops (planted during establishment of the plantation) die back as light penetration decreases. Encroachment by understory vegetation is managed through cutting or spraying chemical herbicides (primarily 41% glyphosate) that is applied several times a year to prevent natural vegetation from competing with the oil palms for nutrients. Therefore, the primary aboveground organic matter returns to the soil are from pruned palm fronds, which are regularly trimmed back and placed in the plantation inter-rows (Figure 6-1). On some plantations, the FFB fibres are also returned to the soil after the fruit has been harvested (Figure 6-1).

a)

b)

c)

d)

d)

e)

Figure 6-1: a) Fruit ripening on palms at a 5Y; b) a recently cleared oil palm plantation prepared for re-planting; c) site 15Y, where the pruned palm fronds can be seen in the inter-rows; d) harvested fruit bunches with the fruit still attached at 3Y; e) site 25Y during the 2010 end of dry season sampling with the 25+ year old palms; and f) the same location at 25Y during the 2012 end of wet season sampling after cutting, chipping and incorporation into the soil of the old palms and planting out of new 3-month old palms. Geographical location for each of the plantations is in Figure 3-1 (p.47).

Table 6-1: Soil properties, location, ownership and generational status of the six oil palm plantations sampled.d.

Plantation Age:	3 years (3Y)	5 years (5Y)	6 years (6Y)	8 years (8Y)	15 years (15Y)	25 years (25Y)
Location	5°31′18.N 118°17′37.E	5°32′52.N 118°08′12.E	5°24′32.N 118°01′08.E	5°33′21.N 118°08′27.E	5°31′08.N 118°17′43.E	5°24′02.N 117°59′18.E
Generation	First	First	First	Second	First	Second/First
Ownership status	Smallholding	Commercial	Commercial	Commercial	Smallholding	Commercial
Original vegetation	Riparian	Mixed dipterocarp	Riparian	Mixed dipterocarp	Riparian	Riparian
Soil type	Tuaran Association	Rumidi Association	Tuaran Association	Rumidi Association	Tuaran Association	Tuaran Association
Substrate	Alluvium	Mudstone & sandstone	Alluvium	Mudstone & sandstone	Alluvium	Alluvium
Soil texture	Silty loam	Silty clay loam	Silty clay loam	Silty loam	Silty clay loam	Silty clay loam
Bulk Density ($g\ cm^{-3}$)	0.94a (0.04)	1.08abc (0.07)	1.29b (0.04)	1.14abc (0.08)	1.10c (0.03)	1.17abc (0.11)
Sand (%)	19.90a (1.05)	11.96a (2.76)	8.06b (1.94)	22.24a (2.22)	7.01b (1.08)	16.89a (1.61)
Silt (%)	53.91a (1.43)	56.88a (0.86)	61.62b (1.29)	55.13a (1.07)	63.87b (0.94)	55.47a (1.12)
Clay (%)	26.19a (0.90)	31.16b (2.61)	30.32b (1.06)	22.63a (1.87)	29.12a (1.21)	27.64a (0.81)
pH_w	4.41a (0.06)	5.91b (0.23)	4.47a (0.06)	4.88ac (0.20)	4.34a (0.08)	5.18bc (0.11)

Notes: Plantations ages are based on the age of the plantation during the 2012 sampling season.
Significant differences in soil variables are indicated by different lower case letters. Standard error in parenthesis. Differences were tested using one-way ANOVA for pH ($F(5, 30.2)=15.386$, $p < 0.001$); bulk density ($F(5, 29.922)=7.264$, $p < 0.001$); sand ($F(5, 66)=35.190$, $p < 0.001$); silt ($F(5, 66)=12.472$, $p < 0.001$); clay ($F(5, 66)=16.208$, $p = 0.006$.

Table 6-2: Concentrations of extractable nitrate and ammonia (g N m^{-2}) and percent water-filled pore space (WFPS) in the six oil palm plantations during the inter-monsoon and end of wet season sampling.

Age	Nitrate (g N m^{-2})				Ammonia (g N m^{-2})				WFPS (%)			
	Inter-monsoon		End of wet season		Inter-monsoon		End of wet season		Inter-monsoon		End of wet season	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3Y	8.72ab	1.96	2.17ab	0.17	16.68a	3.37	4.82	0.56	46abc	1.4	66a	3.3
5Y	109.02a	29.57	1.44a	0.21	112.85b	35.55	8.46	0.78	38a	2.9	91b	2.1
6Y	16.90ab	7.26	2.80b	0.39	45.11ab	6.74	9.13	2.90	50ac	3.7	78c	3.9
8Y	14.48b	7.03	1.17ab	0.19	32.08ab	6.99	8.33	0.61	36b	2.8	85bc	2.5
15Y	2.17b	0.66	2.17ab	0.38	80.27b	16.28	5.73	0.51	52c	3.3	85	1.8
25Y	4.10ab	0.86			25.22ab	5.95			37ab	4.2		

Notes: Differences between plantation age for inter-monsoon and end of wet season sampling are signified by different lower case letters, $p < 0.05$.

N_2O and soil nitrogen transformations were measured across a chronosequence of oil palm plantations that spanned an age range of 3 – 25 years, and incorporated first and second generation plantings within both large estates and smallholdings (). Plantations are described by stand maturity according to their age at the end of wet season sampling. Thus, site 3Y was a three-year old plantation in April 2012 but was only eighteen months old when first sampled in October 2010. Two of the plantations were situated on *terra firme* soils of the Rumidi Association and the remaining four plantations were in the riparian zone of the Kinabatangan River on recent alluvium (). It was not possible to collect data on timing and quantity of fertiliser or herbicide application, although typically in this area 0.5-2.5 kg N is applied per palm in two applications annually (70-350 kg N ha^{-1} y^{-1}) within a 2m radius of the palm trunk ("the palm circle"). Most sites had not received fertiliser for several months prior to sampling, however, at site 5Y (a commercial, five year old plantation on *terra firme* soils), NPK fertiliser was visible at the soil surface during the inter-monsoonal sampling. The effect

of this is apparent in the very high concentrations of soil nitrate (109 ±30 g N m^{-2}) and ammonia (113 ±36 g N m^{-2}) reported for this site at that time (Table 6-2). For the 25-year old plantation (25Y), results are reported only for the first sampling season in 2010. This is because during the inter-monsoon, 25Y was a mature plantation reaching the end of its economic life (Figure 6-1). When the site was revisited at the end of the wet season in 2012, the mature trees had been felled and new seedlings planted out 3 months prior to sampling. Accordingly, it was not possible to examine seasonal differences or the interaction of season with both stand age and location due to the confounding effects of replanting.

6.2.2 Soil analysis

Full details of sample collection and processing, including general soil characteristics, are provided in Chapter 2. Sample preparation and analysis were identical over both years save in the case of extractable nitrate and ammonia. At the end of the wet season, fresh soil samples were placed in pre-prepared bottles of 2M KCl immediately after collection and kept on ice until extraction and freezing. By contrast, during the inter-monsoon, extraction took place approximately 24 hours after collection and storage of soils at ambient temperature. Changes in soil ammonia and nitrate concentrations following sample storage and disturbance have been reported by a number of studies (Arnold, et al., 2008; Kaur et al., 2010; Li et al., 2012). The absence of plant uptake and stimulation of N turnover through soil disturbance and storage is shown to increase extractable inorganic nitrogen (particularly NO_3^-) concentrations and the ratio of NO_3^-:NH_4^+ (Arnold, et al., 2008; Van Miegroet, 1995) Even excluding site 5Y (which had been fertilised during the inter-monsoon), mean soil nitrate and ammonia were three to four times higher during the inter-monsoon than at the end of the wet season. Although these differences may be partly the result of seasonal or inter-annual variation, it is impossible to determine whether the dissimilarity in concentrations over the

two seasons is a real temporal variation or simply an artefact of sample storage and preparation. Accordingly, nitrate and ammonia are excluded from statistical analysis of seasonal variation.

6.2.3 Statistics

A split-plot ANOVA was used to determine differences between location (riparian and *terra firme*) and plantation age across seasons. Variables were tested for multivariate normality using the Kolmogorov-Smirnov test and for homogeneity using Levene's statistic. Where the requirements of normality were not met, variables were log transformed before analysis. For variables that failed the tests of log-normality, non-parametric equivalent tests (trimmed means and bootstrapping) were employed to estimate the interaction effect of between–subject factors (location or age) and within-subjects factor (season). Post-hoc analysis was conducted using Student's *t*-test (two treatments) or one-way ANOVA (three treatments). Calculations were done in SPSS statistical software v.20 (IBM Corp.) and R Development Core Team (2013) v.2.15.3.

6.3 RESULTS

6.3.1 Soil organic matter, nitrate and ammonia availability

Soil moisture differed significantly (t = -12.701, $p < 0.001$) over the two sampling seasons. Mean end of wet-season WFPS (81±1.7%) was approximately double that of the inter-monsoon (43±1.5%). Variability in soil moisture across site age was greater at the end of the wet season ($F(4,55)=11.743$, $p <0.001$) than during the inter-monsoon ($F(5,66)=4.860$, $p = 0.001$). In the dry season, WFPS was positively correlated with SOM ($r = 0.54$, $p < 0.001$), DNRA ($r = 0.46$, $p < 0.001$) and soil ammonia ($r = 0.26$, $p = 0.047$) and negatively correlated

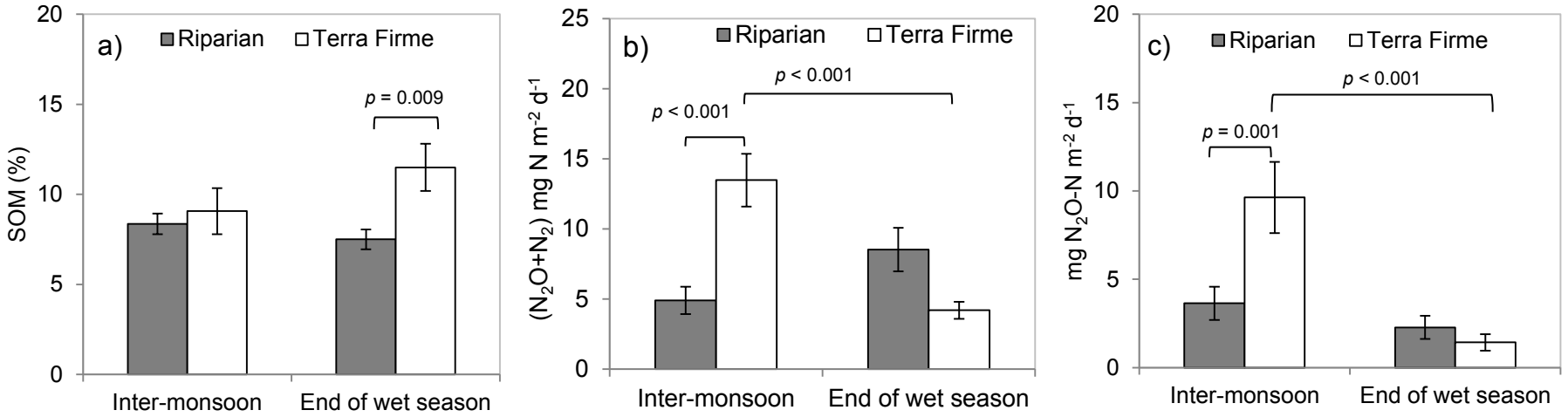
with N_2O emission (r = -0.33, p = 0.004). At the end of the wet season there were similar correlations of WFPS with SOM (r = 0.33, p = 0.009) and ammonia (r = 0.39, p = 0.002) but no relationship with N_2O production. Due to the storage of samples prior to inorganic N extraction during the inter-monsoon, interaction effects were not examined. However, nitrate varied between plantation age during both seasons (Table 6-2). Ammonia concentrations differed significantly between sites during the inter-monsoon. At the end of wet season, nitrate and ammonia were uniformly low across all sites. For both forms of inorganic nitrogen, there was no trend in concentration decline or increase through stand age. Mean soil organic matter (SOM) did not differ between sampling season or location alone, however, there were significant interactions between the two main effects of season and location (F = 4.528, p = 0.038) and between season and stand age (F = 2.909, p = 0.03). *Terra firme* soils held more SOM than riparian soils but the magnitude of this difference varied with season (Figure 6-2). During the inter-monsoon *terra firme* SOM (9.1±1.3%) was only marginally higher than riparian SOM (8.4±0.6%) (t = 0.325, p = 0.746). By contrast at the end of the wet season, SOM in the *terra firme* plantations (11.5±1.3%) was significantly higher than in the riparian plantations (7.5±0.6%) (t = -2.764, p = 0.009).

Table 6-3: Results of the interaction effects of season with location and stand age through the oil palm plantation sites.

		SOM (%)				N_2O (mg N m^{-2} d^{-1})				N_2O+N_2 (mg N m^{-2} d^{-1})				Denitrification$_{pot}$ (g m^{-2} d^{-1})				$DNRA_{pot}$ (mg m^{-2} d^{-1})			
		Inter-monsoon		End of wet season		Inter-monsoon		End of wet season		Inter-monsoon		End of wet season		Inter-monsoon		End of wet season		Inter-monsoon		End of wet season	
Stand age (years)		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3		11.5	0.8	7.8	0.6	2.43	1.63	1.24	0.58	3.31	1.46	4.72	2.12	0.85	0.20	0.62	0.12	4.17	1.34	1.34	0.27
5		10.3	2.2	15.2	1.8	9.07	3.00	1.05	0.39	16.98	2.64	4.41	1.20	0.22	0.08	1.02	0.18	0.54	0.20	1.35	0.11
6		6.2	0.7	6.1	0.3	3.02	1.55	1.23	0.97	5.72	1.80	15.63	3.14	4.83	1.13	1.98	0.33	4.98	0.63	2.15	0.26
8		7.9	1.3	7.8	1.2	10.18	2.81	1.80	0.87	9.97	2.40	3.99	0.28	0.72	0.19	3.35	0.60	6.11	2.54	2.83	0.50
15		7.3	0.8	8.6	1.5	5.45	1.70	4.37	1.51	5.69	1.88	5.24	1.29	0.93	0.32	4.04	0.39	6.93	0.91	1.47	0.32
25		3.0	0.7	-	-	3.23	1.32	-	-	5.17	2.17	-	-	2.61	0.83	-	-	2.54	0.87	-	-
ANOVA	*df*	*F*		*P*		*F*		*P*		*F*		*P*		*df*	*F*	*P*		*df*	*F*	*P*	
Season	1	0.769		0.384		12.309		**0.002**		1.598		0.215		1	2.208	0.145		1	8.011	**0.017**	
Stand age	4	8.793		**<0.001**		3.809		**0.023**		5.509		**0.005**		3	10.076	**<0.001**		3	2.476	0.102	
Stand age x Season	4	2.909		**0.030**		2.775		0.062		7.398		**0.001**		3	13.608	**<0.001**		3	1.791	0.192	
ANOVA	*df*	*F*		*P*		*F*		*P*		*F*		*P*									
Season	1	1.582		0.214		11.551		**0.002**		4.855		**0.033**			4.113	**0.049**			2.610	**<0.001**	
Location	1	2.592		0.113		4.449		**0.043**		4.205		**0.047**			0.193	0.663			1.688	0.138	
Location x Season	1	4.528		**0.038**		5.682		**0.024**		15.411		**0.001**			3.870	0.056			4.652	0.130	

Table 6-4: The effect of season and location on SOM, N_2O emissions, in-situ denitrification (as N_2O+N_2), potential denitrification, potential DNRA and the ratio of N_2O to in-situ denitrification through the oil palm plantations.

Treatment	Soil Organic Matter (%)		N_2O (mg N m^{-2} d^{-1})		(N_2O+N_2) (mg N m^{-2} d^{-1})		$Denitrification_{pot}$ (g N m^{-2} d^{-1})		$DNRA_{pot}$ (mg N m^{-2} d^{-1})		N_2O: (N_2O+N_2)	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
Riparian x Season	-0.996	0.326	-0.915	0.360	1.941	0.051	-0.770	0.441	-5.732	**0.001**	-3.035	0.094
Terra firme x Season	1.631	0.117	-3.700	**<0.001**	-3.571	**<0.001**	2.803	**0.005**	-0.672	0.495	-3.071	0.200
Inter-monsoon x Location	0.325	0.746	3.306	**0.001**	3.670	**<0.001**	-1.946	0.052	-1.669	0.162	0.294	0.783
End of wet season x Location	2.764	**0.009**	-0.421	0.674	-0.679	0.497	1.797	0.079	1.282	0.205	-0.158	0.885



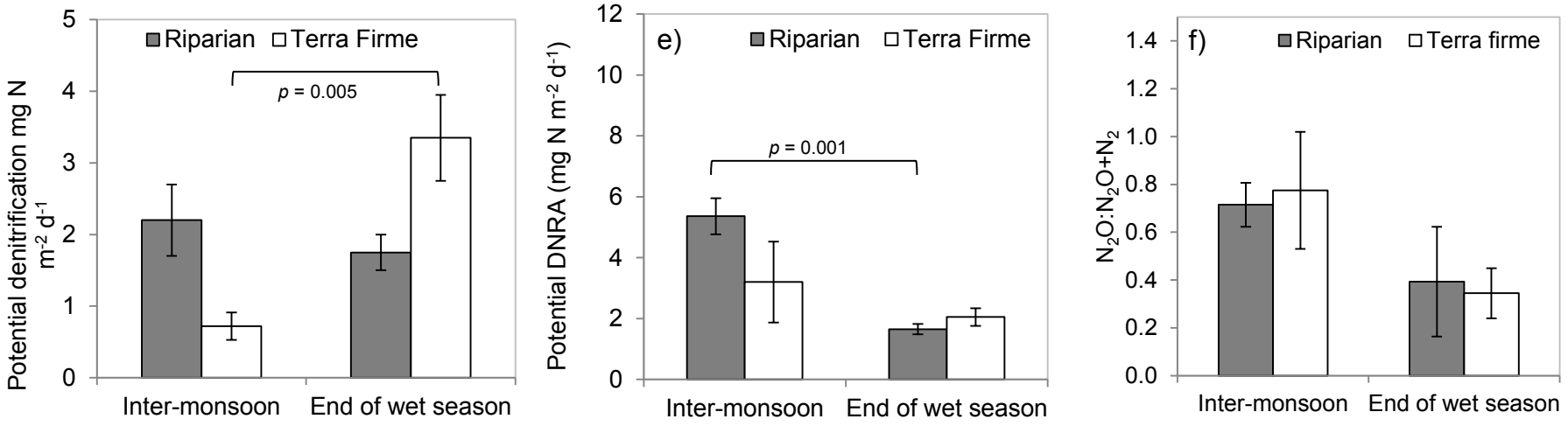


Figure 6-2: Differences in a) SOM; b) in-situ denitrification (as the production of N_2O+N_2); c) N_2O emissions; d) potential denitrification; e) potential DNRA; and f) the ratio of N_2O to in-situ denitrification across season and location. Error bars show standard error of the mean.

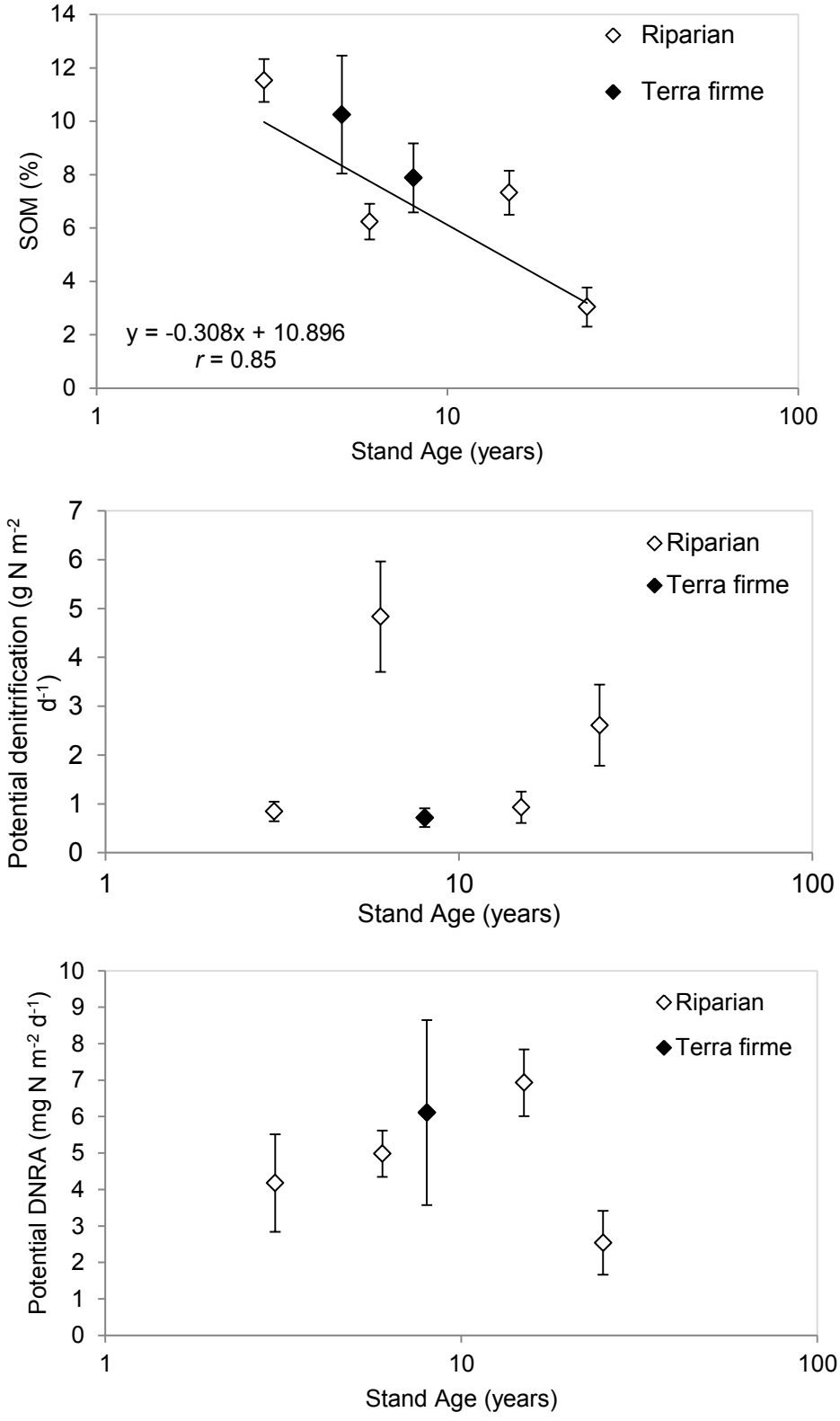


Figure 6-3: Trends through stand age during the inter-monsoon for: a) SOM; b) potential denitrification; and c) potential DNRA. Error bars represent the standard error of the mean.

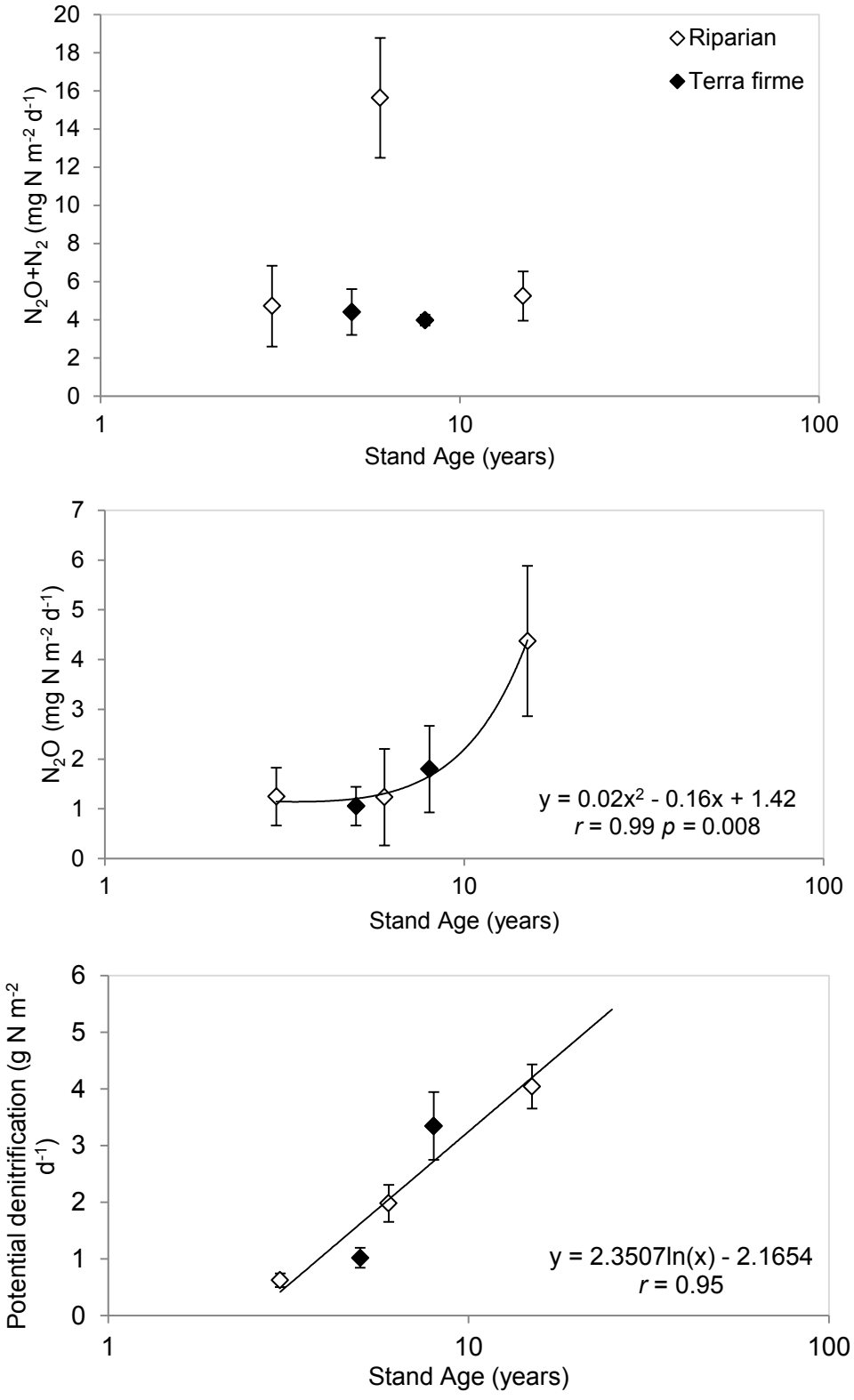


Figure 6-4: Trends through stand age at the end of the wet season for: a) in-situ denitrification (as the production of N_2O+N_2); b) N_2O emissions. Error bars represent the standard error of the mean.

A lack of discrimination between riparian and *terra firme* soils during the inter-monsoon, permitted statistical testing for a declining trend in SOM through stand age. The results of Jonckheere–Terpstra's test confirmed a significant decline in organic matter as plantations matured during the inter-monsoon ($J(72) = 593.5$, $z = -4.803$, $p < 0.001$; Figure 6-3). However, this trend was not repeated during the end of wet season sampling when SOM was more variable across stand age, ($J(60) = 611$, $z = -1.421$, $p = 0.155$). The more mature plantations (i.e. 6 – 15 years in age), displayed little difference in SOM over the two sampling periods. However in the youngest, first-generation, three-year old stand (3Y), SOM was 11.5±0.8% when soils were first sampled in 2010, yet this had declined significantly to 7.8±0.6% when samples were taken eighteen months later in 2012 ($t = -3.315$, $p = 0.007$). By contrast SOM increased in the five year old plantation (5Y) between the two sampling seasons but not significantly so ($t = 1.584$, $p = 0.142$).

6.3.2 Denitrification, DNRA and emissions of N_2O across stand age within riparian and *terra firme* sites

In-situ denitrification (as emission of N_2O+N_2) showed significant interaction effects for both location and stand age with the time of sampling. For the *terra firme* soils, emissions of N_2O+N_2 were three times higher during the inter-monsoon than at the end of the wet season ($t = -3.571$, $p < 0.001$). However, in the riparian sites, there was only a marginal increase in in-situ denitrification at the end of the wet season ($t = 1.941$, $p = 0.051$). There was no correlation between in-situ denitrification and soil texture (i.e. % sand or clay), however there was a weak positive correlation with bulk density at the end of the wet season ($r = 0.30$, $p = 0.004$). During the inter-monsoon, there was also a weak correlation between in-situ denitrification and DNRA ($r = 0.247$, $p = 0.038$). Location had no effect on N_2O+N_2 emissions at the end of the wet season ($t = 0.679$, $p = 0.497$), and hence in-situ denitrification

was plotted through plantation age for that year (Figure 6-4). However, during the inter-monsoon, *terra firme* soils produced nearly three times more N_2O+N_2 on an aerial basis than the riparian sites, (t = 3. 670, $p < 0.001$). Both *terra firme* sites (5Y and 8Y) displayed a trend of increased in-situ denitrification during the dry season although the effect was stronger for 5Y (t = 2.756, p = 0.006) than 8Y (t = 2.040, p = 0.041). Conversely, all riparian sites showed either decreased or no statistical difference in in-situ denitrification during the inter-monsoon. For 6Y, the decrease was highly significant with the rate of 15.63±3.14 mg N_2O+N_2-N m^{-2} d^{-1} at the end of the wet season dropping to 5.72±1.80 mg N_2O+N_2-N m^{-2} d^{-1} during the inter-monsoon (t = -2.267, p = 0.008).

The interaction of location and season on N_2O emissions followed the same pattern as in-situ denitrification. Namely, *terra firme* soils had the highest emissions of N_2O during the inter-monsoon (9.63±1.43 mg N_2O-N m^{-2} d^{-1}) which were significantly higher than riparian emissions at the time of sampling (3.64±2.28 mg N_2O-N m^{-2} d^{-1}; t = 3.306, p = 0.001). Similarly, riparian N_2O did not differ seasonally (t = -0.915, p = 0.360) and was not statistically different from *terra firme* emissions at the end of the wet season (t = -0.421, p = 0.674). The absence of an effect of location at the end of the wet season permitted N_2O to be plotted through stand age (Figure 6-4). The apparent increase as plantations matured was not statistically significant by the Jonckheere–Terpstra's test (J(60)=792.5, z = 0.959, p = 0.338), however, there was a significant increasing quadratic trend through plantation age (r = 0.99, p = 0.008). There was no interaction effect of season and stand age as the main effect of location dominated the differences between sites with 5Y and 8Y releasing 2-4 times more N_2O than the riparian sites during the inter-monsoon. Differences between stand age at the end of the wet season were minimal, although N_2O from 15Y was 2-4 times greater than from other plantations. Ratios of N_2O:(N_2O+N_2) were higher during the inter-monsoon (~0.73)

than during the wet season (~0.37) but differences across season and location were not significant (Figure 6-4).

Potential denitrification displayed no interaction effect of season and location. However, *terra firme* sites had a significantly greater denitrification potential at the end of the wet season ($t = 2.803$, $p = 0.005$), whereas riparian sites showed little difference in rates across season (Figure 6-2). There was also a significant interaction effect of season and stand age with most sites displaying increased potential denitrification at the end of the wet season. An exception to this pattern was site 6Y where rates at the end of the wet season were less than half that of the inter-monsoon. A slight decrease in rates at the end of the wet season was also observed for 3Y (Table 6-3). Rates of both potential denitrification and DNRA in 5Y during the inter-monsoon were lower than in other plantations and are likely to reflect the influence of recent fertilisation which elevated soil nitrate to a mean concentration of 109±30 g N m^{-2}. Accordingly, a 24 hour pre-incubation of the vials for the potential denitrification and DNRA assay may not have been sufficiently long to remove all NO_3^- prior to injection of vials with $^{15}NO_3^-$. Consequently, $^{29}N_2$ production from denitrification of $^{15}NO_3^-$+$^{14}NO_3^-$ averaged more than nine times that of $^{30}N_2$ production when analysed by IRMS. Due to the problem of rate under-estimation at this particular site, it was excluded from the analysis of the interaction effects of stand age and location with season and it is excluded from all further analysis with regard to potential rates of denitrification and DNRA.

Potential DNRA was three orders of magnitude lower than potential denitrification and therefore is likely to play only a minor role in nitrate consumption in these surface soils. Potential DNRA across all sites and in each location, decreased at the end of the wet season. Although the decrease was significant only in riparian plantations ($t = 5.732$, $p = 0.001$). Riparian sites also had higher potential denitrification than *terra firme* plantations during the

inter-monsoon, although this difference fell just short of significance. There was also no difference between riparian and *terra firme* locations at the end of the wet season permitting potential denitrification to be plotted through stand age for both time periods (Figure 6-3; Figure 6-4). During the inter-monsoon, the highest rates were observed in 6Y, although denitrification potential appeared to increase in the more mature (15Y and 25Y) plantations relative to the youngest 3Y (Figure 6-3). However, at the end of the wet season, a noticeable trend of increasing potential denitrification was apparent through stand age (Figure 6-4). There was no difference in DNRA across location during the inter-monsoon (Figure 6-3), however at the end of the wet season riparian plantations had higher DNRA ($t = 2.319$, $p = 0.025$) than *terra firme* plantations. Stand age had no effect on rates of potential DNRA over the two years sampled.

6.4 DISCUSSION

6.4.1 Seasonal differences in N_2O emissions across riparian and *terra firme* plantations

6.4.1.1 DNRA of minor importance to nitrogen cycling and N_2O emissions

DNRA was a less important fate for nitrate than potential denitrification by a factor of one thousand, indicating that this process was probably not an important contributor to N_2O emissions in these soils. Rates of DNRA were much lower than has been reported for some tropical soils. Specifically, the mean rate for these plantations during the inter-monsoon was 0.004 mg kg^{-1} d^{-1} compared to 0.6 mg kg^{-1} d^{-1} in upland Puerto Rican soils where 75% of nitrate turnover was attributable to the process, (Silver et al. 2001). Rates measured in the tropics as a whole span 0.03-2.89 mg kg^{-1} d^{-1}, whereas plantation soils here displayed values at the low end of those reported for temperate soils (Table 2-2). Although rates were low,

DNRA appeared to affect soil NH_4^+ during the inter-monsoon ($r = 0.46$, $p = 0.003$) indicating that there was sufficient soil moisture (and therefore anoxia) for the process to occur. However, WFPS was a controlling factor during the drier weather as rates increased with increasing soil moisture ($r = 0.33$, $p = 0.026$). The difference between rates of denitrification and DNRA potential, most likely, rule DNRA out as a contender in N_2O emissions. Potential rates of DNRA were, however, in a similar range as N_2O and N_2O+N_2 emissions.

6.4.1.2 Differences in N_2O emissions across season and location

The significant interaction of season and location best explained the pattern of N_2O indicating that the temporal dynamics of emissions varied between riparian and *terra firme* sites. Whilst emissions from riparian plantations did not differ seasonally, *terra firme* sites produced approximately six to nine times more N_2O and two to four times more N_2O+N_2 during the inter-monsoon when soils had lower soil moisture. The hole-in-the-pipe model predicts nitrification to be the main process responsible for N_2O flux at WFPS <60% but as soils become wetter, N_2O emissions increase and there is a shift towards denitrification as the main greenhouse gas emitter (Firestone & Davidson, 1989). The lower emissions of N_2O observed here in all soils at the end of the wet season contradict the pattern of higher wet season emissions observed in most other studies of N_2O production in tropical soils (Keller & Reiners, 1994; Verchot, et al., 1999; Kiese, et al., 2008; Garcia-Montiel, et al., 2003). However, extremely high WFPS (mean = 81±1.7%) at the end of wet season may offer one explanation for the temporal variation in emissions. When saturated, soil and atmospheric gas exchange is limited and accordingly reduced diffusivity promotes complete denitrification to N_2 rather than emission of N_2O (Melling, et al., 2007; Hall, et al., 2013). Lower emissions in both *terra firme* and riparian plantations during the end of wet season may be due to reduced gas diffusivity in extremely wet, fine-textured soils on a topographically low floodplain and

greater expression of the N_2O reductase. If this is the case, then the ratio of N_2O:(N_2O+N_2) might be expected to be lower in soils at high WFPS. Table 6-5 confirms that, at the end of the wet season, the proportion of N_2O released (as a percentage of in-situ denitrification) decreased from 73% to 37%. This argument, however, is only valid if it can be assumed that N_2O emissions are largely from denitrification. During the wet season, there is a high probability that this is the case given that reduced O_2 in saturated soils will limit nitrification capacity, particularly for riparian soils that followed the expected pattern of increased in-situ denitrification at the end of the wet season. Correlations of N_2O with other variables across both seasons were low. However, low WFPS (<52%) and higher extractable soil NO_3^- during the inter-monsoon are indicative of nitrification as an important mechanism for N_2O production. A negative correlation (r = -0.33, p = 0.004) of N_2O with WFPS during the drier season also suggests that a decline in N_2O emissions with increased soil moisture may be due to the suppression of nitrification and increased denitrification. Thus, patterns of N_2O emissions during the inter-monsoon are likely a result of coupled nitrification-denitrification under spatially variable redox conditions. Increased rainfall during the wet season may also alter soil O_2 sufficiently to permit greater induction of N_2O reductase relative to dry season conditions. Smith & Tiedje (1979) identified two phases of denitrification following O_2 depletion in soils. During Phase I, there is a linear increase in denitrification until maximum denitrification capacity is reached. During Phase IIa, new enzymes are synthesised and the rate increases again to maximum denitrification capacity. Following this, rates will only increase further with sufficient available carbon to support a growth in the microbial population (Phase IIb). However, as induction of N_2O reductase appears to be slower than induction of other denitrification enzymes following conditions of anaerobisis (Firestone & Tiedje, 1979), lower rainfall frequency will shorten the time that soils remain anoxic. As a

result, the length of time that soils remain anaerobic may be insufficient to induce N_2O reductase under Phase I thereby resulting in higher N_2O emissions. Furthermore, continued anaerobisis during the wet season may stimulate induction and/or growth of new reductase under Phase II, thereby reducing N_2O emissions. Thus, induction of existing and new N_2O reductase under favourable redox conditions may be an important determinant of N_2O emissions and deserves further attention.

Table 6-5: The proportion of N_2O released from in-situ denitrification in plantation soils during the inter-monsoon and end of wet season

	Season	Plantation Age						Seasonal average
		3Y	5Y	6Y	8Y	15Y	25Y	
$N_2O:(N_2O + N_2)$	Inter-monsoon	0.74	0.53	0.53	1.02	0.96	0.63	0.73
	End of wet season	0.26	0.24	0.08	0.45	0.84	-	0.37

Emission rates of N_2O at the end of the wet season (1.05±0.39 – 4.37±1.51 mg N_2O-N m^{-2} d^{-1}) are at the top end, or exceeded, those reported for other oil palm plantations. For example, on peat soils, observed rates range from 0.02 – 1.4 mg N_2O-N m^{-2} d^{-1} in Sarawak, Malaysian Borneo (Melling, et al., 2007) and 0.03-1.34 mg N_2O-N m^{-2} d^{-1} in Sumatra, Indonesia (Ishizuka, et al., 2005). During the inter-monsoon however, rates from these plantations were higher, ranging from 3.02-10.18 mg N_2O-N m^{-2} d^{-1}. This equates to an annual rate of 8.9 – 37.1 kg N ha^{-1} y^{-1}, which is considerably higher than the 1-5 kg N ha^{-1} y^{-1} more generally observed in tropical forests (Verchot, et al., 1999; Verchot, et al., 2006; Breuer, et al., 2000; Werner, et al., 2007). This is also higher than rates reported for tropical agricultural soils such as coffee plantations (4.3-5.8 kg N ha^{-1} y^{-1}) (Hergoualc'h et al., 2008) and banana plantations (8.1-27.5 kg N ha^{-1} y^{-1}) (Veldkamp & Keller, 1997), although some very high rates have been reported for fertilised oil palm plantations within Sabah. For example, 60 km south of the field sites near Lahad Datu, Skiba et al. (2012) found emissions to be 156 kg N

ha^{-1} y^{-1} within the palm circle where fertiliser is applied. Recent fertilisation (as evidenced by extremely high nitrate and ammonia) at plantation 5Y is a likely cause of the very high emissions of N_2O and N_2O+N_2 observed at this site during the inter-monsoon. However, the *terra firme* plantation 8Y also displayed high N_2O and N_2O+N_2 despite no evidence of recent fertilisation and relatively modest soil ammonia and nitrate. The reason for the high emissions at 8Y relative to the riparian sites is unclear although there are a number of possible explanations. It is likely that the low temporal resolution to sample collection does not adequately reflect the periodicity of climatic factors that can have a dramatic effect of N_2O emissions. For example, during the inter-monsoon when soils are relatively dry, rainfall events can increase trace gas emissions in the hours following a wetting event, (Zhu et al., 2013; Ishizuka et al., 2010). The magnitude of a "hot moment" of biogeochemical activity may also be greater during the dry season as lower soil moisture for extended periods permits the build-up of nutrients and labile carbon that become readily available to microorganisms upon soil re-wetting (McClain, et al., 2003). For *terra firme* soils that are more freely draining than riparian soils, variability in soil moisture is greater allowing nitrification in periods of drier weather and denitrification following rainfall events. For the same reasons discussed above, attenuation of N_2O peaks when soil moisture is high during the wet season may also occur for the riparian soils where higher WFPS (as a result of topographic location) may keep soils saturated for longer periods relative to *terra firme* sites. It is possible therefore that the lower magnitude of increased inter-monsoonal N_2O emissions and relatively modest increase in denitrification at the end of the wet season observed in riparian sites is constrained by nitrate and/or carbon limitation under conditions of sufficient soil moisture.

In some tropical studies, high inter-annual variability of N_2O release has also been reported. For example, N_2O emissions differed seven-fold over sampling years in the wet tropical

forests of Queensland, Australia (Kiese, et al., 2003). Although differences in soil nitrate and ammonia observed here over the two sampling seasons may be due to differences in sample preparation, it may also be the case that variations in rainfall (either seasonally or inter-annually) have a profound effect on N cycling and consequently N_2O release. Further work at higher temporal resolution is needed to examine this possibility.

6.4.2 Chronosequence analysis along stand age

In many cases, differences between sampling season precluded pooling of data across years for chronosequence analysis. However, when no difference was found to exist between riparian and *terra firme* sites, results were plotted along stand age to permit some general hypotheses about changes in soil properties and N processing to be formulated. In-situ denitrification during the wet season displayed no trend through plantation age (Figure 6-4). For organic matter, nitrous oxide emissions and potential denitrification and DNRA, there were possible trends through stand development that deserve further consideration, but the results need to be interpreted with caution.

The only significant trends in stand age detectable were for SOM during the inter-monsoonal sampling period and N_2O and potential denitrification during the end of wet season sampling (Figure 6-3). Unlike Haron et al. (1998), who found increasing carbon through stand age on mainland Malaysia, the trend here was one of decreasing SOM as plantations matured. The conversion of tropical forest to plantation agriculture almost invariably results in losses of soil carbon (Sommer, et al., 2000; Murty, et al., 2002; Silver, et al., 2005; Skiba, et al., 2012), although the recovery of these losses following conversion is more equivocal. For example, Frazao et al (2012) found a decline in soil carbon through oil palm plantation age, however, when adjustments were made to account for clay content, no obvious trend was apparent. Similarly, Ishizuka et al. (2005) found no trend in soil carbon through plantations aged 3-15

years in Indonesia, and Smith et al. (2012) saw no significant effect of plantation age on SOM in plantations between 11-34 years. An assumption of decreased SOM in the more mature plantations of this study requires caution, not least because the trend was not repeated at the end of the wet season. Although stand age does appear to affect SOM, the recovery of soil carbon will be dependent on factors such as soil texture, plantation management practices and topographic position. All plantations here were on level ground at low (<100m a.s.l.) elevation minimising the effect of slope. However there were differences in soil texture (), and not all plantations had the same management regime. For example, given the mechanisation of FFB collection in commercial plantations *vis-à-vis* manual collection in smallholdings, SOM may be affected by edaphic or land use factors not accounted for by this study.

Regression of N_2O emissions through plantation age at the end of the wet season suggests a trend of increasing N_2O emissions with stand development. The low temporal resolution of measurements in this study highlights that caution is needed in attributing observed trends in nitrous oxide production to plantation age. However, in Sumatra, a decrease in mean emissions from 0.28±0.13(SD) in young to 0.06±0.05(SD) mg N_2O-N m^{-2} d^{-1} in mature oil palm stands was reported (Ishizuka, et al., 2005). Whilst contrary to the current observations, the five plantations (i.e. 3, 3-5, 5, 15 and 15 years in age) sampled in Ishizuka et al. (2005) were located on four differing soil types and only seven soil gas chambers were deployed once during the dry season. Further measurements are required to decipher any pattern of N_2O emissions through plantation age, although as mechanisms of N_2O production are similar regardless of location or crop, it is possible to formulate some general predictions. Of prime importance to nitrous oxide emission within any agricultural system is the rate and timing of fertiliser application. As plantations mature, application rates of N fertiliser increase thereby

increasing the potential for N_2O emissions with stand age. Furthermore, whether emissions increase, decline, or remain constant, through stand age will depend on how plantation establishment affects soil N cycling and soil moisture. If rates of mineralisation and nitrification are not affected through, for example, losses of soil carbon then emission rates might be expected to be similar across plantation age. Similarly, if organic matter inputs increase with age then mineralisation and nitrification may increase and with them the rate of N_2O release. In these plantations, there was no difference in gross mineralisation or gross nitrification across stand age when rates were measured at the end of the wet season. However, there was a trend of increasing potential denitrification. N_2O emissions are therefore potentially similar through stand age and are unlikely to decline as plantations mature, although again the temporal limitation of these results is emphasised highlighting the necessity of additional measurements to confirm this interpretation. The increase in potential denitrification as stands matured at the end of the wet season, together with an increase in emission of N_2O are suggestive of a greater potential for N losses in mature plantations. However, this trend was only partially observed during the inter-monsoon, highlighting the need for caution in attributing the observed trends to stand age alone.

6.5 CONCLUSION

Denitrification was a more important fate for NO_3^- than DNRA, which likely played only a minor part in N cycling within these soils and was of minor importance to N_2O production. There were, however, notable differences in nitrogen cycling across both season (wet versus dry) and location (i.e. riparian or *terra firme*). Specifically, N_2O emissions were higher during the inter-monsoon than at the end of the wet season, although this was only significant for the *terra firme* plantations. In-situ denitrification was also higher for *terra firme* sites

during the inter-monsoon and contradicted the usual pattern of higher wet season emissions. Nitrification is likely to be a significant N_2O producing process during the inter-monsoon when WFPS<52%. However, denitrification rates may also be elevated by provision of available substrate from nitrification. Therefore, the most probable explanation for higher N_2O and N_2O+N_2 during the inter-monsoon are conditions of simultaneous nitrification and denitrification. Meanwhile, at the end of the wet season under conditions of higher soil moisture (WFPS = 81±1.7%), emissions of N_2O are primarily from denitrification, which is limited by nitrate availability.

Contrary to other studies, SOM decreased significantly through plantation age during the inter-monsoon. N_2O emissions and potential denitrification during the wet season also appeared to increase as plantations matured. However, a lack of similar trends across seasons highlights the need for caution when interpreting this reduction as a function of plantation age. Ultimately, the pattern of N_2O emissions through stand age will depend on how plantation establishment affects soil nitrogen cycling and soil moisture. Although, as N processing rates do not appear to decline, and fertiliser inputs increase as palms mature, N_2O emissions are unlikely to decline through the life of the plantation. Further measurements at higher temporal resolution are needed to confirm this assumption of increasing N_2O emissions as plantations mature.

CHAPTER 7: THE EFFECT OF TROPICAL FOREST CONVERSION TO OIL PALM PLANTATION ON NITROGEN CYCLING: PROCESS RATES IN LOWLAND SOILS

7.1 INTRODUCTION

Within the last four decades, global demand for palm oil has seen 16×10^6 ha of the lowland tropics converted to oil palm plantations, primarily in Southeast Asia (FAOSTAT, 2013). The oil palm requires a low-lying, humid tropical environment and is grown in areas that would formerly have been occupied by biodiverse, moist tropical forest. A number of recent publications have focused on aboveground biodiversity loss and changes to ecosystem function following tropical forest conversion to oil palm agriculture (Fitzherbert, et al., 2008; Foster, et al., 2011; Azhar, et al., 2013; Wilcove & Koh, 2010; Brühl & Eltz, 2010). Fewer studies have concentrated on belowground processes (Lee-Cruz, et al., 2013; Ishizuka, et al., 2005; Haron, et al., 1998). However, land use change is likely to result in alterations to soil chemistry and physical structure that impact microbial cycling, with important consequences for ecosystem function and atmospheric pollution.

Chapter 2 highlighted the fact that microbial N transformations are sensitive to physiological constraints triggered by the quality of organic carbon (Bowman & Focht, 1974; Weier, et al., 1993; Silver, et al., 2001; Cookson, et al., 2006), the degree of soil wetness or anoxia (Linn & Doran, 1984; Sierra, 1997; Breuer, et al., 2002; Pett-Ridge & Firestone, 2005; Cookson, et al., 2006), soil temperature (Sierra, 1997; Breuer, et al., 2002) and pH (Bremner & Shaw, 1958; Stevens, et al., 1998). Clearing forest vegetation decreases shading, increases soil temperatures and increased the vulnerability of soils to erosion from high surface runoff

following rainfall events. Vegetation removal affects both the quantity and quality of available organic matter returns to the soil and can result in lower C:N ratios following reductions in soil carbon, and to a lesser extent, nitrogen (Murty, et al., 2002; Ishizuka, et al., 2005). Other common physical changes to soil characteristics from plantation agriculture include soil compaction (from machinery or row planting), and increased water-filled pore space (WFPS) due to reduced plant uptake and transpiration. On a global scale, tropical forests are the largest natural source of N_2O (a greenhouse gas with 300 times the global warming potential of CO_2), contributing ~ 8-21% (1.3-3.5 Tg N y^{-1}) to the total budget of 16.4 Tg N_2O-N y^{-1} (IPCC, 2001). In soils, N_2O is produced primarily though microbial nitrification and denitrification both of which are sensitive to soil redox conditions. Autotrophic nitrification is an aerobic process, whereas dissimilatory processes such as denitrification and nitrate ammonification (DNRA), require anaerobisis (Knowles, 1982; Burgin & Hamilton, 2007; Norton & Stark, 2011). Thus, higher soil moisture contents and faster rates of decomposition in plantations, relative to forests, may increase soil anoxia thereby altering the amount and relative contribution of denitrification and nitrification to N_2O emissions.

Soil carbon losses of between 20-50% have been observed following conversion of forest land to tillage and annual cropping use (Sommer, et al., 2000; Murty, et al., 2002; Silver, et al., 2005; Germer & Sauerborn, 2008). However, the impact of perennial tree crop plantations on soil C and N is less clear and may be species and soil specific (Russell et al., 2007; Forrester et al., 2013). Few studies have compared gross rates of nitrogen transformation in tropical forests with plantations, although observations within a *Cordia alliodora* plantation in Costa Rica, found that gross mineralisation was reduced by up to 50% in comparison with forest (Silver et al., 2005). Significantly, in the Costa Rican study, gross nitrification did not decline

following plantation establishment and no microbial assimilation was measured in plantation soils (Silver et al., 2005). Accordingly, the potential for N losses following forest conversion to plantation agriculture remained high. Both net mineralisation and net nitrification rates have also been reported to decline in oil palm plantations compared with the secondary and primary forests of Central Sumatra (Ishizuka, et al., 2005). Conversely, a decline in soil carbon, and to a lesser extent soil nitrogen, will lower C:N ratios which often negatively correlate with rates of gross mineralisation (Booth, et al., 2005). Therefore, a reduction in the C:N ratio coupled to increased soil temperatures suggests that rates of decomposition and N mineralisation will be enhanced in plantation soils relative to forests.

The contradictory nature of the literature highlights the need to clarify the changes to soil N biogeochemical cycling following the establishment of oil palm plantations on previously forested land. A further 410-570 million ha of lowland tropical forest are potentially suitable for conversion (Fitzherbert, et al., 2008). Therefore, a greater understanding of the changes to nitrogen biogeochemistry will enable quantification of the effects of future plantation establishment within this region. The aim of this chapter is to determine the effects of land use change from forest to oil palm plantation on soil microbial nitrogen transformations. Nitrogen mineralisation, nitrification, NH_4^+ and NO_3^- consumption, potential denitrification, potential DNRA were measured in mature secondary forests and oil palm plantations ranging from 3 – 15 years of age at the end of the wet season in 2012. It is hypothesised that rates of nitrogen turnover will decline in plantations relative to forests as a result of decreased N availability. Rates of N_2O and N_2O+N_2 emission are also reported for both the inter-monsoon in 2010 and the end of wet season in 2012. As Chapter 6 reported higher rates of N_2O emission during the inter-monsoon than during the wet season, it is expected that forests will display similar trends to plantations. In particular, *terra firme* forests are expected to have

significantly greater emissions of N_2O during the inter-monsoon relative to riparian plantations. Replicate samples were therefore collected from sites in forests and plantation soils over two differing substrates to examine the magnitude of the impact that plantation establishment has on alluvial (riparian) and residual mudstone and sandstone (*terra firme*) soils in the lowlands of Sabah, Borneo (Figure 3-1, p. 47).

7.2 MATERIALS AND METHODS

7.2.1 Site selection and data pooling

Sites selected for comparison of nitrogen cycling rates consisted of two mature forest sites (MDF and SRF2) and five oil palm sites (3Y, 5Y, 6Y, 8Y and 15Y) (Table 7-1). Chapters 4 and 6 identified significant differences in nitrogen cycling within forests and oil palm plantations respectively that were located on differing substrates. Accordingly, a replicated comparison of nitrogen process rates under both land uses (i.e. forests and plantations) was conducted on both the alluvial and mudstone and sandstone substrate (i.e. riparian and *terra firme*). As this Chapter re-uses data for the forested sites that has already been reported in Chapter 4, the MDF and SRF2 were chosen as the most suitable representatives of the original pre-disturbance state following the observation that, generally, secondary forests sampled for this study appeared to be following a trajectory of recovery towards a state more akin to that of primary forests. Similarly, the data reported in this Chapter for the chosen oil palm sites forms part of the same dataset previously reported in Chapter 6 which examined trends through plantation age.

Table 7-1: Physical characteristics (0-10 cm) for the forests and oil palm plantations located on *terra firme* and riparian soils.

Site		Clay (%)	Sand (%)	Bulk Density (g cm^{-3})	pH_w	WFPS (%)
Forests						
MDF	*Terra firme*	24.12b (1.05)	25.27c (2.21)	0.87b (0.08)	5.10ab (0.10)	67.74b (0.83)
SRF2	Riparian	25.45b (0.79)	14.88ac (1.29)	1.06 (0.08)	5.09ab (0.11)	53.77a (4.05)
Plantations						
5Y	*Terra firme*	31.16 (2.61)	11.96ab (2.76)	1.08 (0.07)	5.90a (0.23)	90.51c (2.11)
8Y	*Terra firme*	22.63b (1.87)	22.24bc (2.12)	1.14 (0.08)	4.88bc (0.20)	85.13c (2.47)
6Y	Riparian	30.32a (1.06)	8.06a (1.94)	1.29a (0.04)	4.47c (0.06)	78.34bc (3.87)
3Y	Riparian	26.19 (0.90)	19.9bc (1.05)	0.94b (0.04)	4.41c (0.06)	65.48ab (3.29)
15Y	Riparian	29.12 (1.21)	7.01a (1.08)	1.10b (0.03)	4.34c (0.08)	85.09c (1.81)

Notes: Significant differences are indicated by different lowercase letters ($p < 0.05$). Standard error in parenthesis.

Table 7-2: Results of statistical testing for significant differences between plantations located on *terra firme* (5Y, 8Y) and riparian (3Y, 6Y and 15Y) soils.

	Terra firme (5Y & 8Y)		Riparian (3Y, 6Y & 15Y)	
	t	*p*	*F*	*p*
Gross mineralisation (g N m^{-2} d^{-1})	-1.630	0.129	1.587	0.247
Gross nitrification (g N m^{-2} d^{-1})	0.077	0.941	2.377	0.150
NH_4^+ consumption (g N m^{-2} d^{-1})	-1.792	0.098	0.640	0.539
NO_3^- consumption (g N m^{-2} d^{-1})	-0.143	0.890	1.705	0.211
Net mineralisation (g N m^{-2} d^{-1})	-1.420	0.181	0.080	0.923
Net nitrification (g N m^{-2} d^{-1})	0.221	0.831	0.955	0.404
Denitrification (g N m^{-2} d^{-1})	-	-	24.241	<0.001
DNRA (mg N m^{-2} d^{-1})	-	-	0.161	0.852
NH_4^+ (g N m^{-2})	0.140	0.890	1.802	0.406
NO_3^- (g N m^{-2})	-0.956	0.350	3.309	0.191
Carbon (Mg N ha^{-1})	-0.834	0.413	0.098	0.907
Nitrogen (Mg N ha^{-1})	-0.734	0.471	1.607	0.216

Prior to pooling data, the three riparian plantations on alluvium (3Y, 6Y and 15Y) and the two *terra firme* plantations on mudstone and sandstone (5Y and 8Y) were tested for significant differences in N processing rates (Table 7-2). This confirmed that for all process variables

(with the exception of potential denitrification in the riparian plantations), the results could be pooled.

7.2.2 Soil analysis

Mineralisation, nitrification and NH_4^+ and NO_3^- consumption were measured on intact cores by isotope pool dilution (Kirkham & Bartholomew, 1954; Hart, et al., 1994a). Mean residence time (MRT) for inorganic N was estimated by dividing the NH_4^+ and NO_3^- pool concentrations by the gross mineralisation and gross nitrification rate respectively, and assuming transformation rates remained constant over the time period (Verchot, et al., 2002). Microbial immobilisation of NH_4^+ was estimated by subtracting gross nitrification from gross NH_4^+ consumption and assuming no volatilisation losses and no plant uptake during the incubation period (Davidson, et al., 1991). Emissions of N_2O and N_2O+N_2 were determined using soil gas chambers (Section 3.5.2.1). Potential rates of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) were estimated by addition of ^{15}N to anaerobic soil slurries using the modified method of Trimmer et al. (2003) as described in Lansdown et al. (2012). Full details of soil sample collection and processing, including general soil characteristics are provided in Chapter 3.

7.2.3 Statistical analysis

Analyses of the effect of land use change from forest to plantation was carried out using two-way analysis of variance (ANOVA) with soil type (riparian and *terra firme*) and land use (forest and plantation) as the factors. Tests for multivariate normality were conducted using the Kolmogorov-Smirnov D statistic and for homogeneity using Levene's test. Parameters with heterogeneous variance or non-normal distributions were log transformed prior to analysis using the univariate general linear model in SPSS (v.21). To examine differences in

the magnitude of conversion effects on riparian and *terra firme* soils, post hoc analysis was conducted using the Student's *t*-tests (for normal distributions) or Mann-Whitney U test (non-normal data). A one-way ANOVA was also conducted on the four treatments that incorporated both land use and soil type (i.e. riparian forest, riparian plantations, *terra firme* forest, *terra firme* plantations). Values are reported here as means (±1 SE) and significance level is $p < 0.05$ unless otherwise stated.

7.3 RESULTS

7.3.1 Soil carbon and nitrogen

There was no discernable effect of plantation establishment on soil carbon or nitrogen in the *terra firme* sites. However, riparian soils generally held less C and N than *terra firme* soils. Similarly, riparian plantations had significantly less C and N than forested sites in the same location (Table 7-3). The C:N ratio did not differ across land use, or soil type.

Ammonia was the dominant form of inorganic N ($NH_4^+ + NO_3^-$) in both forest and plantation soils. Extractable nitrate was low across all sites, ranging from 1.58±0.14 g N m^{-2} in the *terra firme* oil palm to 2.90±0.62 g N m^{-2} in the *terra firme* forest. Soil NO_3^- in the forested sites did not differ, either between soil type or land use. However, riparian plantations held more soil nitrate than *terra firme* plantations. Extractable ammonia decreased from forest to plantation in the riparian sites and increased from forest to plantation in the *terra firme* sites. The ratio of nitrate to ammonia was 0.21±0.02 in the *terra firme* plantations compared to a ratio of 0.55±0.16 in the *terra firme forests* but the difference between both was not significant. Riparian plantations meanwhile had a significantly higher (0.59±0.11) ratio of nitrate to ammonia when compared to the riparian forest (0.17±0.03).

Table 7-3: Carbon and nitrogen contents of soils sampled across land use type (forest and oil palm plantation) and across soil substrate (*terra firme* mudstone and sandstone and riparian alluvium).

	Carbon (Mg C ha^{-1})		Nitrogen (Mg N ha^{-1})		C:N		NH_4^+ (g N m^{-2})		NO_3^- (g N m^{-2})		NO_3^-:NH_4^+	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Terra firme												
Forest	35.23	5.44	3.12	0.44	9.77	0.40	6.62b	0.98	2.90	0.62	0.55ab	0.16
Plantation	34.43a	3.40	3.20a	0.27	10.42	0.33	8.40a	0.48	1.58a	0.14	0.21bc	0.02
Riparian												
Forest	32.74a	3.51	2.90a	0.17	11.06	0.68	10.91a	1.06	1.75	0.24	0.17c	0.03
Plantation	21.04b	0.98	2.21b	0.09	9.52	0.31	6.56b	1.02	2.39b	0.19	0.59a	0.11

Notes: Difference lowercase letters indicate significant differences ($p < 0.05$) between the four treatments (i.e. *terra firme* forest ($n = 12$), *terra firme* plantation ($n = 24$), riparian forest ($n = 12$) and riparian plantation ($n = 36$)).

7.3.2 Nitrogen cycling and turnover in forest and oil palm soils

In general, rates of gross mineralisation and NH_4^+ consumption were higher in oil palm sites than in forested sites, whereas gross nitrification and NO_3^- consumption were higher in forests than in oil palm (Figure 7-1). Specifically, one-way ANOVA confirmed that gross mineralisation in the *terra firme* oil palm (13.71±2.08 g N m^{-2} d^{-1}) was significantly higher than in both the *terra firme* (5.78±2.14 g N m^{-2} d^{-1}) and riparian (4.85±1.21 g N m^{-2} d^{-1}) forests. Mineralisation in the riparian oil palm (8.58±1.21 g N m^{-2} d^{-1}), was also higher than both forested sites but the difference was not significant. Despite the lack of significance in the differences across land use for riparian sites (Table 7-5), two-way analysis of variance confirmed (1) that plantations had significantly higher gross mineralisation than forests, and (2) that there was no effect of soil type (Table 7-4). Gross mineralisation was positively correlated with C ($r = 0.40$, $p = 0.006$), N ($r = 0.44$, $p = 0.002$), and WFPS ($r = 0.31$, $p = 0.037$). Higher rates of mineralisation in the oil palm plantations were also reflected in short (< 1 day) mean residence times for NH_4^+ (Table 7-6). NH_4^+ consumption was highly correlated with NH_4^+ production ($r = 0.87$, $p < 0.001$). Although differences between all four treatments were marginal ($F = 2.842$, $p = 0.049$) and post-hoc tests were not significant

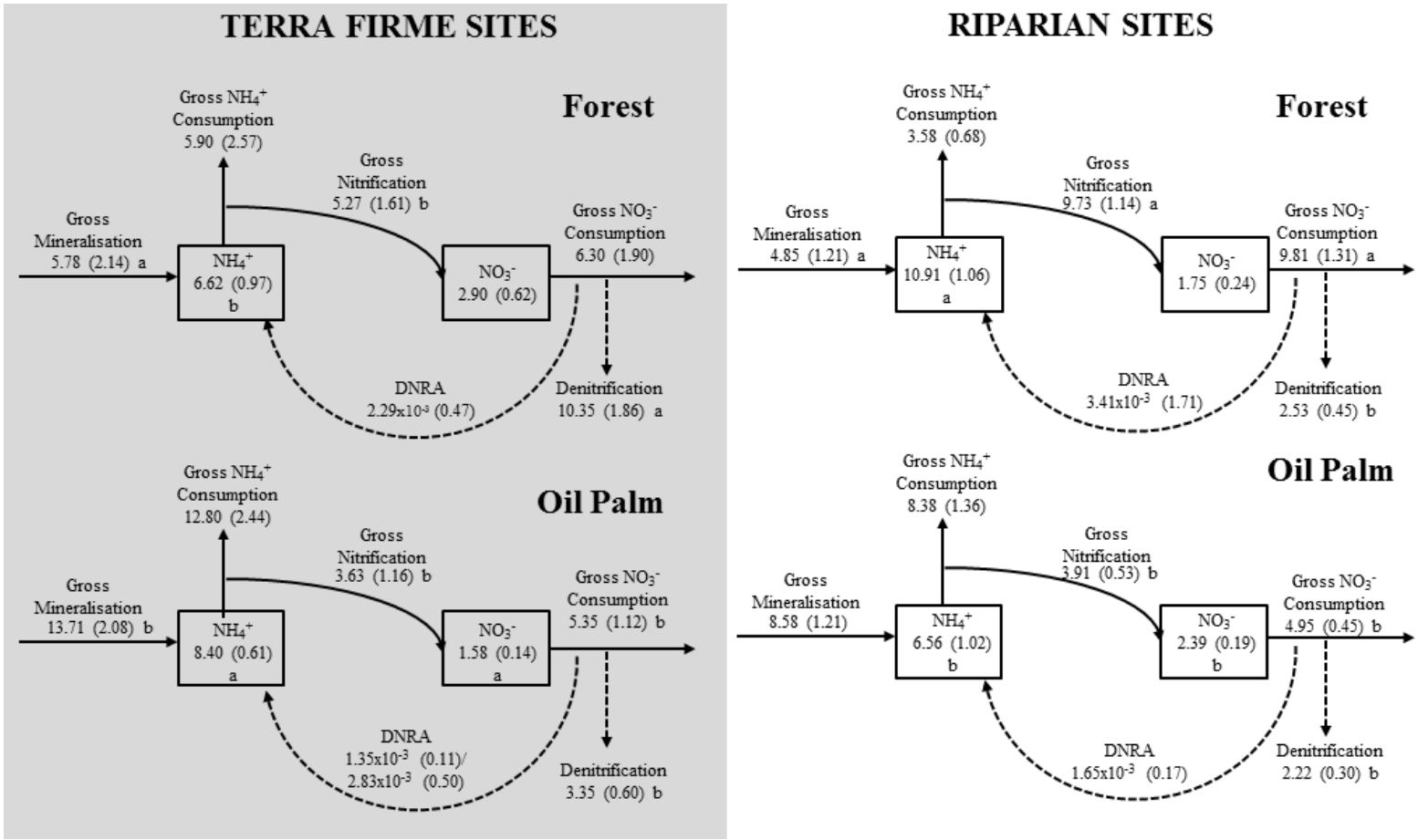


Figure 7-1: Rates of gross mineralisation, gross nitrification, NH_4^+ consumption, NO_3^- consumption and potential denitrification (g N m^{-2} d^{-1}) in forest and oil palm plantations located on *terra firme* and riparian soils. NH_4^- and NO_3^- pools are in g N m^{-2}. As rates of mineralisation, nitrification and nitrate and ammonia consumption are in-situ gross rates (solid lines) and denitrification and DNRA are potential rates (broken lines), this figure does not show a formal budget for nitrogen transformations within each of the land uses. Different lowercase letters indicate significant differences following comparison of the four means (for each parameter) using one-way ANOVA. Standard error in parenthesis.

Table 7-4: Results of two-way ANOVA on nitrogen process rates (g N m^{-2} d^{-1}) across land use (i.e. forests and plantations) on differing soil type (i.e. *terra firme* and riparian soils).

	Gross mineralisation		NH_4^+ consumption		Gross nitrification		NO_3^- consumption	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Land use	7.893	**0.008**	5.951	**0.019**	13.264	**0.001**	7.587	**0.009**
Soil type	2.129	0.152	1.972	0.169	5.325	**0.026**	2.164	0.149
Land use x soil type	1.022	0.318	0.192	0.664	4.161	**0.048**	3.426	0.072

Table 7-5: Results of post-hoc analysis for the magnitude of difference in process rates following land use change in riparian and *terra firme* soils.

	Terra Firme		Riparian	
	t	*p*	*t*	*p*
Gross mineralisation (g N m^{-2} d^{-1})	-2.391	**0.027**	-1.483	0.152
Gross nitrification (g N m^{-2} d^{-1})	0.848	0.410	5.283	**<0.001**
NH_4^+ consumption (g N m^{-2} d^{-1})	-1.761	0.094	-3.146	**0.005**
NO_3^- consumption (g N m^{-2} d^{-1})	0.455	0.656	4.498	**<0.001**

Table 7-6: Mean residence times (MRT) of ammonia and nitrate in *terra firme* and riparian soils across land use type. Standard error in parenthesis.

	Terra firme		Riparain	
	Forest	Plantation	Forest	Plantation
NH_4^+ MRT (days)	1.14 (0.25)	0.61 (0.10)	2.25 (0.16)	0.76 (0.15)
NO_3^- MRT (days)	0.55 (0.25)	0.44 (0.19)	0.18 (0.13)	0.61 (0.10)

(Figure 7-1), results of the two-way ANOVA for NH_4^+ consumption confirmed significant differences between land use but not soil type (Table 7-4). Additionally, when riparian and *terra firme* sites were compared separately, differences in NH_4^+ consumption across land use were greater in riparian than in *terra firme* sites (Table 7-5).

Gross nitrification rates ranged from 22 to 64% of gross mineralisation in the plantations, compared to 91% in the *terra firme* forest, and 200% in the riparian forest (Table 7-7). One-way ANOVA confirmed that nitrification in the riparian forest (9.73±1.14 g N m^{-2} d^{-1}) was significantly higher than in both plantation soils and the *terra firme* forest (Figure 7-1). Rates of nitrification in the *terra firme* forest (5.78±2.14 g N m^{-2} d^{-1}) were also higher than in *terra firme* and riparian plantations but the differences were not significant. The decline of gross nitrification in riparian plantations relative to riparian forests was much greater than the decline in nitrification following land use change in the *terra firme* sites. As such there was a significant interaction effect between land use and soil type which reflected the much higher rate of nitrification displayed in the riparian forest (Table 7-4).

NO_3^- turnover rates were less than one day in all locations, but the very high rates of nitrification displayed in riparian forests resulted in a NO_3^- mean residence time approximately half that of other sites (Table 7-6). Gross NO_3^- production correlated positively with NO_3^- consumption ($r = 0.96$ $p < 0.001$) and negatively with WFPS ($r = -0.31$, $p = 0.041$). NO_3^- consumption also exceeded production in all soils across both land uses and was greater

in forested sites than in plantations but, unlike NO_3^- production, there was no interaction effect with soil type. The magnitude of the difference in NO_3^- production and consumption within land use type was only significant for riparian sites (Table 7-5). Subtracting gross nitrification from gross NH_4^+ consumption gives an estimate of NH_4^+ immobilisation in the soils, which correlated with gross nitrification (Figure 7-2) and increased in plantations relative to forests (Table 7-7).

Table 7-7: Rate of NH_4^+ immobilisation and the ratio of gross nitrification to gross mineralisation (Nit:Min) and gross nitrification to potential denitrification (Nit:Denit) across *terra firme* and riparian forests and plantations. Standard error in parenthesis.

	NH_4^+ immobilisation ($g\ N\ m^{-2}\ d^{-1}$)	Nit:Min	Nit:Denit
Terra firme			
Forests	0.62 (1.19)	0.91 (0.34)	0.51 (0.12)
Plantations	9.17 (0.92)	0.27 (0.24)	1.08 (0.27)
Riparian			
Forests	-6.15 (1.30)	2.01 (0.18)	3.84 (0.58)
Plantations	4.48 (0.87)	0.46 (0.14)	1.76 (0.23)

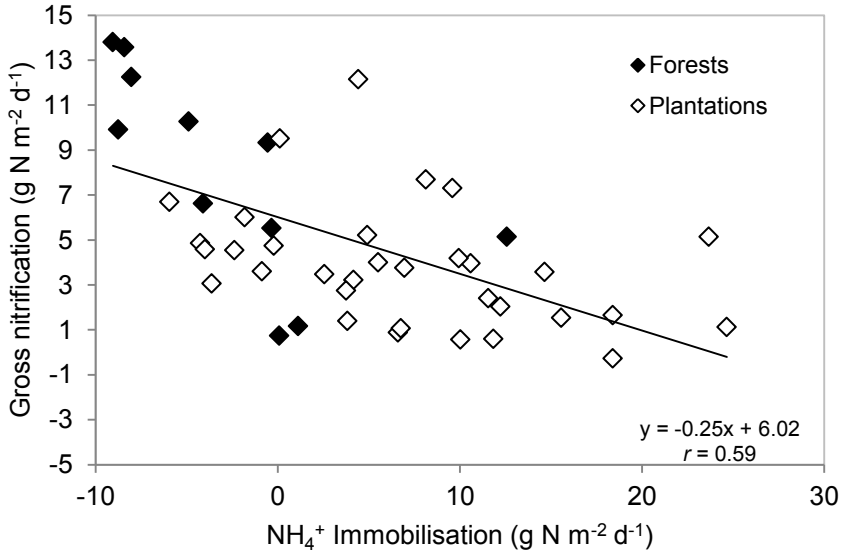


Figure 7-2: Relationship between rates of gross nitrification and NH_4^+ immobilisation in forest and platnation soils. Solid line represents the fitted linear regression

A decline in potential denitrification from forest to oil palm in *terra firme* sites was matched by an increase from riparian forest to riparian oil palm (Table 7-5). However, as there were significant differences in potential denitrification within the riparian sites, results were not pooled for further analysis of trends across land use and soil type. Potential denitrification was positively correlated with organic matter ($r = 0.32$, $p = 0.004$), nitrification ($r = 0.32$, $p = 0.04$) and nitrate consumption ($r = 0.40$, $p = 0.009$) and negatively correlated with clay content ($r = -0.28$, $p = 0.013$). For the most part, gross nitrification exceeded the rate of potential denitrification (Table 7-7). Although in the *terra firme* forest, in-situ nitrification was approximately 50% of denitrification. Whilst this is obviously unsustainable in the long term, short periods of elevated nitrogen processing (i.e. hot moments) may sustain losses of nitrate in the long term. Furthermore, the potential rate of denitrification gives only an indication of the maximum potential rate of nitrogen loss under favourable conditions, it does not provide an estimate of the actual rate of denitrification at the time sampled. Rates of potential denitrification were three orders of magnitude higher than rates of potential DNRA. Due to significantly different rates of DNRA in 5Y and 8Y, results were not pooled for the *terra firme* plantations. Furthermore, as there were no significant differences in DNRA rates across land use and soil substrate, regardless of whether 5Y or 8Y was chosen as the representative site for *terra firme* plantations, and there was no interaction effect, results of the two-way analysis of variance are not reported in Table 7-4.

7.3.3 N_2O emissions

N_2O emissions were highly variable across sampling location and, as such, results could not be pooled. There were also differences in emission rates between seasons. Soils in the inter-monsoon generally displayed higher fluxes of N_2O across all sites than at the end of the wet

season (Figure 7-3), however differences in emission rates were only significant for *terra firme* sites (Table 7-8). N_2O emissions in the riparian forest were below detection limits during the wet season. N_2O+N_2 emissions were different across season in *terra firme* sites but, whilst the forest site displayed higher wet season emissions, plantation soils emitted more N_2O+N_2 during the inter-monsoon (Table 7-8). Of the riparian sites, only 6Y emitted significantly more N_2O+N_2 at the end of the wet season.

For plantations, there was little variability between season in the amount of N_2O emitted relative to N_2O+N_2 (Figure 7-3). However, forest sites had extremely high ratios of N_2O:N_2O+N_2 during the inter-monsoon coupled to much lower emissions of N_2O in comparison with 5Y and 8Y (Figure 7-3).

Table 7-8: Results of related sample Wilcoxon signed-rank test for differences in N_2O and N_2O+N_2 emissions between the inter-monsoon and end of wet season sampling in *terra firme* and riparian forests and plantations.

	N_2O (mg N m^{-2} d^{-1})		N_2O+N_2 (mg N m^{-2} d^{-1})	
	z	*p*	*z*	*p*
Terra firme sites				
MDF	-2.432	**0.015**	2.667	**0.008**
5Y	-2.589	**0.010**	-2.824	**0.005**
8Y	-2.589	**0.010**	-2.118	**0.034**
Riparian sites				
SRF2	-1.826	0.068	1.752	0.116
3Y	-0.051	0.959	0.941	0.347
6Y	-1.260	0.208	2.667	**0.008**
15Y	-0.157	0.875	-0.078	0.937

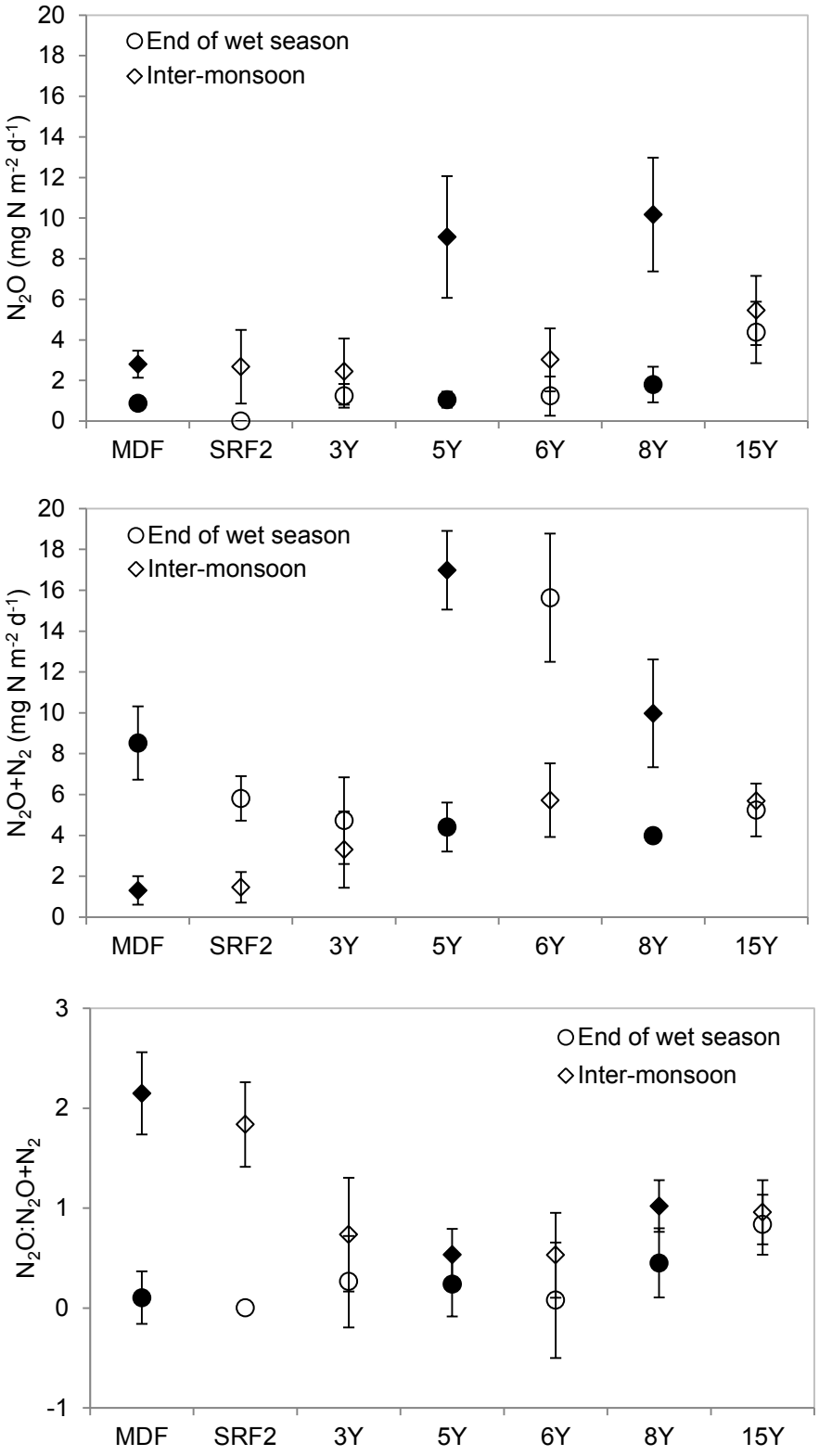


Figure 7-3: Mean rates of a) N_2O emission; b) N_2O+N_2 emission; and c) the ratio of N_2O to N_2O+N_2 across *terra firme* (black circles and diamonds) and riparian (white circles and diamonds) forests and plantations sampled during the inter-monsoon and at the end of the wet season. Error bars represent the standard error of the mean.

7.4 DISCUSSION

7.4.1 The effect of forest conversion to plantation on nitrogen cycling process rates

There were significant differences in microbial nitrogen cycling between forests and oil palm plantations in the lowland soils sampled for this study. Specifically, rates of mineralisation and NH_4^+ consumption in plantations were approximately double those observed in forested sites on the same substrate. Higher rates of NH_4^+ turnover in plantations were also matched by shorter ammonia residence times. Meanwhile, rates of gross nitrification and NO_3^- consumption declined in plantations relative to forests and nitrate residence times were variable. N mineralisation rates are related to factors including the initial organic matter quantity and quality (e.g. ratios of C:N, lignin:N or lignin:cellulose), soil temperature, texture and moisture. The removal of complex forest vegetation and its replacement with oil palm monoculture can result in alterations to WFPS and higher soil temperatures (Ramdani, et al., 2014; Melling, et al., 2007). In addition, gross mineralisation is often positively correlated with soil C and N and negatively correlated with the C:N ratio (Booth, et al., 2005). Thus, increased rates of gross rates of mineralisation recorded in *terra firme* oil palm plantations, relative to forested sites, is likely to be a result of higher WFPS and soil temperatures without a concurrent decrease in soil carbon or nitrogen. At the time of writing, there were no published reports of gross mineralisation and nitrification rates in oil palm plantations which could be compared with these results. However, both mineralisation and nitrification rates are at the high end of those reported in other tropical regions (Table 2-1, p.22). Rates of mineralisation across all sites (9.34±1.1 μg N g^{-1} d^{-1}) were ~35% greater than in *Cordia alliodora* plantations and forests (5.91 μg N g^{-1} d^{-1}) in Costa Rica (Silver et al., 2005) but similar to a *Eucalyptus saligna* plantation (~10-13 μg N g^{-1} d^{-1}) in Hawaii (Garcia-Montiel & Binkley, 1998). Whereas, mean nitrification rates (5.15±0.51 μg N g^{-1} d^{-1}) were similar to

those reported in Silver et al. (2005) (4.75 μg N g^{-1} d^{-1}), but lower than in Hawaii (~0.4-1.5 μg N g^{-1} d^{-1};Garcia-Montiel & Binkley, 1998). Unlike the Costa Rican study, rates of mineralisation and NH_4^+ consumption in this thesis did not decline in plantations relative to forests (Silver, et al., 2005). Furthermore, although gross nitrification was lower in plantations, strong NH_4^+ consumption suggests that there was no reduction in the microbial biomass following forest conversion. The substantially higher estimates of NH_4^+ immobilisation and short NH_4^+ residence times in the plantations also support this interpretation. If immobilisation of NH_4^+ remains strong, it may obstruct the rate of gross nitrification by conserving N within the microbial biomass. The negative relationship between nitrification and ammonia immobilisation, therefore, suggests that nitrification in the plantations was affected by NH_4^+ availability (Figure 7-2). Gross nitrification rates in the plantations were also constrained by high soil moisture levels which deplete soil O_2 and inhibit both ammonia and nitrite oxidation. In combination, low soil oxygen and high NH_4^+ consumption may depress the rate of nitrification in plantations relative to forests. Although in most cases, NH_4^+ consumption exceeded nitrification, in the riparian forest gross nitrification was notably higher than NH_4^+ consumption. One reasons for this may be that as nitrification and mineralisation are estimated from separate cores, spatial heterogeneity in process rates may contribute to the discrepancy that shows greater nitrification consumption of ammonia to be greater than total ammonia consumption.

NO_3^- consumption was greater than NO_3^- production across all sites and concentrations of soil nitrate were low. In wet soils, denitrification and DNRA are the most likely consumers of available nitrate. However, rates of potential DNRA were three orders of magnitude lower than denitrification, indicating that the latter process is probably dominant in these soils. Correlations of potential denitrification and N_2O+N_2 production with most indices of nitrogen

cycling were low across all locations. However, the potential to denitrify ranged from 25-200% of gross nitrification across all sites with the highest rates in the *terra firme* forest. Although potential denitrification in the *terra firme* forest exceeded rates of nitrate production by almost 100%, it should be noted that Figure 7-1 does not provide a formal budget of nitrogen transformations at the time of sampling. Rather, rates of gross mineralisation, nitrification, and nitrate and ammonia consumption are in-situ estimates, whereas denitrification and DNRA are potential rates of N turnover under optimal environmental conditions. However, in all sites consumption of nitrate exceeded production, an imbalance in the nitrogen budget that, save in the case of the *terra firme* forest could not be explained wholly by denitrification. Possible reasons for this high rate of nitrate consumption may be nitrate retention or loss through processes that are not measured for this thesis such as leaching or abiotic retention. Alternatively, the addition of nitrate to measure rates of nitrification and nitrate consumption may have stimulated (and thus over-estimated) the latter process as explained in Section 4.4.2 above. Rates of N_2O emission were also below detection in the riparian forest and, whilst this is somewhat surprising given the high capacity for nitrification, it may again be that nitrate is lost through leaching or transformed through alterative microbial or abiotic N consuming pathways.

7.4.2 N_2O emissions and the ratio of N_2O:(N_2O+N_2) across wet and dry seasons

N_2O emissions at the end of the wet season differed little between forest and plantation sites, though a quadratic trend of increasing emissions with plantation age was observed ($r = 0.99$, $p = 0.008$; Figure 6-4, p.145). For riparian soils, there was little seasonal difference in emission rates despite WFPS at the end of the wet season (79±2%) declining to 44±1% during the inter-monsoon. By contrast, all three *terra firme* sites had significantly higher N_2O emissions during the inter-monsoon. In the plantations, higher dry season emission is attributed to

coupled nitrification-denitrification as a result of spatially variable redox conditions which is supported by high N_2O+N_2 emissions during the inter-monsoon (Section 6.4.1.2). For the *terra firme* forest, N_2O+N_2 emissions were lower during the inter-monsoon than during the wet season. Furthermore, both forested sites, emitted more N_2O than N_2O+N_2 following the addition of the acetylene block during the inter-monsoon, highlighting the probability that nitrification rather than denitrification is responsible for N_2O emissions when forest soils are at lower WFPS. By contrast, the ratio of $N_2O:N_2O+N_2$ production in the plantations was similar across both seasons.

Emissions of N_2O from these plantations (3.84±1.41 – 37.14±10.24 kg N ha-1 y^{-1}) were higher than rates more generally reported for tropical agriculture. For example, oil palm plantations on peat soils emitted 0.07 – 5.1 kg N ha^{-1} y^{-1} in Malaysia and Indonesia (Melling, et al., 2007; Ishizuka, et al., 2005). Approximately 50 km south of the study location, near Lahad Datu, Fowler et al., (2011) reported rates of 3 kg N ha^{-1} y^{-1} for unfertilised plantations and 513 kg N ha^{-1} $y^{-}1$ for fertilised plantations. Although fertilised soils within the palm circle emitted extremely high rates of N_2O, when spatial variability within the plantation was taken into account, a mean rate of 4.4 kg N ha^{-1} y^{-1} was reported. This is considerably lower than N_2O emissions in the Kinabatangan plantations, however, it should be noted that mean rates reported in this thesis are not adjusted to take account of spatial or temporal variability of emission rates. In comparison to the plantations, primary forests near Lahad Datu emitted 3.1 kg N ha^{-1} $y^{-}1$ (21.9 μg N_2O-N m^{-2} h^{-1}), which was lower than that reported by the same authors for secondary (31.8 μg N_2O-N m^{-2} h^{-1}) and disturbed forests (52.9 μg N_2O-N m^{-2} h^{-1}) (Fowler, et al., 2011). By contrast, emission rates from the secondary forest sampled for this thesis (below detection – 10.23±2.44 kg N ha^{-1} y^{-1}) were lower than reported for the adjacent oil palm plantation and are within the range reported for primary forests on Peninsular

Malaysia (0.42 – 27.07 kg N ha^{-1} y^{-1}; Itoh, et al., (2010)), and lower than secondary forests on peat in Indonesia (13.4 kg N ha^{-1} y^{-1}; Hadi, et al., (2005)). Although N_2O was only measured over a few days in each season, these results suggest that the conversion of forest to plantation may change soil conditions (e.g. WFPS), thereby altering nitrogen process rates such as nitrification and denitrification and emissions of N_2O. This is in agreement with several researchers that have found higher emissions of N_2O following the replacement of forests with oil palm plantations (Melling, et al., 2007; Fowler, et al., 2011; Skiba, et al., 2012). Although our findings largely confirm that of others, more work is needed to improve estimated budget of N_2O emissions as a result of land use change. In particular, these results highlight differences in emission rates through seasons even within the wet tropics that should be addressed. Notable differences in emissions from *terra firme* and riparian soils were also apparent with seasonal interaction effects that highlight the importance of soil moisture in controlling rate of nitrate production and loss. In contrast to the large body of literature on riparian biogeochemistry in temperate regions, articles on denitrification and N_2O emissions from tropical riparian zones are sparse. Accordingly, further work at greater temporal resolution is needed to confirm the hypotheses that lower soil moisture in forests during the inter-monsoon results in nitrification as the main N_2O forming process. Whereas, in *terra firme* plantations, greater soil moisture as a result of reduced plant uptake and transpiration, may lead to a coupling of nitrification and denitrification with increased N_2O emissions relative to forests. Alternatively, the higher emissions of N_2O in forests during the inter-monsoon relative to the wet season may still be primarily from denitrification, however lower soil moisture may affect the ability of soils to reduce N_2O. Specifically, the response of the N_2O reductase appears to be slower than the other denitrification enzymes induced upon anaerobisis (Firestone & Tiedje, 1979). Therefore, lower rainfall frequency will shorten the

time that soils remain anoxic, reduce the efficacy of N_2O reductase and result in higher N_2O emissions. The impact of the changes to nitrogen cycling following land use change, therefore, may differ between *terra firme* and riparian sites and is deserving of further investigation. As initiatives such as that of the United Nations REDD (reducing emissions from deforestation and forest degradation) programme are put into practice throughout the tropics, accurate assessments of N_2O emissions are required to validate the approach taken by governmental organisations to mitigate the effects of global warming. Although static chambers provide a cost effective and versatile measure of N_2O flux from soils, technological advances such as that provided through laser technology are now available to provide accurate measurements at high resolution. Nevertheless, static chambers may still provide a useful addition to N_2O emission data where process understanding is still lacking.

7.4.3 The relative importance of riparian and *terra firme* conversions

The increase in gross mineralisation rates following conversion was statistically more significant in *terra firme* plantations than in riparian plantations (Table 7-5). Conversely, differences in rates of NH_4^+ consumption, nitrification, NO_3^- consumption and denitrification between forest and plantations were all greater in riparian than in *terra firme* sites. However, as increases in ammonia consumption and decreases in nitrification were statistically more significant following conversion of the riparian sites, it could be inferred that the effect of land use conversion on losses of nitrogen will be of less impact where plantations are established on riparian soils relative to *terra firme* soils. In support of this, the effect of plantation establishment on N_2O emissions appeared to have a greater impact in *terra firme* sites relative to those in the riparian zone.

For emissions of greenhouse gases, therefore, riparian zone conversion appears from these results to have less impact than converting *terra firme* forests to oil palm plantations.

However, greenhouse gas emissions are not the only environmental consequence of an altered nitrogen cycle in agricultural soils. Removal and reduction of vegetation and root biomass in riparian zones may increase bank erosion and alter stream chemistry with an associated increase in leaching or export of pollutants to the aquatic ecosystem. Furthermore, the application of inorganic fertiliser to the riparian zone may reduce N contact time with soils, increasing the possibility of nitrate leaching and ultimately determining whether the riparian zone acts as a NO_3^- sink or source. Finally, riparian zones are also of fundamental importance to biodiversity conservation in the region as highlighted by the creation of the Wildlife Sanctuary along the Kinabatangan River riparian zone in 2005.

7.5 CONCLUSION

This chapter sought to examine the effects of plantation establishment on N process rates in riparian and *terra firme* soils. The results show that gross mineralisation rates increase in plantations relative to forests, most likely following changes in climate (i.e. temperature and WFPS). However, NH_4^+ consumption was also preserved in plantations indicating that the microbial immobilisation of ammonia remained strong and, in combination with higher WFPS, possibly decreased rates of nitrification. Potential denitrification showed no consistent decline or increase following land use change, but rather, was lower in *terra firme* plantations and higher in riparian plantations relative to forests on the same substrate. The *terra firme* forest was the only site to have a rate of denitrification that was higher than the rate of nitrification, indicating the potential for high NO_3^- losses in this one location. For all other sites, both forests and plantations, denitrification was, most likely, the primary NO_3^- consumptive fate as rates of potential DNRA were three orders of magnitude lower than

potential denitrification. However, in the riparian forest, N_2O emissions below detection limit suggest that another microbial or abiotic process may affect NO_3^- consumption in this site.

N_2O emissions varied little across land use and soil type during the wet season. During the inter-monsoon, *terra firme* sites emitted significantly more N_2O than riparian sites, highlighting potential differences in N_2O forming processes within riparian and *terra firme* soils. Furthermore, the contribution that nitrification and denitrification make to N_2O production might differ between forests and plantations. Specifically, lower fluxes of N_2O and ratios $N_2O:(N_2O+N_2)> 1$ in forested sites indicate that nitrification may plays an important role in N_2O production during the inter-monsoon. In contrast, coupled nitrification-denitrification may be responsible for the higher fluxes of N_2O in plantations soils as evidenced by lower ratios of $N_2O:(N_2O+N_2)$. Alternatively, lower soil moisture may reduce induction of N_2O reductase and result in higher N_2O emissions.

The effect of land use change on NH_4^+ consumption, nitrification and NO_3^- consumption was statistically more significant in riparian than in *terra firme* plantations. However, greater NH_4^+ immobilisation and lower NO_3^- production in riparian plantations relative to riparian forests suggest that the effect of land use conversion on losses of nitrogen will be reduced where plantations are established on riparian soils relative to *terra firme* soils. The establishment of plantations on *terra firme* sites also appeared to have a greater impact on N_2O emissions relative to those in the riparian zone. However, as riparian zones provide a number of important ecosystem functions, the impact of establishing plantations within the riparian zone extends beyond the effect on greenhouse gas emissions. These results highlight the potential changes to nitrogen cycling when forests are converted to oil palm plantations. As more land is sequestered for palm oil production, further work will be needed to manage

plantations effectively and sustainably, and to minimise N losses through atmospheric emissions and leaching.

CHAPTER 8: CONCLUSIONS AND FUTURE WORK

8.1 INTRODUCTION

The principal objective of this thesis has been to examine the effect of land use change from forest to oil palm plantation on nitrogen cycling within the lowland tropics of Sabah, Malaysia. Chapter 1 introduced the subject and provided a rationale for the work undertaken. Chapter 2 reviewed the literature on nitrogen cycling in the lowland tropics and identified research gaps, which the thesis sought to address. Specifically, the aim of the thesis was to:

1. Determine whether secondary forests follow a trajectory of increased nitrogen availability with the time since disturbance, and if so, to establish whether mature secondary forests represent an appropriate baseline by which to gauge the effect of land use conversion on N process rates (addressed in Chapter 4).
2. Quantify the magnitude of spatial variability of soil properties indicative of nitrogen cycling within oil palm plantations using geostatistical analysis (addressed in Chapter 5).
3. Determine the effect of plantation age (time following establishment) and seasonal (wet versus dry) variation on denitrification, nitrate ammonification and N_2O emissions (addressed in Chapter 6).
4. Compare nitrogen status and nitrogen cycling process rates in tropical forests with those of oil palm plantations (addressed in Chapter 7).

This final chapter synthesises the results presented in Chapters 4 to 7 within the framework of the thesis aims and makes recommendations on the direction of future work in this area.

8.2 MAJOR RESEARCH FINDINGS

The principal findings of Chapters 4 to 7 are summarised as follows:

1. The secondary forests appear to be following a trajectory of nitrogen recovery through secondary forest development. As such, later successional forests display characteristics of increasing "openness" to nitrogen cycling as they recover from disturbance.
2. Management practices result in spatial variability of soil properties within plantations highlighting the need to incorporate this variability into future sampling designs.
3. Plantation location (i.e. riparian or *terra firme*) affected whether seasonal differences in N_2O emissions and in-situ denitrification were apparent. The age of the plantation had little effect on most variables, however SOM decreased during the inter-monsoon and N_2O increased at the end of the wet season as plantations matured. Rates of DNRA were approximately three orders of magnitude lower than potential denitrification and were much lower than has been reported for many tropical soils.
4. Replacement of tropical forest with oil palm plantation has a significant effect on nitrogen process rates. Mineralisation increased and nitrification decreased in plantations relative to forests, although strong consumptive processes suggest that the microbial biomass was preserved post-disturbance. The effect of land use change on N_2O emissions appeared of greater impact in *terra firme* than riparian plantations, however, the conversion of riparian zones to palm oil production may have additional environmental costs that need to be considered.

8.2.1 Chapter 4: Recovery of secondary forests post-disturbance

This chapter used a chronosequence of forest disturbance to determine whether nitrogen status and cycling properties recovered though secondary forest succession. As the overriding aim of this thesis was to compare nitrogen biogeochemistry in forests with plantations, it was first necessary to establish an appropriate baseline against which the effects of land use change could be compared. Younger successional forests had lower soil carbon and nitrogen storage relative to mature forests, which, for the most part, appeared to follow a trajectory of increasing accumulation with time since disturbance. However, soil texture also had an impact on carbon storage and nitrogen accumulation and loss (through denitrification and N_2O emission), thereby highlighting the importance of edaphic properties on soil fertility.

Although all forest soils conformed to the same soil textural class (i.e. silty clay loams), even a modest increase in the sand fraction resulted in significant reductions in soil carbon and nitrogen, foliar N, in-situ denitrification and N_2O emission. These findings emphasise the complicating factors inherent in space-for-time substitutions, whereby differences in unaccounted for variables, such as land use history, may establish sites on independent trajectories of recovery rather than a successional continuum. Only four secondary forests formed part of this study, therefore some of the uncertainty in attributing observed trends to successional development may be alleviated through greater sampling resolution.

Nitrogen processing rates were less variable across the secondary forests than carbon and nitrogen storage. There were no differences in rates of gross mineralisation and nitrification, gross NH_4^+ and NO_3^- consumption, DNRA, denitrification or N_2O emission through any of the riparian sites. However, in-situ denitrification followed the pattern of carbon and nitrogen storage, resulting in greater atmospheric losses of N as soil nitrogen status recovered through forest succession. The least disturbed forest on *terra firme* soil tended to have lower gross

nitrification and higher rates of denitrification and N_2O emission than riparian sites. Greater losses of N through denitrification and N_2O emission signify a greater potential for N loss from the most mature site; a fact confirmed by soils significantly enriched in $\delta^{15}N$.

At the time of sampling (i.e. the end of wet season), denitrification and N_2O flux represented only a very small (i.e. <1%) proportion of the total N being cycled within the soil and ratios of NO_3^- to NH_4^+ were lower than 1. Small losses of N relative to the overall N turnover in these soils suggest that these forests were conservative of N. However, a comparison of nitrous oxide emissions during the wet season with those of the dry season illustrates the temporal variability of nitrogen losses, which were approximately 3-20 times greater during the inter-monsoon. It is likely that, during the inter-monsoon, greater emissions of N_2O result from the nitrification process and an increase in the rate of denitrification fuelled by a supply of NO_3^-. High rates of mineralisation, nitrification, and potential denitrification show that these forests have the ability to cycle large quantities of nitrogen and are significant emitters of N_2O. Furthermore, rates of potential DNRA were very low in comparison to potential denitrification, signifying that this process was not important in conserving nitrogen within these soils. Within the riparian forest, some other mechanism (e.g. leaching or abiotic retention) may have been responsible for NO_3^- conservation or loss as rates of potential denitrification were lower than the rate of nitrate consumption.

Overall, within these secondary forests, the picture is of increasing carbon and nitrogen storage and greater nitrogen loss indicative of a more open N cycle with recovery since disturbance. The successional state of the forest therefore has important implications for global carbon and nitrogen cycles. Secondary forest regrowth represents a major global carbon sink; however, where regrowth is limited by availability of nitrogen, carbon storage may be constrained. How tropical forests respond to increasing nitrogen deposition is a major

source of uncertainty in global carbon and nitrogen modelling. For example, increased N deposition in young N-limited secondary forests may stimulate biomass accumulation thereby enhancing carbon storage. Late successional forests are likely to respond to increased N inputs through elevated N losses from denitrification and N_2O emissions. Conversely, a slowdown in the rate of mineralisation following nitrogen enrichment may reduce CO_2 respiration and lead to nutrient retention as organic matter accumulates within the soil. Although reducing microbial respiration will increase carbon storage, elevated N deposition may have negative consequences such as degradation to soil fertility or reductions in ecosystem biodiversity. There are too few studies of N and C interactions within the tropical region to form any firm conclusion as to how these regions will respond to increasing nitrogen deposition. Yet, the importance of tropical secondary forests to global C and N cycling requires a better understanding of soil response to nitrogen additions.

8.2.2 Chapter 5: Spatial variability of nitrogen cycling in plantations

This chapter examined the spatial variability of nitrogen cycling indices within a 15Y old oil palm plantation and sought to relate them to plantation management practices. Specifically, the application of palm fronds to plantation inter-rows resulted in higher soil carbon beneath frond inputs. However, relatively modest increases in soil C and N relative to the amount of organic matter returned suggests that the vast majority of carbon is respired at the soil surface during decomposition and is consistent with the high rates of mineralisation reported for the plantations within this thesis. As might be expected, the practice of applying fertiliser to an area 2 m radius around the palm trunk (i.e. “the palm circle”) increased soil ammonia. Equally, soil carbon was significantly higher in the palm circle, most likely as a result of the high root density within this region. Soil nitrate was also higher in the palm circle but results were not significant.

Although denitrification may keep nitrate concentrations low under palm fronds that receive a fresh supply of organic matter, the distribution of soil $\delta^{15}N$ suggests that the highest rates of N processing occur within the palm circle. Specifically, the application of inorganic fertiliser to the palm circle with a $\delta^{15}N \approx 0‰$ would be expected to lower soil ^{15}N relative to the remainder of the plantation. However, the opposite trend was observed, namely soil $\delta^{15}N$ was significantly higher in the palm circle relative to the remainder of the plantation, indicating that the main ^{15}N fractionating processes such as nitrification and denitrification were likely to be greater in this region. These findings indicate that microbial process rates are likely to be spatially variable within plantations as a result of management practice and that future sampling design, particularly post-fertilisation, should account for this spatial variability. Finally, the results of the kriging interpolation confirmed that for most variables (pH_w excluded), spatial independence was reached over distances much smaller than the mean sampling distance employed for the plantations in this thesis.

8.2.3 Chapter 6: Temporal variability of N cycling in plantations

Chapter 6 looked at the temporal variability of nitrogen processing in plantations, both seasonally and through stand age. Although lowland tropical regions have less climatic variability than most temperate regions, inter-annual variability can be significant. For *terra firme* (but not riparian) plantations, N_2O and in-situ denitrification were higher during the inter-monsoon when soils were drier. Riparian sites meanwhile showed little temporal variability across season. The differing effect of season on process rates suggests that N_2O emissions are greater in *terra firme* sites during the inter-monsoon as a result of coupled nitrification and denitrification, whereas denitrification may be limited by nitrate availability in the riparian sites. These differences in process rates across substrate informed the

statistical design for Chapter 7, which compared N cycling in oil palm plantations with forests.

For the most part, differences in substrate precluded analysis of process rates through plantation age. However, trends were observed for SOM during the inter-monsoon and for N_2O emissions at the end of the wet season. Although, as these trends were not universally true across both seasons, caution must be exercised in attributing observed patterns to stand age. Nevertheless, a lack of difference in rates of mineralisation and nitrification, together with increasing fertiliser applications through stand age suggest that N_2O emissions are not likely to decrease as plantations mature.

The plantations sampled in this thesis spanned an age range of 3 months to 25 years; however, the 3-month-old plantation was excluded from most analyses due to the difficulty in separating the effect of re-planting from the effect of seasonal variability. As a result, the youngest plantation reported on (3Y) was already over one year old at the time samples were first taken during 2010. Many tropical studies on land use change report elevated rates of nitrogen processing in the months following initial disturbance (Matson, et al., 1987; Reiners, et al., 1994; Neill, et al., 1999; Burton, et al., 2007). Therefore, the effect of plantation establishment and replanting on nitrogen cycling in the shot-term immediately following disturbance needs to be determined. Furthermore, this thesis did not address the effect of continued re-planting on the long-term fertility and nitrogen status of the soil. In the Kinabatangan region, most plantations are first or second generation in age, however in future, the effect of continued tree cropping on soil fertility should be considered.

8.2.4 Chapter 7: Comparing process rates in plantations with forests

The data presented in Chapter 4-6 informed the statistical design employed in Chapter 7. Namely, Chapter 4 established the two most mature secondary forests as the appropriate baseline by which to assess the impact of land use change on nitrogen cycling. Chapter 5 confirmed the validity of the assumption of spatial independence of sampling design within the plantation environment. An effect of substrate type on N process rates identified in Chapter 6 dictated that *terra firme* forests and plantations should be considered separately to forests and plantations in the riparian zone. Accordingly, a replicated comparison of nitrogen process rates under both land uses (i.e. forests and plantations) was conducted on both the alluvial and mudstone and sandstone substrate.

This chapter shows that replacing tropical forests with oil palm plantations can result in significant changes to soil nitrogen processing. Rates of mineralisation, nitrification and inorganic nitrogen consumption displayed marked differences in plantations relative to forests, irrespective of soil type. For gross mineralisation, conversion had the effect of doubling process rates, most likely a result of higher temperatures and/or greater soil moisture in the plantations. Meanwhile, gross nitrification declined in plantations relative to forests, probably due to higher NH_4^+ immobilisation and reduced soil O_2 through higher soil moisture. The retention (and increase) in NH_4^+ immobilisation within the plantations indicate that the microbial population was unlikely to have declined in these soils following conversion to plantation. Rates of potential denitrification showed no consistent trend with plantation establishment and rates of potential DNRA did not vary across land use or soil type. DNRA, through all sites, was three orders of magnitude lower than potential denitrification and was much lower than rates reported in some tropical soils where DNRA is of similar importance to denitrification in its consumption of NO_3^-. Although these results are surprising, the low

$C:NO_3^-$ ratios found in these soils may favour denitrification over DNRA as the main dissimilatory nitrate consuming process and should be investigated further. Although denitrification was the primary NO_3^- consuming process in the majority of sites sampled, for the riparian forest, rates of NO_3^- consumption were greater than rates of potential denitrification. Accordingly, an alternative process such as abiotic immobilisation may be responsible for the very high rates of NO_3^- consumption and negative N_2O flux in the riparian forest.

Chapter 6 identified that *terra firme* plantations had higher emissions of N_2O during the dry season, which was attributed to coupled nitrification and denitrification. *Terra firme* forests also displayed higher rates of N_2O emission during the dry season, however, emission rates were lower for forests than plantations and were more likely to be due to nitrification or delayed induction of N_2O reductase in drier soils. The exact mechanism of N_2O emission requires further investigation, but it may be that the effect of conversion is to alter the relative proportions of nitrification and denitrification to emissions of N_2O.

The effect of plantation establishment on N_2O emission appeared to be relatively more important for *terra firme* than riparian soils, particularly during the inter-monsoon. Thus, the environmental impact, through greenhouse gas emission, of land conversion may be greater for *terra firme* than for riparian forests.

8.3 RECOMMENDATIONS FOR FUTURE WORK

The abundance of secondary forests continues to increase throughout the tropical regions of the world, therefore evaluating the effect of disturbance and their subsequent recovery is vital to understanding how they may respond to future anthropogenic change. The pattern of

increasing nitrogen accumulation and loss with secondary forest recovery suggests that young secondary forests should be considered separately to late-successional and old-growth forests when determining their response to nitrogen deposition, ability to sequester carbon or strength as an N_2O source. For example, N_2O emissions from forests were correlated with soil carbon and nitrogen, nitrate availability and the ratio of NO_3^-:NH_4^+. Therefore, secondary forests are likely to have lower emissions of N_2O than adjacent primary forests but increase emissions in response to N accumulation through successional progression. In the absence of alternative nutrient limitation (e.g. P or base cations), the loss of N in young secondary forests indicates that elevated N inputs may accelerate N cycling recovery and increase C storage in plant biomass. However, the impact of N additions on soil fertility (e.g. increased acidity, mobility of P) also needs to be taken into account, as do edaphic factors such as soil texture when predicting successional forest response to increasing N deposition. The ability of secondary forests to act as a carbon sink has gained increasing attention over recent years, but C cycling models are only just beginning to incorporate factors such as nitrogen status and alleviation of N-limitation through increased anthropogenic nitrogen inputs (Yang, et al., 2010). The altered N status of disturbed and re-generating tropical forests, therefore, highlights the importance of incorporating these differences (*vis-à-vis* old-growth forests) in large-scale biogeochemical models.

Integration of management practices into future sampling designs is emphasised by the observation of spatial variability of N cycling indices within the oil palm plantations. Specifically, the spatial distribution of soil $\delta^{15}N$ suggests that N process rates (e.g. nitrification and denitrification) are greater within the palm circle. As a result, N_2O emissions are likely to be greater where fertiliser is applied, thereby informing the placement of soil gas chambers for an accurate quantification of N_2O flux at the landscape scale. The analysis of

process rates across wet and dry seasons also emphasises the necessity of incorporating temporal variability into estimates of N gas emissions from both plantation and forest soils. However, soil type (i.e. riparian and *terra firme*) may be an important determinant of emission rate. Specifically, the results in this thesis suggest that riparian locations have less seasonal variability in denitrification and N_2O emission rates than *terra firme* sites. For *terra firme* plantations, factors such as soil temperature and moisture are likely to be important determinants of nitrogen cycling and N_2O flux. Conversely, soil texture was a significant contributing factor to N availability and loss in riparian sites. Therefore, the primary controls on nitrogen cycling may differ as a result of site location, thus informing model parameterisation where rates are to be scaled-up. Although an attempt was made to determine the effect of plantation age on N process rates, to a certain extent, the difference in soil substrate through the plantations frustrated efforts. Nevertheless, some trends were apparent such as increasing N_2O emissions with plantation maturity, which would benefit from additional investigation at greater temporal resolution. Studies of temporal variability should also incorporate changes that occur immediately after, and during the first few months, following plantation establishment. Furthermore, the effect on soil fertility of re-planting oil palms on a 25-year rotation is unclear. However, the observed trend of decreasing soil organic matter with plantation age may be indicative of declining nutrient returns with important implications for long-term crop productivity.

The Oxidant and Particle Photochemical Processes (OP3) Project conducted in Sabah during 2008 highlighted the importance of oil palm agriculture to N_2O and NO_x emissions. However, published results from that project are primarily from measurements conducted above the ground using atmospheric research aircraft and tower-based micrometeorological instruments (Hewitt, et al., 2009; Fowler, et al., 2011). By contrast, few published studies

have made soil-based measurements of nitrogen cycling within oil palm plantations (Haron, et al., 1998; Ishizuka, et al., 2005; Melling, et al., 2007; Kimura, et al., 2012; Schroth, et al., 2000; Banabas, 2007; Skiba, et al., 2012). Seemingly, this is also the first study to compare gross nitrogen cycling rates in forests with plantations. The results presented in this thesis show that establishment of plantations can significantly affect soil N storage and rates of N cycling. Forests and plantations were classified either as *terra firme* or riparian and, for the most part, the effect of land use change was to increase emissions of N_2O in the *terra firme* (but not riparian) sites. Meanwhile *terra firme* plantations retained soil C and N to a greater extent than riparian plantations. Specifically, reductions in soil nitrogen were only significant when riparian sites were converted to plantations. There was also a considerable, but non-significant, reduction in soil carbon when plantations were established on riparian (but not *terra firme*) soils. This infers that the conversion of *terra firme* soils is of greater importance for N_2O emissions, whereas riparian conversion will result in higher emissions of CO_2 through losses of soil C following land use change. Quantitatively, the greater global warming potential of N_2O and the high emission rates from these soils suggests that converting *terra firme* sites will be of greater significance for greenhouse gas emissions than the conversion of riparian sites. However, there are other important environmental consequences of converting riparian zones to agriculture such as buffering surface water pollutants, intercepting sediments, and providing wildlife habitat. In conclusion, the key variables identified by this thesis that should be considered in future assessment of greenhouse gas emissions include:

1. The successional stage and C and N status (i.e. soil C, N and NO_3^-:NH_4^+) of forests;
2. The interactive effects of soil texture on N status and loss;

3. The seasonal control on N availability and N_2O emissions through alterations to soil temperature and moisture in *terra firme* locations;
4. The spatial variability in N process rates within oil palm agriculture as a result of uneven fertiliser application and organic matter returns throughout plantations; and
5. The temporal variability of N_2O emissions through plantation maturity.

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