MICROBIAL NUTRIENT DYNAMICS AND MINERALIZATION IN DEGRADED SUBTROPICAL FOREST ECOSYSTEMS UNDERGOING RECOVERY

By

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I certify that the thesis entitled "***Microbial nutrient dynamics and mineralization in degraded subtropical forest ecosystems undergoing recovery***" submitted by Miss Kusum Maithani, for the degree of Doctor of Philosophy in Botany of the North-Eastern Hill University, Shillong, embodies the record of original investigation by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. The work has not been submitted for any degree of any other University.

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CHAPTER 1

INTRODUCTION

Forest provides a critical pathway for exchange of carbon dioxide between the atmosphere and land, and covers about one-third of world's total land area. Forests are also responsible for about two-third of global photosynthesis on land surface. Besides, forests have been intimately linked with human society and its socio-economic development. Due to explosion in human population in recent years, there has been tremendous pressure on forest resources. The over-exploitation of forest resources has led to forest degradation and depletion in forest cover.

Ecological implications of land degradation and deforestation in the tropics are far reaching. At the global scale, degradation of natural resources leads to loss of gene pools and biological diversity, exacerbation of climatic change through the increase of green-house gases in the atmosphere and altered hydrological cycles. At the local scale, natural resource degradation leads to lack of economic growth, increased impoverishment of the population and urban migration. Forest cutting has been reported to cause a net loss of soil organic carbon. This loss affects the soil fertility level of the highly weathered tropical soils and plant regeneration in the degraded sites. Soil organic matter is an important regulator of numerous environmental constraints to productivity. Mineralization of decomposing residue is a major source of plant nutrition in highly weathered soils with little inherent mineral fertility (Sanchez *et al.* 1989).

The activities of microorganisms and soil fauna serve to promote soil aggregation (Oades 1984), leading to reduced erosion (Lal 1986) and greater moisture infiltration (Lavelle 1988). Other important benefits resulting from the maintenance of the soil organic matter include nutrient retention and storage (Russell 1973), increased buffering capacity in low activity clay soils (Swift & Sanchez 1984) and an increase in their water holding capacity. However, a decrease in total soil organic matter as a result of land management and increased soil aeration is an universal phenomenon in both temperate and tropical regions. Degradation of the soil system through soil organic matter loss results from soil tillage (Follett & Schimel 1989) and clearing of natural vegetation (Srivastava & Singh 1989). Even simple land perturbations are associated with a decline in soil organic matter (Nye & Greenland 1960). However, the carbon loss from the system is gradual and is not easily detectable. One of the current models of SOM dynamics *viz.*, CENTURY, divides SOM into three fractions with different turnover times; the active (0.14 yr), slow (5 yrs) and passive (150 yrs) fractions (Parton *et al.* 1989). The active fraction with short turnover time appears to contain primarily the living soil microbial biomass and microbial products.

Information on changes in microbial biomass following vegetation removal is valuable not only because it provides an indication on slower, less easily detectable SOM changes but also because the microbial biomass contributes to soil fertility. The microbial biomass represents an important labile pool of soil nutrients and plays an active role through immobilization in preventing nutrient leaching (Vitousek & Matson 1984). The microbial biomass accounts for only 1-3% of soil organic-C but it acts as medium through which all organic material that enters the soil must pass (Jenkinson 1977). From the

viewpoint of soil productivity, the soil biomass controls the major processes involved in nutrient transformation and cycling, soil organic matter maintenance and macroaggregation for favourable water and aeration characteristics. Changes in microbial population in response to variation in soil conditions have important implications for nutrient cycling in forest ecosystems. According to Luizao *et al.* (1992), the amounts of SOM and microbial biomass in the humid tropical rain forest soils are substantial. Soil microbial biomass C and N fluxes are far greater than the cycling of C and N through litterfall (Arunachalam *et al.* 1996b). Slashing and burning reduces microbial biomass and mineralization in the top soil and is associated with a shift in forms of mineral-N in favour of nitrate.

Studies on soil biological processes including soil microbial biomass have engaged considerable attention in recent years and numerous methods have been proposed to estimate soil microbial biomsss. Physiological and biochemical methods are most frequently used, although these methods have their particular limitation and significance. Chloroform-fumigation (a physiological method) has been widely accepted for soil microbial biomass estimation; the fumigation is either followed by incubation or direct extraction depending upon the soil pH and humic nature.

Values of microbial biomass can provide one of the most satisfactory estimates of the restoration of soil microbial populations. Several workers argue that soil biomass measurements are not uniform across systems and may only be relative, however, as an ecological marker, relative biomass change over time should be sufficient for predicting the ecosystem recovery after disturbance.

The microbial biomass measurements may provide the information needed for ecosystem level monitoring of disturbance and recovery

(Smith & Paul 1990). Srivastava *et al.* (1989) found a direct relationship between coal-mine spoil age and microbial biomass and suggested that microbial biomass is a critical factor in the recovery of the mine spoils and can be taken as a functional index for soil redevelopment.

Improving soil fertility is one of the most common tactics to increase agricultural and forest productivity. Maintaining high level of available N and P, the two most limiting nutrients in soil, remains a major challenge to ecologists and land managers. The availability of N and P in soil is largely controlled by biologically mediated processes such as mineralization and immobilization.

Mineralization is a process of nutrient release from the organically bound materials into inorganic or plant-available forms. Mineralization, is therefore, an important factor regulating production in non-fertilized forest ecosystems. Many studies have reported increased loss of N and other elements from forest ecosystem following tree felling. It has been suggested that these losses are caused either by increased N mineralization rate or reduced N uptake by plants after disturbance (Vitousek & Melillo 1979). Increased N minerlization following disturbance has been attributed to changes in microclimate, substrate quality or both. Matson & Vitousek (1981) reported that microclimate was more important in controlling mineral-N flux in a one-year old clear-cut site, and substrate quality was more important in 4-year old site.

Wide variations occur in N mineralization among soils of different vegetational types, even when they are in close proximity and develop from similar parent material. It has also been suggested by Rice & Pancholy (1972) that the ionic form of mineral-N in soil may indicate the stage of secondary succession.

A better understanding of nutrient transformation within forest ecosystem requires data on microbial nutrient immobilization and mineralization patterns. A survey of available literature reveals that although microbial nutrient studies have engaged the attention of a large number of researchers in other countries, studies on these aspects from India are limited. Though some studies relating to microbial nutrients (Singh *et al.* 1989, 1991, Srivastava & Singh 1989) are reported from Indian subcontinent, they are restricted to dry tropical deciduous forests and savanna. Studies relating to microbial nutrient dynamics and mineralization in disturbed humid subtropical forests undergoing natural recovery have not been undertaken so far.

OBJECTIVES

The objective of the present research was to study microbial immobilization and mineralization patterns in an aggrading human-impacted humid subtropical forest ecosystem. The specific objectives of the study were as follows:

1. To study the changes in chemical and biological properties of soil and diversity and dominance patterns of fungi during revegetation of the disturbed forest.
2. To study the relative changes in microbial biomass C, N and P in forest stands undergoing recovery after tree cutting.
3. To study the N minerlization pattern during forest regrowth.
4. To study the influence of resource quality (litter and roots) on mineral-N dynamics in regrowing forest communities.

5 To evaluate the relative influence of soil pH, temperature and moisture on N mineralization.

To achieve the above-mentioned objectives, data on microbial population, microbial biomass C, N and P dynamics and mineral-N flux as influenced by season, soil characteristics, stand age, and resource quality were collected on a seasonal basis in forest regrowths of three different ages in a humid subtropical forest of Meghalaya. The data obtained on these aspects have been analysed to characterize the pattern of nutrient restoration during revegetation of the tree-cut sites, and the role of microbial biomass in soil organic matter and nutrient dynamics during the recovery of degraded forests.

The thesis is divided in to 10 chapters. The data collected on various aspects such as soil, vegetation, microbial population, microbial C, N and P, N mineralization, etc. are presented in Chapters 3-9. Chapter 1 gives a general introduction to the whole study. Chapter 2 presents the review of literature published on the subject matter of the thesis and related aspects. Chapter 3 includes the details pertaining to location, geology, climate, soil, species richness and diversity of the selected study sites. Soil and microbial population dynamics have been discussed critically in Chapter 4. The details relating to microbial biomass C, N and P dynamics and *in situ* N mineralization pattern have been given in Chapters 5 and 6. Influence of leaf litter and fine and coarse roots on N mineralization has been detailed in Chapters 7 and 8. Results of *in vivo* studies on the effects of soil pH, temperature and moisture on N mineralization have been discussed in Chapter 9. The results presented in chapters 4-9 have been critically discussed in detail in the individual chapters. However, the major findings of the whole study have also been briefly discussed in an integrated manner in chapter 10 (General discussion). This is followed by a brief summary and references.

CHAPTER 2

REVIEW OF LITERATURE

The first and foremost effect of disturbance on forest ecosystem is the loss of complexity in community structure. Subsequently, some important ecosystem functions such as productivity and nutrient cycling are adversely affected. Tree cutting in the forest causes accelerated soil erosion and soil compaction, as a consequence of which most of the essential nutrients are lost from the site, textural condition deteriorates and revegetation on these nutrient-poor sites becomes difficult (Bormann & Likens 1981). Nwoboshi (1980) reported 10-20% reduction in soil nitrogen pool in teak plantations of Nigeria as a result of moderate canopy thinning. Raghubanshi (1990) showed that harvesting of Bamboo plantation depletes soil organic C and N by 13 and 20%, respectively. The most important consequence of tree felling in the forest ecosystem (the most common human activity in this part of the country) is soil degradation which is a serious problem. Restoration of such a degraded soil, both in terms of its structure and quality is quite tedious, because it is directly exposed to insulation by which top soil becomes dry and compact. Majority of the nutrient stock is leached due to heavy rainfall. The immediate effects of these disturbances are not known. However, it is implied that such a poor quality soil may not favour the growth and establishment of the original vegetation and microbial community. Due to these reasons, the degraded system has to be given the utmost care for its regeneration *i.e.* site should be protected and managed

properly.

Matson & Smith (1993) studied the status of detrital organic matter and soil carbon dioxide in regenerating forests following tree cutting in west Virginia. They reported substantial increase in the soil organic matter in these forests. If the forest is allowed for natural recovery, the ecosystem invariably tends to restore its original species composition, structure and function. Marion & Black (1988) reported an increase in total soil C with stand age. This they attributed to plant-derived organic matter and microbial products. Arunachalam *et al.* (1996a) also reported an increase in the litter and fine-root input with stand age in a subtropical humid forest ecosystem. Microbial population and activity also showed a steady increase with the vegetation regrowth and improvement of soil fertility (Henrot & Robertson 1994). It is the microbial population which plays a vital role in organic matter decomposition and nutrient mineralization in forest soils. The functioning of microbial population with special reference to their role in improving the degraded soils, can be easily detected by measuring the soil microbial biomass in terms of nutrient immobilization and mineralization.

Recent studies on tropical forest ecosystem as related to disturbance (Luizao *et al.* 1992, Henrot & Robertson 1994) have established that microbial diversity and its biomass decrease as a result of disturbance. The microbial population in soil is greatly influenced by physico-chemical characteristics of soil and vegetal cover (Mishra & Sharma 1977). Loss of fungal species diversity due to disturbance has been reported by Alexander (1977) and Jha *et al.* (1992a & b).

According to Srinivasan (1995), biodiversity is widespread among natural population of microorganisms. Correct identification and isolation of microbes are essential for conserving their gene pools and for biotechnological progress. He also pointed out that a fuller

knowledge of microbial taxonomy as well as physiology is helpful in getting a better insight into the complexities of microbial biodiversity and also in identifying potential endangered species.

The microbial community has been given little emphasis during the past decade in analysing their diversity and biomass dynamics (Dormaar *et al.* 1984). Nevertheless, a few studies have been carried out in this regard by Jha *et al.* (1992a & b) in subtropical and tropical forests of north-east India. They reported loss of fungal species and their biomass in disturbed forest.

SOIL MICROBIAL BIOMASS DYNAMICS

Microbial biomass is defined as the living part of the soil organic matter (SOM) excluding plant roots and soil animals larger than $5x10^3$ μm^3 in size. According to soil chemists, the biomass is the small but labile fraction of SOM that contributes significantly to plant nutrition. Microbiologists envisage microbial biomass as a largely dormant population with two unique features: an enormous richness of species and an ability to survive hard times. The microbial biomass plays a dual role in soil: as an agent for nutrient transformation and also as a reservoir for N, P and S.

Values of microbial biomass can provide satisfactory estimates of the restoration of soil microbial population (Raghubahshi & Singh 1992). Microbial biomass serves as an ecological marker for predicting ecosystem recovery after disturbance (Ross *et al.* 1982, 1984). Powlson & Jenkinson (1981) and Powlson *et al.* (1987) argued that soil biomass measurements can give an early indication of changes in SOM long before these changes in total soil-C and N can be detected. The principal function of microbial biomass is to accumulate and conserve nutrients in biologically active form and subsequently to release them in soil. Thus it acts as sink and source of nutrients for plant growth (Jenkinson & Ladd 1981, Singh *et al.* 1989). Brookes & McGarth (1984)

reported that microbial biomass also serves as a sensitive indicator of soil toxicity.

METHODOLOGIES

In view of increasing importance being attached to the study of microbial biomass, several methods have been proposed for estimating it in soil. These include chloroform fumigation incubation (Jenkinson & Powlson 1976), substrate-induced respiration (Anderson & Domsch 1978), direct microscopy (Van Veen & Paul 1979), ATP determination (Nannipieri *et al.* 1978) methods and direct estimation of biomass through chloroform-fumigation extraction method (Vance *et al.* 1987). Among these, chloroform fumigation incubation (CFI) (Jenkinson & Powlson 1976) and chloroform fumigation extraction (CFE) (Vance *et al.* 1987) have been widely accepted as the most reliable methods for the biomass estimation. CFI method is the most basic technique which is used for the calibration of other methods. However, there are certain limitations with the CFI method. Vance *et al.* (1987) reported that the application of this method is limited to soil with pH above 4.5, and it also gives unacceptable results in soils that have recently received large additions of substrate (Jenkinson & Powlson 1976). These limitations of CFI method are largely overcome by fumigation extraction method. However, K_{EC} factor applied to calculate microbial biomass from the carbon additionally made extractable by the fumigation is still controversial. CFE method has also been found to be reliable for the estimation of microbial biomass N (Brookes *et al.* 1985). Methods have been also developed to measure the amount of phosphorus held in the soil microbial biomass (Brookes *et al.* 1982).

SEASONALITY OF MICROBIAL BIOMASS

Season influences microbial number (Diaz-Ravina *et al.* 1993) and its mass (Lynch & Panting 1980) either directly, by inducing microbial

response to soil changes or indirectly, by influencing plant metabolism. Not much information is available on the fluctuations of the microbial biomass within seasonal and annual cycles (Diaz-Ravina *et al.* 1995). Pattern of seasonal fluctuations in tropical forest ecosystems differs from that in temperate forests. In tropical forest soils the peak microbial biomass is observed during winter (Luizao *et al.* 1992, Maithani *et al.* 1996a) when the temperature goes down and becomes unfavourable for microbial activity. On the contrary, during rainy season which is favourable period for organic matter decomposition, microbial activity is at its peak thus, resulting in lower value for soil microbial biomass. However, for temperate forest soils peak microbial biomass has been recorded during summer or spring (Diaz-Ravina *et al.* 1993). Von Lutzow *et al.* (1992) recorded the highest biomass N in autumn and/or spring and lowest during summer. Ding Ming Mao *et al.* (1992) found seasonal fluctuations in the collective microbial biomass (bacteria+fungi) in the tropical soils, while Soderstrom (1979) reported fluctuation in only fungal biomass in pine forest soils. In case of agricultural soils, several authors (Lynch & Panting 1980, Van Gestel *et al.* 1991) observed clear annual variation in the microbial biomass, while Patra *et al.* (1990) found no significant temporal change. Microbial biomass in soil is influenced by rainfall and temperature gradients and also by pH (Campbell *et al.* 1973, Brookes *et al.* 1986, Sarathchandra *et al.* 1989, Kaiser *et al.* 1992, Baath *et al.* 1995). Besides climatic factors, soil texture also influences the microbial biomass. Christensen & Sorensen (1985) and Christensen (1988) reported that clay soil contains greater soil biomass. Merckex *et al.* (1985), Van Veen *et al.* (1985) and Maithani *et al.* (1996a) obtained a significant positive relationship between microbial biomass and clay content.

Soil moisture content is another important factor which regulates the decomposition of litter and fine roots *vis-a-vis* the status of

soil biomass. Bottner (1985), Kieft *et al.* (1987), Van Gestel *et al.* (1993) and Clein & Schimel (1994) have explained the dynamics of microbial biomass in relation to drying and rewetting of soils. Higher evapotranspiration from the top soil results in the drying of soil, which causes reduction in soil biomass (Linn and Doran 1984). Subsequent rewetting causes a rapid release in water potential. This 'upshock' releases microbial nutrients by cell lysis or death (Bottner 1985). In field situations, the drying and rewetting processes are cyclic over the seasonal pattern. The drying phase of the soil is mainly during winter and summer, when the plant and microbial growth is hampered due to unfavourable conditions (Singh *et al.* 1989). On the other hand, microbial growth and activity is favoured due to rewetting of soil during rainy season, and therefore, there could be a greater release of microbial nutrients through increased mineralization. The mineralized nutrients are readily absorbed by the plants due to their peak vegetative growth during the rainy season. There seems to exist some kind of synchrony between microbial biomass dynamics in soil with nutrient mineralization and its uptake by the plants. Temperature also has an enduring effect on the dynamics of soil microbial population and its biomass (Sarathchandra *et al.* 1989). However, little work has been done on the interaction between temperature and organic matter decomposition (Novak 1974).

The plants growing over the soil also influence microbial biomass dynamics, as they improve the status of soil fertility through detrital input and decay (cfs. Vogt *et al.* 1986). Both litter and fine roots are of significance in any ecosystem functioning especially in terms of nutrient cycling. It has been established that the fineroot turnover is faster than the litter in tropical forest ecosystems (cfs. Vogt *et al.* 1986, 1991). Decomposition of organic detritus on the forest floor is mainly caused by the microbes (Singh & Gupta 1977) and therefore, it is expected that any change in the quality and

quantity of detritus may influence the dynamics of soil microbial biomass.

IMPORTANCE OF MICROBIAL BIOMASS IN SOM AND NUTRIENT DYNAMICS

Numerous studies on the measurement of microbial biomass C, N and P in different natural as well as disturbed ecosystems have shown that the soil biomass contains important labile pools of C and mineral nutrients (Anderson & Domsch 1980, Smith & Paul 1990, Diaz-Ravina *et al.* 1993, 1995) which are liberated by the death of microorganisms. Microbial biomass C ranges between 60-2000 ug g^{-1} for various tropical forests (Singh *et al.* 1989, Luizao *et al.* 1992, Henrot & Roberston 1994), while for the temperate forests it ranges between 132-880 ug g^{-1} (Diaz-Ravina *et al.* 1995). The dynamics of N in soil are closely associated with that of C (Jenkinson 1988, Billore *et al.* 1995), as most of the N exists in organic compounds and heterotrophic microbes. The microbial biomass N values range between 42-191 ug g^{-1} for coniferous soils (Martikainen & Palojarvi 1990, Diaz-Ravina *et al.* 1995) and 9-239 ug g^{-1} for tropical forest soils (Vitousek & Matson 1988). Diaz-Ravina *et al.* (1988) reported that microbial biomass N ranges between 132-240 ug g^{-1} for broadleaved deciduous forest and between 42-242 ug g^{-1} for evergreen forests.

The microbial biomass P pool in the soil varies between 5.2-67.2 ug g^{-1} for arable land, grassland and pine forest of temperate region (Brookes *et al.* 1984). Diaz-Ravina *et al.* (1995) reported that microbial biomass P estimates range between 41 and 187 ug g^{-1} for the temperate forest soils in Spain. However, in the tropical dry deciduous forest the microbial biomass P varied between 8.7 and 28.3 ug g^{-1} (Srivastava & Singh 1988, Srivastava 1992a). Many workers have reported a close positive relationship between microbial biomass and soil properties (Anderson & Domsch 1989, Billore *et al.* 1995, Arunachalam *et al.* 1996b). Microbial biomass C is positively

correlated to total soil C (Ayanbaba *et al.* 1976, Singh *et al.* 1989, Maithani *et al.* 1996a).

The contribution of microbial biomass C to the total soil organic-C ranges between 1.5-5.3% for tropical forest soils (Theng *et al.* 1989, Luizao *et al.* 1992) and 1.8-2.9% for temperate forests (Vance *et al.* 1987). However, the percentage of microbial biomass N to total soil N may range from 3.4-5.9% for forest soils (Martikainen & Palojarvi 1990). Brookes *et al.* (1984) reported that the contribution of microbial biomass P to total soil P ranges from 2.4 to 3.0% for grasslands and 1.2-4.7% for woodlands.

MINERALIZATION

Mineralization rates generally correlate with the productivity of ecosystems (Adams *et al.* 1989). *In situ* methods for the measurement of mineralization rate have not been widely used in agricultural systems. In contrast, much of the interest in forest soil is associated with studies of nutrient cycling in field situations. The first study of *in situ* N mineralization in forest soil was by Lamee (1967). *In situ* methods have now been applied by several workers in different forest ecosystems as given below:

(i) Mature forests of different productivity status (Adams & Attiwill 1986, Raison *et al.* 1987),

(ii) Forests along a gradient of N availability (Aber *et al.* 1985, Nadelhoffer *et al.* 1984),

(iii) Forests along a successional gradient (Lamb 1980) and

(iv) Forests subjected to disturbance (Vitousek & Denslow 1986, Vitousek & Matson 1985, Matson & Boone 1984).

These studies have shown that N mineralization in mature forests ranges from 10 kg ha^{-1} yr^{-1} in cool temperate forests to 800 kg ha^{-1} yr^{-1} in tropical forests. Effects of disturbance on element cycling and nutrient loss in forest ecosystem has been a matter of long

standing concern among forest scientists. Element losses following disturbance have also been used to characterize the degree of homeostasis in forest biogeochemical cycles (Bormann & Likens 1979), and can be a useful measure of ecosystem level stability.

Among the various elements, N has been given a special emphasis in forest ecosystem studies owing to following reasons:

(i) N is most often a limiting element to forest regrowth. Its substantial loss following disturbance slows down forest regrowth (Vitousek *et al.* 1982).

(ii) Following disturbance, loss of nitrate usually increases more than any other ion (Likens *et al.* 1970).

(iii) Increased losses of nitrate in disturbed forest can cause increased losses of cations, since the supply of mobile anions control cation leaching (Nye & Greenland 1960).

(iv) Increased nitrification can either directly (Bremner & Blackmer 1978) or indirectly (Firestone 1982) increase the rate of nitrous oxide production and volatilization.

N uptake in disturbed forests equals or slightly exceeds net N mineralization and both uptake and mineralization are generally 10-100 times greater than annual losses (Stone *et al.* 1979). A severe disturbance reduces the ability of plants to take up mineralized N. At the same time, removal of the canopy can increase the rate of N mineralization by increasing soil temperature and moisture, by increasing the frequency and intensity of drying and rewetting cycle in the forest floor (Campbell *et al.* 1973, Van Gestel *et al.* 1991), by increasing the availability of substrate for mineralization (Rice 1979) and by decreasing resource competition between heterotrophs and mycorrhizae (Gadgil & Gadgil 1975). Altogether, these processes cause N mineralization in excess of the requirement of the regrowing vegetation. The amount of this excess mineralized N varies among the

sites depending upon, (i) the extent to which plant N uptake is decreased by the disturbance, (ii) the amount of increase in the rate of mineralization caused by the disturbance (Vitousek 1981) and (iii) the rate of N mineralization prior to disturbance. Ammonia volatilization and denitrification reduce nitrate leaching by causing gaseous losses of N. Clay fixation holds N in a form where it may slowly be made available to the developing vegetation. The remaining processes delay nitrate losses by temporarily holding N in a form where it is either unavailable (immobilized) or it is taken up by the plants. Regrowing plants eventually reestablish the intra-system N cycle by taking up as much N as is mineralized.

Seasonality in nutrient mineralization is governed by the rainfall (Zak & Grigal 1991) and air temperature gradients prevailing in the locality. Moisture-limited seasonality of N mineralization has been reported in dry tropical forests (Singh *et al.* 1991), in Scotish highlands (Morecroft *et al.* 1992) and in Taiga forests (Clein & Schimel 1995). Powers (1990) have emphasized on the influence of altitude on N mineralization. Rates of mineralization like other biological and chemical processes are influenced by the environmental conditions *e.g.* mineralization rates tend to be low at low temperatures (Floate 1970). As the temperature falls with the increase in altitude (Barry 1981), mineralization rate and hence N availability to the plant is expected to show a declining trend with it. However, contrary to this, high N concentration has been found in plants growing at higher altitudes (Korner *et al.* 1986, Morecroft 1990). Morecroft *et al.* (1992) reported a peak in N mineralization rate in spring and autumn in temperate ecosystems.

Turner *et al.* (1993) reported highest N mineralization and nitrification during summer for mixed old-growth coniferous forests. However, in the field conditions, various environmental factors interact together in a highly complex manner in controlling N

mineralization. Notable factors are soil moisture, temperature, substrate quality and quantity. All these factors vary both spatially as well as temporally and therefore, experiments under controlled laboratory conditions have been considered better to study N mineralization processes. The field processes are difficult to measure because controlling factors are not static, and consequently, the results based on laboratory experiments may not compare well with field observations and there is a strong need for field research to complement laboratory studies.

NITRIFICATION AND DENITRIFICATION

Several studies have been carried out on nitrification in acid forest soils. Klein *et al.* (1983) reported nitrate formation in a forest soil with pH as low as 3.6-5.1. They found that addition of ammonium to soil incubation did not increase nitrate production. Schimel *et al.* (1984) reported that nitrifying activity in a Sierran forest soil (pH 5.8) was neither affected when NO_2^- oxidation was experimentally inhibited, nor it increased by the addition of ammonium. They concluded that potential for heterotrophic nitrification was greater than the autotrophic nitrification.

Johnsrud (1978) isolated heterotrophic bacteria and fungi from four acid forest soils (pH 3.9-5.7) that were capable of producing nitrate in a glucose-ammonimum inorganic salt medium. Lang & Jagnow (1986) did not find any autotrophic nitrifying bacteria from a beech forest soil (pH 3.5) in Germany, however, about 1/4th of the fungi and heterotrophic bacteria that were isolated, were capable of producing nitrate and nitrite. These studies indicated that in acidic soils nitrification is inhibited and if it occurs, it is due to the activity of heterotrophic microbes rather than the autotrophic bacteria.

The nitrate-N formed through nitrification may be lost to the atmosphere by conversion to gaseous forms of N. Gaseous loss of N from

the forest soil is not well understood and has only recently received attention. In forest soils, N loss as gas is most likely in the form of nitrous oxide and nitrogen. Nitrous oxide is produced in the soil mainly as an intermediate product of denitrification (Blew & Parkinson 1993).

Keeney (1980) in his comprehensive review on N availability in forest soils reported that virtually no information exists on the amount of denitrification that might be occurring in the forests. However, after the publication of this review, a few reports have come on this aspects from a old-growth white pine stand (Roberston & Tiedje 1984), from a black spruce stand (McKeeney *et al.* 1984) and from a 120-year old white spruce forest (Blew & Parkinson 1993).

Denitrification has, however, proved to be a difficult process to study because of high spatial and temporal variations in activity (Robertson & Tiedje 1984). One of the most important environmental variables affecting denitrification is the water content of the soil. Broadbent & Clark (1965) identified two soil water regimes under which denitrification occurs. In the first the soil is water-logged and losses are rapid. In the second, the soil is well drained but slow losses occur from the anaerobic zones within the generally well-aerated soil matrix.

RESOURCE QUALITY AND MINERAL-N FLUX

Recent studies show that the type of vegetation present on a given site influences the availability and cycling of the nutrients. The specific influence of vegetation, in isolation from other factors such as climate, soil, time and topography (Jenny 1980, Van Cleve *et al.* 1991) has been difficult to assess because of the property of the species to grow on different sites. Harmer & Alexander (1986) have demonstrated variation in nutrient availability in soil due to

difference in plant species growing over it. The extent to which these differences are attributable to the differences in litter quality or to the other factors mediated by the vegetation such as nutrient uptake by roots, rhizosphere effects and herbivory, is not fully understood.

In a study on Northern Vancouver Island, Prescott *et al.* (1993) found substantially lower N availability in forest floors of mixed forest of western red Cedar and western Hemlock than in adjacent mixed forests of western Hemlock-Anabilis forest. They suggested that cause of difference in N supply between these two forest types was the presence of Cedar in Cedar-Hemlock forest. Pure leaf litter from these forests had lower initial N concentration and decomposed more slowly than mixed hemlock and fir needle litter.

Alban (1969) and Turner *et al.* (1993) studied the species-specific influence on N mineralization and nitrification in old-growth forest stands. Their results showed that nitrification and nitrate concentration in soil differed due to the tree species growing on it thus indicating species-specific effect on ammonium and nitrate production and uptake within the forest type.

Pronounced small scale heterogeneity in N in the soil around plants has been reported by Jackson *et al.* (1989). They have shown that plants possess a potential mechanism *e.g.*, root proliferation and changes in nutrient uptake kinetics, for exploring soil heterogeneity. The growth, death and decomposition of fine roots are the major processes in the C and nutrient circulation in forest ecosystems (Edwards & Harris 1977, McClaugherty *et al.* 1982, Arunachalam *et al.* 1996c).

Chemical composition of detritus determines its decomposition and nutrient release patterns (Swift *et al.* 1979). The concentrations of N, lignin and polyphenol in plant material are the generally recognized plant factors controlling N mineralization rates of plant

materials added to the soil (Haynes 1986). According to Stevenson (1986), N concentration has to be greater than a critical level (15-25 g ha^{-1}) and then only net-mineralization can take place. However, Melillo *et al.* (1982) and Palm & Sanchez (1991) showed that despite having N concentrations above the critical level nitrogen mineralization may be low because of the higher lignin and polyphenol concentrations in the plant material. Melillo *et al.* (1982) and Muller *et al.* (1988) found that lignin concentration of plant material was a much better predictor for plant residue decomposition rate rather than the N concentration.

Gower & Son (1992) found a good relationship between the lignin/N concentration of foliar litter and N mineralization rate in soil under the vegetal cover of five tree species. They suggested that lignin/N in fresh leaf litter may be an important positive feedback mechanism that influences N availability. Pastor *et al.* (1987) demonstrated that spruce needle litter with its high lignin and low N content is slow to decompose on account of which N availability in boreal forests is reduced.

The literature reviewed in the foregoing pages clearly reveal the conspicuous lack of knowledge relating to the role of microbial biomass in SOM and nutrient turnover through immobilization and mineralization processes in forest ecosystems in general, and disturbed forests in particular. Moreover, under Indian situations such type of work has been conducted only with reference to dry tropical ecosystems. Thus the present study was designed to evaluate the relative importance of microbial biomass in nutrient immobilization and mineralization in soil during the recovery of a disturbed humid subtropical forest ecosystem of Meghalaya, India.

CHAPTER 3

STUDY SITE: LOCATION, CLIMATE, FLORISTIC RICHNESS AND SPECIES DIVERSITY

LOCATION

The study was carried out in three adjacent forest stands located at Upper Shillong (latitude 25°34'N, longitude 91°56'E, altitude 1900 m asl), 12 km south of Shillong, the capital of Meghalaya (Figure 3.1). All three stands are part of a subtropical humid forest, which was once regarded as sacred grove ('Shillong peak sacred grove'). The grove was disturbed from time to time by cutting of trees. The three forest stands where the study was carried out represent the disturbed sacred grove where the trees were cut 7, 13 and 16 years ago before the commencement of the study in 1993. These stands have been designated in this thesis as 7-, 13- and 16-year old regrowths. Each stand covers an area of about 25-40 ha and they are located on a gentle slope facing south to south-east.

GEOLOGY

The shillong plateau is situated at a height of about 1500 m above the alluvial plain of the Brahmaputra valley. A major portion of the Shillong area is formed by the Archaean gneisses and granites. The gneisses are finely banded, grey to pink in colour and contain microcline, biotite, subordinate quartz and plagioclase. The intrusive granites are mostly porphyritic with flesh-coloured microclines, some

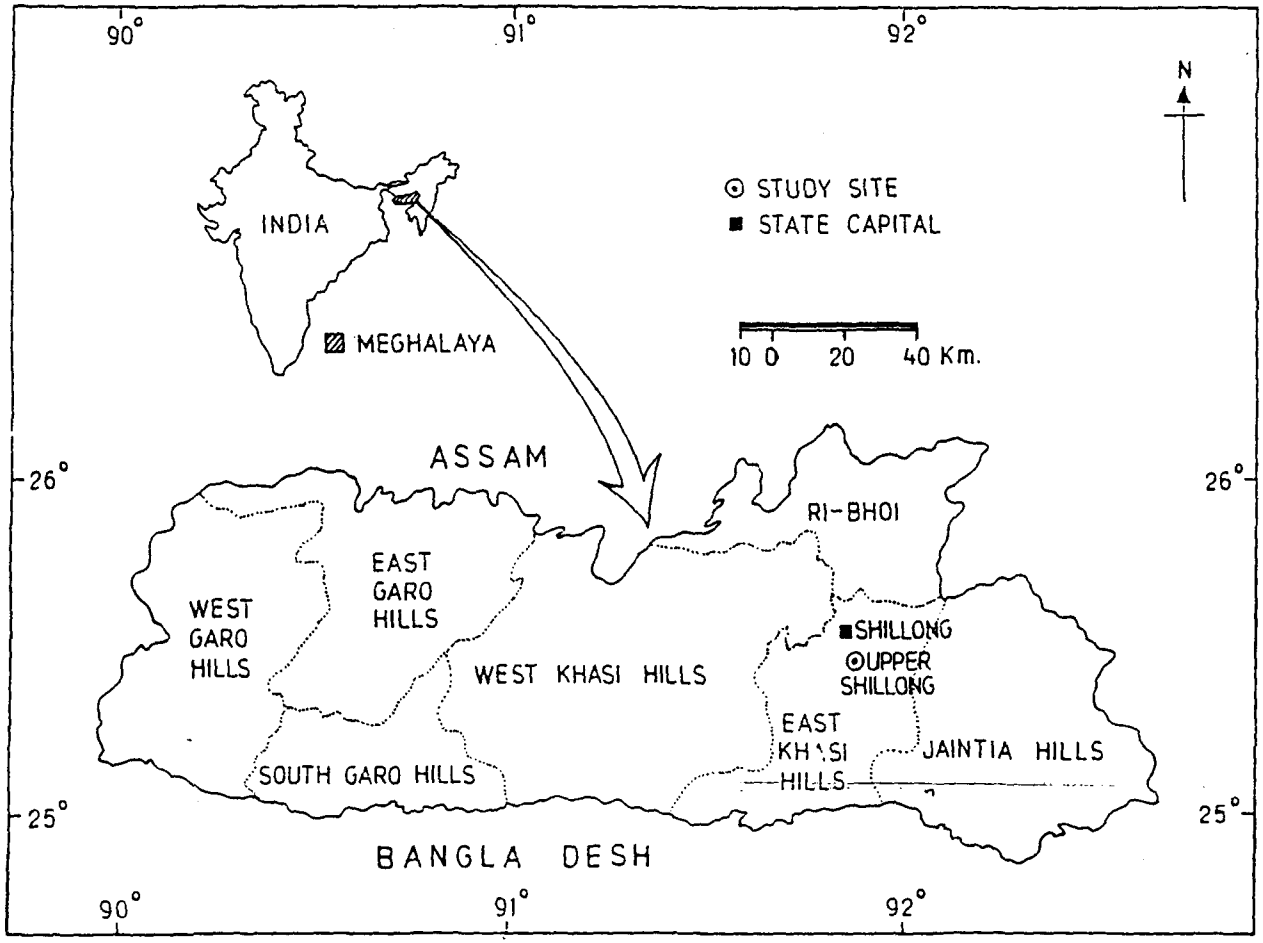

Figure 3.1: Map showing location of the study site.

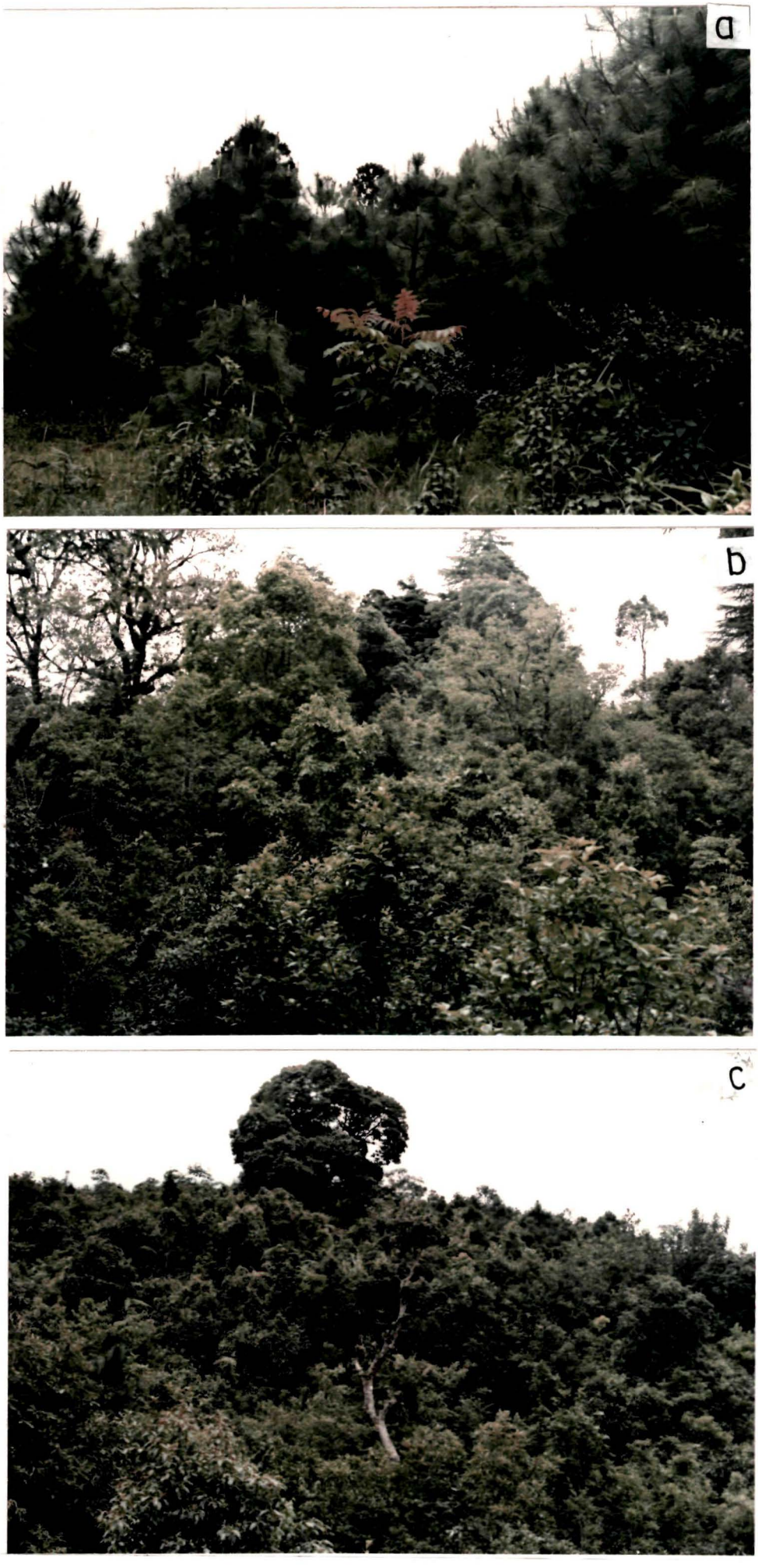

Plate 3.1: Over-view of (a) 7-year old regrowth (b) 13-year old regrowth (c) 16-year old regrowth.

acid plagioclase, orthoclase and biotites. The granites also intrude the Shillong schists, but are less frequent in the Shillong quartzites (Pascoe 1950).

CLIMATE

The climate of Meghalaya is monsoonic with an average annual rainfall of 2500 mm. The area is characterized by four distinct seasons. The period from mid-November to February represents winter. The period from March to mid-May with small amount of rainfall represents the spring. May to September with about 85% of total annual rainfall (Figure 3.2) represents rainy season and period between October and mid-November represents autumn.

Mean maximum and minimum temperatures are 16° and 22°C, respectively.

MICROCLIMATE OF THE STUDY SITES

METHODS

The microclimate of the three forest regrowths was studied by measuring light intensity, relative humidity and air temperature at seasonal interval during the year 1993 and 1994. All the parameters were measured close to the ground surface in each stand at 12.00 hrs. Light intensity was measured using a lux meter (LUBRON LX-101). The relative humidity and air temperature was measured using a hygrometer and thermometer, respectively. Soil temperature was measured by using a soil thermometer (ELITE) without disturbing the soil.

FOREST MICROCLIMATE

Light intensity and air temperature were significantly higher ($P<0.01$) in 7-year old regrowth than 13- and 16-year old regrowths.

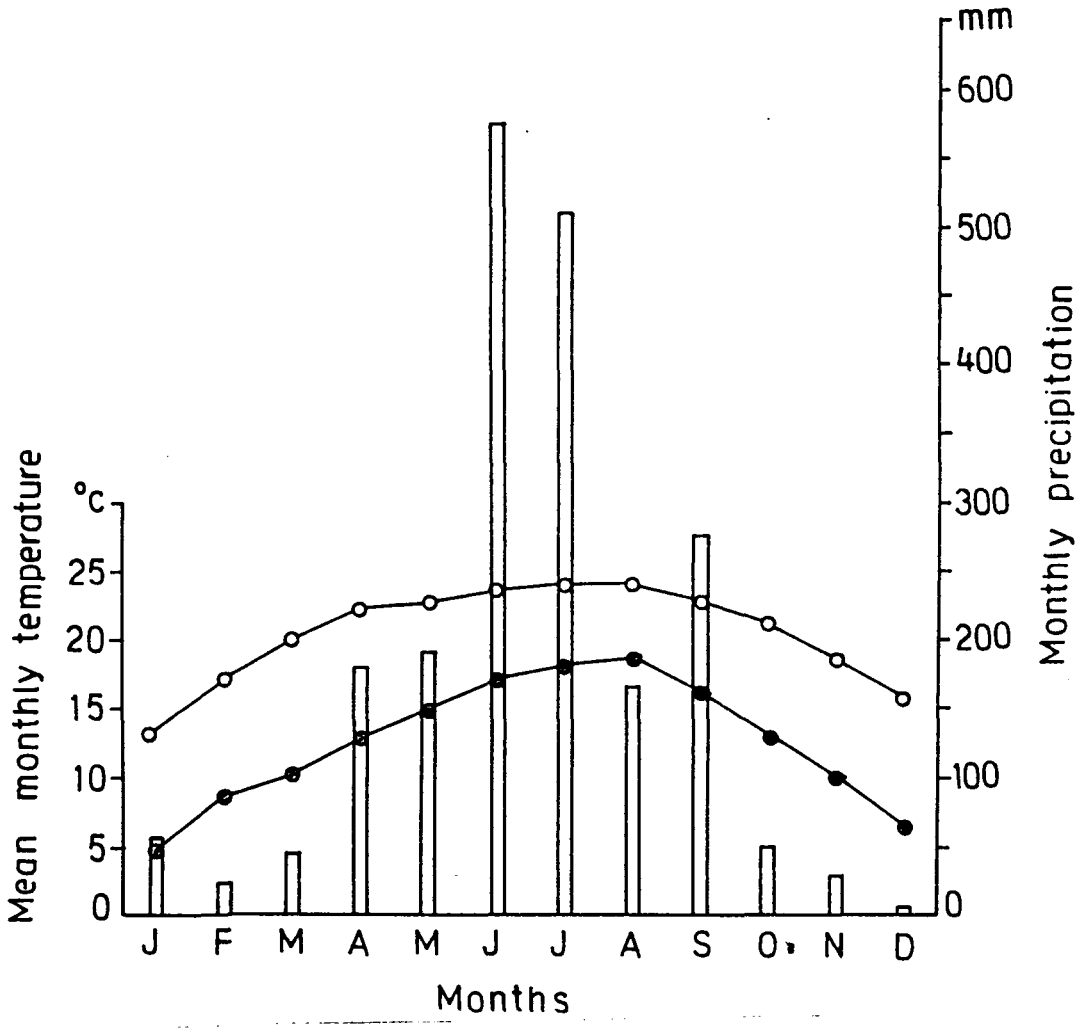

Figure 3.2: Climatograph of the study area (□) total monthly precipitation, mean monthly maximum (○) and minimum (●)air temperature.

On the contrary, relative humidity was more in the 16-year old regrowth than the other two younger regrowths. A definite seasonal trend was observed in both light intensity and air temperature. The maximum values were recorded during spring and minimum during winter (Figure 3.3). However, in the case of relative humidity the lowest value was observed during spring and highest during rainy season (Figure 3.3).

All the three regrowths recorded lower soil temperature during winter and higher during rainy and post-rainy (autumn) seasons (Figure 3.4). Soil temperature was higher in the surface soil layer (0-10 cm) than in the subsurface soil layer (10-20 cm). Three-way ANOVA revealed significant difference in soil temperature due to season, soil depth and regrowth age. The 16-year old regrowth recorded lower soil temperatures than the other two younger stands.

FLORISTIC RICHNESS AND SPECIES DIVERSITY IN THE STUDY SITES

METHODS

In all three forest sites, vegetation analysis was done during July-August (1993), as these months represented the period of peak vegetative growth. An area of 50 m x 50 m was demarcated in each stand and ten quadrats each of 10 m x 10 m were randomly laid to study the tree and shrub components, while ground vegetation was studied by laying ten 1 m x 1 m quadrats. Nomenclature of the plant species follows Hooker (1872-1897). Density, basal cover and importance value index (IVI) of the woody and herbaceous species were calculated according to Misra (1968).

Community indices such as Sorensen's similarity index, species richness index and Shannon-Wiener diversity index were computed as follows:

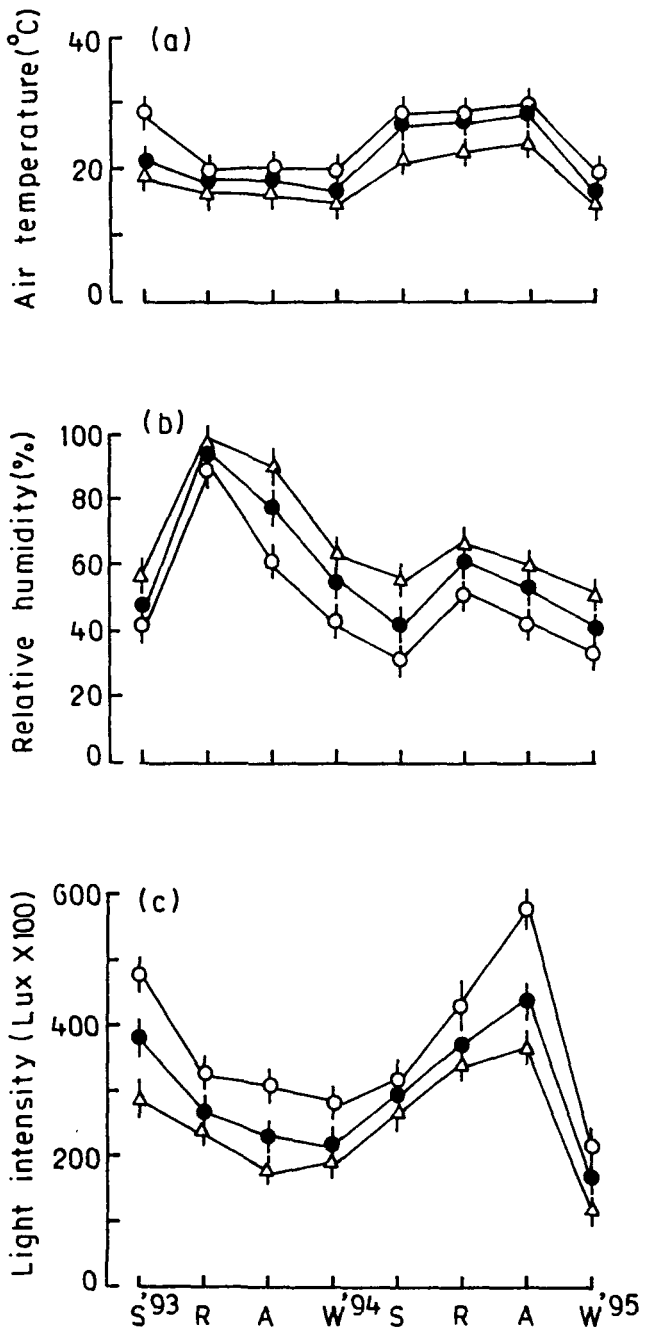

Figure 3.3: Seasonal variation in microclimate of the three study site. S-spring; R-rainy; A-autumn; W-winter (○-○-10, ● 10-20 cm)

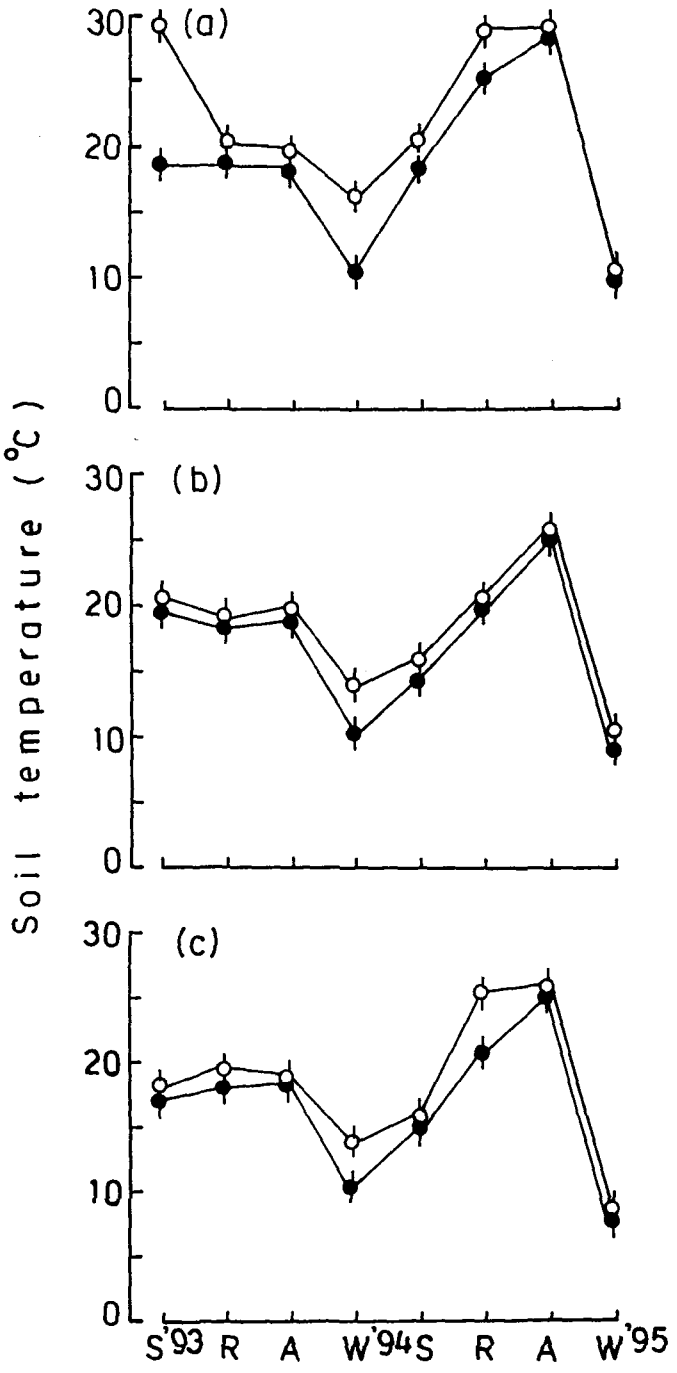

Figure 3.4: Seasonal variation in soil temperature in two soil depths (o 0-10 and • 10-20 cm) in (a) 7-, (b) 13- and (c) 16-year old regrowths. S-spring; R-rainy; A-autumn; W-winter.

Similarity index (Sorensen 1948): $\frac{2C}{A+B} \times 100$

where, A = Number of species in stand A, B = Number of species in stand B, C = Number of species common to both stands A and B

Species richness index (Magurran 1988): $\frac{S-1}{\log_e N}$

where, S = Number of species, N = Number of individuals

Diversity index (Shannon & Wiener 1963):

$$\sum - \left(\frac{n_i}{N}\right) \log_e \left(\frac{n_i}{N}\right)$$

where, n_i = Importance value for each species,
N = Total importance value

SPECIES COMPOSITION

The plant species were distributed in three distinct layers in 13- and 16-year old regrowths, whereas in 7-year old regrowth there were only two strata. The 7-year old regrowth was composed of a few sparsely distributed young trees of *Pinus kesiya*, *Schima wallichii*, *Schima khasiana* and *Corylopsis himalayana*, while densely growing perennial grasses and early successional weeds dominated the ground layer. *Quercus dealbata* was present only as saplings in the 7-year old stand, while it had a remarkably higher dominance (IVI=204) in the 13-year old stand. In the 16-year old stand along with *Q. dealbata*, *Rhododendron arboreum* and *Quercus griffithii* were also present in the canopy layer (Table 3.1). The sprouts of the constituent broadleaved species formed the sub-canopy layer in the older regrowths. Out of 10 tree species identified, *P. kesiya* and *Q. dealbata* were present in all the three forest regrowths.

Shrub species like *Litsea elongata* and *Rhus semi-alata* were

Table 3.1. Species composition and importance value indices (IVI) of different vegetation components in the broadleaved forest regrowths of three different ages.

Age of the forest regrowth	Vegetation component/ Plant species	IVI
7-yr old	**Tree species**	
	Pinus kesiya	148.12
	Schima wallichii	66.57
	Schima khasiana	55.21
	Corylopsis himalayana	29.73
	Shrub species	
	Litsea elongata	102.60
	Osbeckia stellata	85.94
	Rubus ellipticus	60.78
	Rhus semi-alata	50.67
	Herbaceous species	
	Imperata cylindrica	41.87
	Eupatorium adenophorum	27.70
	Arundinella bengalensis	24.03
	Anemone rivularis	15.65
	Ranunculus diffusus	15.24
	Commelina benghalensis	13.66
	Anaphilis araneusa	12.84
	Brunella vulgaris	10.64
	Oxalis corniculata	10.55
	Ambrosia artimissifolia	10.08
13-yr old	**Tree species**	
	Quercus dealbata	214.06
	Castonopsis kurzii	35.55
	Pinus kesiya	20.82
	Myrica esculenta	18.44
	Litsea khasiana	11.57
	Shrub species	
	Litsea elongata	108.99
	Gaultheria fragrantissima	38.65
	Osbeckia stellata	33.47
	Symplocos spicata	27.81
	Rhus semi-alata	26.59
	Eurya japonica	25.13
	Breynia retusa	18.36
	Lonicera macrantha	13.93
	Symplocos sismuntia	7.04
	Herbaceous species	
	Pteridium aquilinum	79.41
	Smilax blumeii	51.85
	Hemiphragma heterophylla	48.91
	Osbeckia crinata	43.02
	Rubus acuminatus	26.18
	Hedyotis orcinella	26.03
	Asparagus racemosus	10.65

Table 3.1. continued.

Site	Vegetation component/ Plant species	IVI
16-yr old	**Tree species**	
	Quercus dealbata	71.77
	Rhododendron arboreum	62.81
	Quercus griffithii	36.72
	Schima khasiana	30.58
	Pinus kesiya	25.97
	Castonopsis kurzii	17.46
	Corylopsis himalayana	16.89
	Lindera caudata	15.94
	Schima wallichii	14.27
	Litsea khasiana	7.48
	Shrub species	
	Litsea elongata	139.44
	Viburnum foetidum	82.66
	Rhus semi-alata	77.64
	Herbaceous species	
	Lycopodium clavatum	52.22
	Commelina benghalensis	51.61
	Selaginella bisulcata	40.55
	Oxalis corniculata	27.22
	Hedyotis orcinella	25.73
	Gleichenia longissima	19.27
	Hypericum japonicum	16.00
	Rubia cordifolia	14.76
	Ranunculus diffusus	14.32
	Pteridium aquilinum	12.22
	Plantago major	10.12

Note: Only those herbaceous species which have IVI more than 10 are listed here.

abundant in all the three forest regrowths. Grasses like *Imperata cylindrica*, *Arundinella bengalensis* and early successional weeds like *Eupatorium adenophorum* dominanted the herbaceous vegetation in the 7-year old stand, while, *Pteridium aquilinum* and *Smilax blumeii* were the dominant herbaceous species in the 13-year old stand. *Commelina benghalensis* and *Lycopodium clavatum* had the maximum IVI (52) in the 16-year old stand (Table 3.1).

In terms of Sorensen's similarity index, tree and herbaceous components (22.22 and 19.10%, respectively) were less similar in 7- and 13-year old stands as compared to 13- and 16-year old stands (53.33 and 20.00%, respectively). The shrub species showed greater similarity between 7- and 13-year old stands (46.15).

DENSITY

The tree density increased from 180 ha^{-1} in the 7-year old stand to 1140 ha^{-1} in the 16-year old stand. The basal area of the woody vegetation also showed a similar trend (Table 3.2). Density of herbaceous species was highest in the 7-year old stand; after this stage of vegetation recovery it declined significantly ($P<0.01$) in the 13-year old stand and then again increased in the 16-year old stand.

SPECIES RICHNESS AND DIVERSITY

The number of tree species increased from 4 in the 7-year old regrowth to 5 and 10 in the 13- and 16-year old regrowths, respectively. Consequently, the tree species richness and Shannon-Wiener diversity indices (Table 3.2) increased with the increasing age of the regrowth. There were 4, 9 and 3 shrub species in 7-, 13- and 16-year old regrowths, respectively. However, the number of herbaceous species declined sharply from 33 in the 7-year old stand to 12 in the

Table 3.2. Density (plants ha^{-1}), basal area (m^2 ha^{-1}) and species richness and diversity indices in three forest regrowths

Age of forest regrowth	Vegetation component	Density	Basal area	Species content	Species richness index	Species diversity index ($\bar{H}$)
7-yr	Trees	180	3.10 (10-25)	4	0.58	1.22
	Shrubs	500	0.31	4	0.48	1.35
	Herbs	439000	1.17	33	2.46	2.94
13-yr	Trees	480	19.85 (10-30)	5	0.65	0.98
	Shrubs	780	0.62	9	1.20	1.92
	Herbs	55000	0.29	9	0.73	1.26
16-yr	Trees	1140	44.21 (10-40)	10	1.28	2.09
	Shrubs	300	0.43	3	0.35	1.06
	Herbs	143000	0.54	12	0.93	1.83

Values in parentheses are DBH (cm) range.

- absent

16-year old stand. These trends resulted in higher herbaceous species diversity (2.94) in 7-year old stand and that for the shrub species (1.9) in the 13-year old stand. The overall diversity for the community was highest in the 7-year old regrowth, which then declined to a lower level in the 13-year old regrowth and again increased markedly in the 16-year old regrowth.

CHAPTER 4

PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES OF SOIL

INTRODUCTION

Tree regeneration in a disturbed forest community is influenced by temporal and spatial variations in a wide variety of soil physico-chemical properties (Thorhaug 1980). Soil moisture regime strongly influences tree seedling regeneration in the forest after disturbance (Arunachalam *et al.* 1996b). Other factors of soil complex such as pH, organic matter and nutrient contents also influence plant growth and succession on degraded sites (Aweto 1981, Pandey & Singh 1985). Loss of soil carbon after forest cutting has been reported by Edwards & Ross-Todd (1983), Miller & Sirois (1986) and Nakane *et al.* (1983). Nutrient regeneration in soil during revegetation in forest fallows have been studied by Aweto (1981) and in landslide affected areas by Pandey & Singh (1985) in the tropical region and by Ramakrishnan & Toky (1981) and Mishra & Ramakrishnan (1984) in the humid subtropics.

Soil is inhabited by a diverse group of microorganisms *viz.* bacteria, fungi, actinomycetes, arthropods and protozoa etc. Out of these, bacteria and fungi play a more important role in nutrient cycling and in influencing the biological properties of soil. Soil also influences the population and activity of microorganisms. It has been reported that soils with relatively higher organic matter usually develop larger microbial population. Organic matter as well as quantity and activity of microorganisms represent sensitive indicators

of soil genesis (Powlson *et al.* 1987). The population composition and activity of microorganisms are largely regulated by soil physico-chemical properties and by climate and vegetation. Recent studies by Jha *et al.* (1992a & b) and Henrot & Robertson (1994) have shown the influence of canopy cover on microbial population dynamics and composition of microbial communities in forest ecosystems. However, an integrated approach to the dynamics of microbial population and edaphic variables in degraded forest ecosystems has been missing in most studies, although such an approach may give greater insight into microbial population dynamics as influenced by SOM and nutrient build-up during revegetation in the disturbed sites.

The present chapter deals with the seasonal and spatial variations in bacterial and fungal populations and some important edaphic variables during forest regrowth following selective tree cutting. Further, an attempt has been made to evaluate the relative influence of soil and climate on microbial population dynamics.

METHODS

SOIL SAMPLING

Soil samples were collected in January, April, July and October during 1993 and 1994. In each stand, ten replicate samples were collected using a steel corer (6.5 cm diameter) from two soil depths (0-10 and 10-20 cm). The replicated samples of a given depth were thoroughly mixed to obtain one composite sample. The samples were air-dried, sieved through a 2 mm mesh sieve to remove stone particles and then passed through 0.5 mm mesh screen. The screened samples were stored in polythene bags for physico-chemical analyses.

ISOLATION OF SOIL BACTERIA AND FUNGI

During each sampling date, soil samples were also collected asceptically in sterililzed polythene bags from each site and from the two soil depths and were immediately used for the isolation of bacteria and fungi

Soil bacterial population was estimated by Waksman's (1952) method using the nutrient agar medium (Difco Laboratories 1953) and 10^5 dilution. Fungal population was estimated by dilution plate method (Johnson & Curl 1972) using Rose Bengal agar medium (Martin 1950) and 10^3 dilution in water. The Petridishes were incubated at 30±1°C for 24 h and at 25±1°C for 5 days for bacteria and fungi, respectively. The fungi were identified following Gilman (1957) and their percentage relative abundance was calculated as follows:

$$\text{Relative abundance (\%)} = \frac{\text{Total no. of individual fungus}}{\text{Total no. of all fungi}} \times 100$$

Fungal species diversity was calculated using the diversity index given by Shanon & Weiener (1963). The formula is given in Chapter 3, however, species number has been used in place of IVI.

SOIL ANALYSIS

Soil texture and bulk density (BD) were determined by Bouyoucos hydrometer method and gravimetric method, respectively (Allen *et al.* 1974). Water holding capacity (WHC) was determined by Keen's box method by using copper cups of 5.6 cm internal diameter and 1.6 cm height (Piper 1942). Soil moisture content (SMC) was determined gravimetrically by taking 10 g of fresh seived soil, and pH was determined electrometrically by a digital pH meter (SYSTRONICS-3[illegible]) in 1:2.5 suspension of soil in deionized water (Anderson & Ingram 1993). Cation exchange capacity (CEC) was determined after extracting the

exchangeable bases from the soil with 1 M ammonium acetate (pH 7.0) followed by the replacement of ammonium-N with potassium chloride and distillation with magnesium oxide (Allen *et al.* 1974).

Organic carbon was determined by rapid titration method (Walkley & Black 1934). Soil organic matter content was obtained by multiplying the organic carbon concentration by 1.724 assuming that the soil organic matter contains 58% of carbon (Allen *et al.* 1974). Total Kjeldahl nitrogen (TKN) was determined by digesting air-dried soil samples with concentrated sulphuric acid using Kjeltabs (TECATOR) as catalysts, on a block digester. Distillation and titration were done simultaneously in a TECATOR KJELTEC AUTO 1030 ANALYSER. Available-P was determined after extracting soil P in 0.5 M sodium bicarbonate solution by ammonium-molybdate blue method (Anderson & Ingram 1993). Each analysis was performed in triplicate and the final results are expressed on oven-dry weight basis.

STATISTICAL ANALYSIS

The data were statistically analysed using ANOVA (fixed effects model) to study the effect of the age of the forest regrowth, season, year and soil depth on microbiolgical and edaphic variables. Linear regressions were worked out according to Zar (1974), wherever necessary.

RESULTS

PHYSICO-CHEMICAL PROPERTIES OF SOIL

Physical properties: The texture of the soil was sandy loam, sandy clay loam and clay loam in the 7-, 13- and 16-year old regrowths, respectively. The proportion of clay particles increased significantly

($P<0.01$) from 7-year old to 16-year old stand, while the percentage of sand particles showed a reverse trend. Bulk density of the soil though did not vary much with stand age, it showed an increasing trend with soil depth (Table 4.1). Water holding capacity (WHC) of the soil increased significantly ($P<0.01$) with the regrowth age (Figure 4.1a). In all the three regrowths, WHC was maximum in the surface soil layer (0-10 cm) and it declined significantly ($P<0.01$) with the increase in soil depth. Three-way ANOVA revealed significant ($P<0.01$) differences in soil moisture content due to season, year and soil depth in the three forest stands. Moisture content was more during the rainy season and less during spring and winter. However, in the subsurface layer moisture content was maximum during winter and spring (Figure 4.2). The soil moisture content increased with the regrowth age.

Chemical properties: Cation exchange capacity (CEC) was least in the 7-year old stand (8.7 meq $100g^{-1}$) and it increased significantly in the 13- and 16-year old stands, where CEC values were 12.7 and 17.2 meq 100 g^{-1}, respectively (Figure 4.1b). There was little seasonal variation in the soil pH (Table 4.2) in all the three forest regrowths. The pH though not significantly different, was generally lower in the surface soil layer than the subsurface layer.

Soil organic carbon (SOC) was minimum during rainy season and maximum during autumn. The concentration of SOC declined with the increase in soil depth, whereas a reverse trend was observed with increasing stand age (Table 4.3). Total Kjeldahl nitrogen (TKN) did not vary markedly between seasons and years, but varied significantly ($P<0.01$) with soil depth and stand age. The concentration of TKN was higher in the surface soil layer which then gradually declined in the subsurface layer. Maximum concentration of TKN was observed in the 16-year old regrowth and minimum in the 7-year old regrowth (Table 4.4).

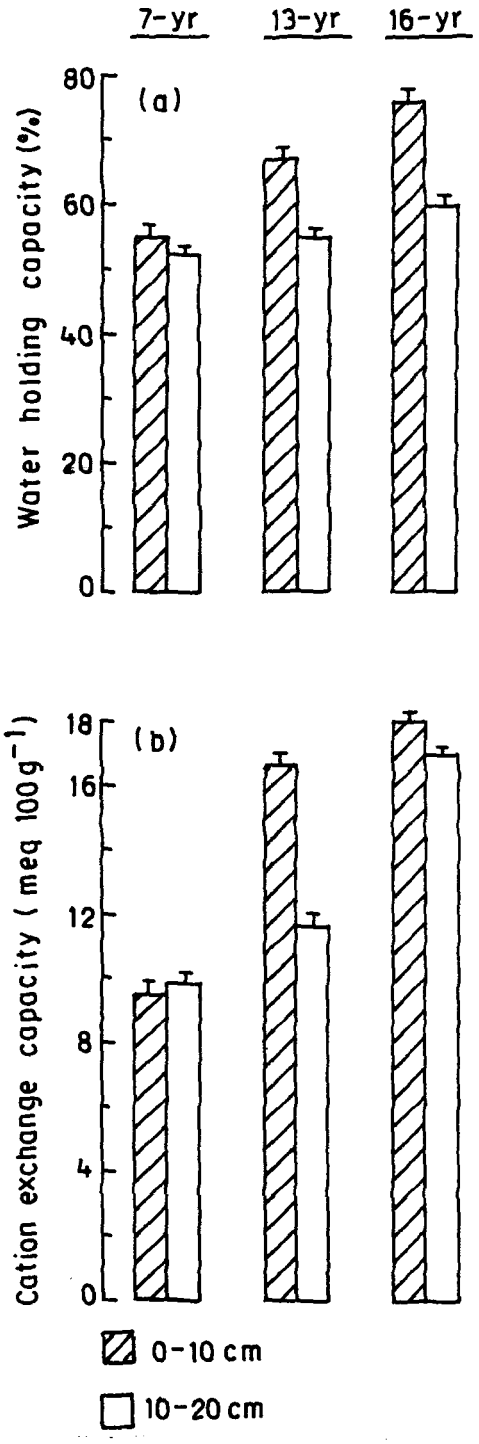

Figure 4.1: Water holding capacity and Cation exchange capacity of soils in two depths (▨ 0-10 and □ 10-20 cm) in forest regrowths of three different ages.

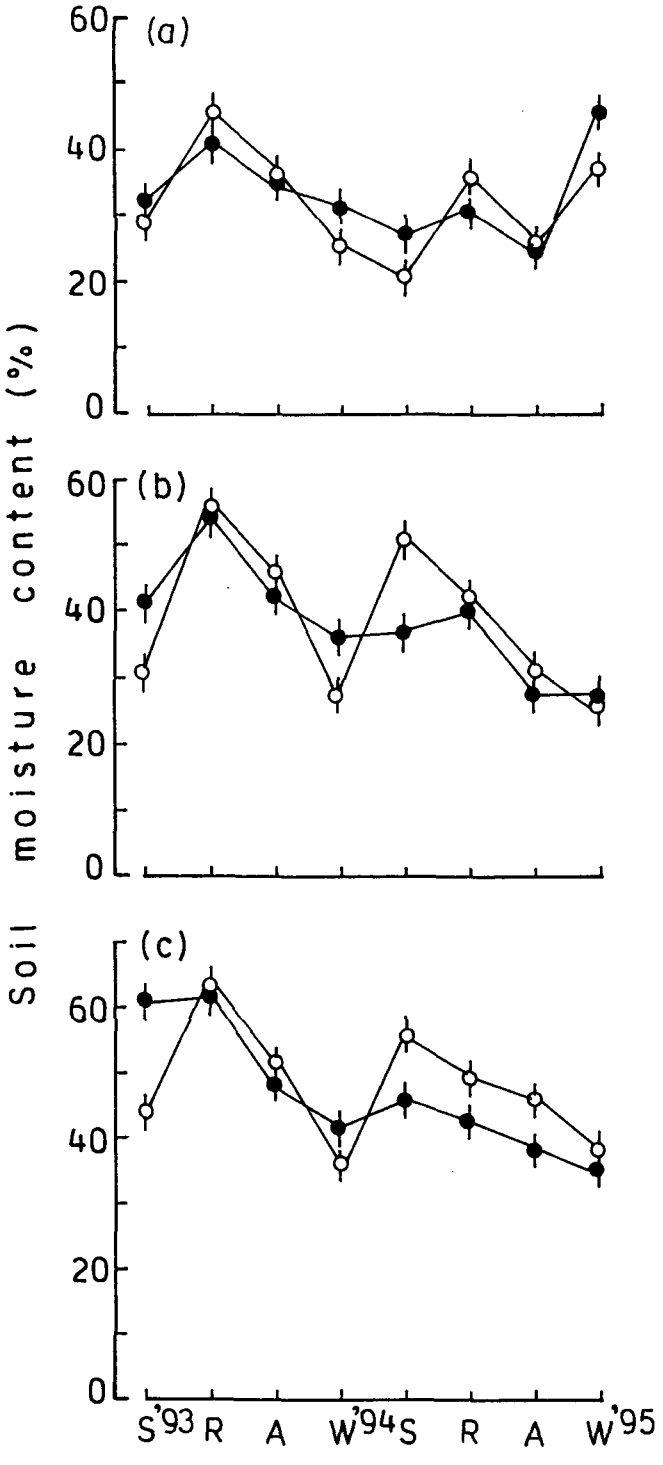

Figure 4.2: Seasonal variation in soil moisture content at two soil depths (o 0-10 and • 10-20 cm) in (a) 7, (b) 13 and (c) 16-year old regrowths. S-spring; R-rainy; A-autumn; W-winter.

Table 4.1. Texture and bulk density of soil in three forest regrowths.

Age of the forest regrowth	Soil Depth (cm)	Proportion of soil particles				Bulk density ($g\ cm^3$)
		Sand (%)	Silt (%)	Clay (%)	Textural class	
7-yr	0-10	75.25 ±0.25	14.74 ±0.47	10.11 ±0.14	SL	1.32 ±0.03
	10-20	68.04 ±0.05	16.18 ±0.13	15.87 ±0.07	SL	1.39 ±0.02
13-yr	0-10	53.51 ±0.46	23.32 ±0.39	23.26 ±0.23	SCL	1.41 ±0.01
	10-20	42.33 ±0.44	29.06 ±0.90	27.32 ±0.17	CL	1.43 ±0.01
16-yr	0-10	35.64 ±0.91	33.64 ±0.05	30.89 ±0.13	CL	1.42 ±0.02
	10-20	31.09 ±0.18	33.20 ±0.09	34.63 ±0.62	CL	1.46 ±0.01

SL-Sandy loam, SCL-Sandy clay loam, CL-Clay loam, C-Clay.

± SEM (n=3)

Table 4.2. Seasonal variation in soil pH in forest regrowths of three different ages.

Age of the forest regrowth	Soil depth (cm)	1993-94					1994-95				
		S	R	A	W	Mean	S	R	A	W	Mean
7-yr	0-10	5.41 ±0.01	5.15 ±0.05	5.25 ±0.01	5.35 ±0.04	5.29	5.45 ±0.01	5.17 ±0.03	5.33 ±0.02	5.47 ±0.15	5.36
	10-20	5.52 ±0.01	5.39 ±0.02	5.63 ±0.13	5.11 ±0.05	5.41	5.85 ±0.20	5.28 ±0.21	5.57 ±0.01	5.73 ±0.02	5.61
13-yr	0-10	5.05 ±0.05	4.72 ±0.02	4.41 ±0.05	5.24 ±0.12	4.86	5.21 ±0.01	5.20 ±0.05	5.18 ±0.21	5.42 ±0.01	5.25
	10-20	5.20 ±0.01	5.06 ±0.01	4.81 ±0.06	5.00 ±0.03	5.02	5.27 ±0.02	5.24 ±0.05	5.31 ±0.02	5.45 ±0.01	5.32
16-yr	0-10	5.17 ±0.01	4.44 ±0.03	4.50 ±0.05	5.68 ±0.06	4.95	4.80 ±0.01	5.18 ±0.05	5.14 ±0.01	5.06 ±0.01	5.05
	10-20	5.10 ±0.01	5.05 ±0.02	4.50 ±0.05	5.49 ±0.09	5.04	5.00 ±0.01	5.38 ±0.09	5.24 ±0.15	5.31 ±0.05	5.23

S-spring, W-winter, R-rainy, A-autumn

± SEM (n=3)

Table 4.3. Seasonal variation in soil organic carbon (%) in the three forest regrowths.

Age of the forest regrowth	Soil depth (cm)	1993-94					1994-95				
		S	R	A	W	Mean	S	R	A	W	Mean
7-yr	0-10	3.07 ±0.03	3.81 ±0.01	3.57 ±0.04	3.95 ±0.12	3.60	3.65 ±0.01	3.85 ±0.01	3.65 ±0.01	3.61 ±0.00	3.69
	10-20	2.59 ±0.26	2.73 ±0.02	2.70 ±0.01	2.94 ±0.03	2.74	2.95 ±0.00	2.94 ±0.02	2.61 ±0.00	2.91 ±0.04	2.85
13-yr	0-10	5.08 ±0.12	5.05 ±0.02	4.78 ±0.16	6.92 ±0.18	5.46	5.32 ±0.17	5.40 ±0.04	5.10 ±0.04	5.65 ±0.01	5.37
	10-20	3.04 ±0.33	3.60 ±0.04	3.34 ±0.01	3.75 ±0.61	3.43	3.72 ±0.01	3.89 ±0.03	3.80 ±0.17	3.92 ±0.01	3.83
16-yr	0-10	6.26 ±0.01	6.31 ±0.01	5.56 ±0.03	7.29 ±0.00	6.36	6.16 ±0.01	6.29 ±0.09	5.91 ±0.08	6.09 ±0.03	6.11
	10-20	5.96 ±0.04	6.25 ±0.02	5.53 ±0.01	6.54 ±0.07	6.07	6.10 ±0.01	6.19 ±0.08	5.91 ±0.01	6.03 ±0.03	6.06

S-spring, W-winter, R-rainy, A-autumn

± SEM (n=3)

Table 4.4. Seasonal variation in total Kjeldahl nitrogen (%) of soil in forest regrowths of three different ages.

Age of the forest regrowth	Soil depth (cm)	1993-94					1994-95				
		S	R	A	W	Mean	S	R	A	W	Mean
7-yr	0-10	0.34 ±0.01	0.35 ±0.01	0.38 ±0.02	0.36 ±0.01	0.36	0.35 ±0.02	0.36 ±0.00	0.37 ±0.00	0.39 ±0.01	0.37
	10-20	0.28 ±0.00	0.28 ±0.00	0.30 ±0.00	0.30 ±0.02	0.29	0.26 ±0.00	0.27 ±0.01	0.24 ±0.02	0.29 ±0.02	0.27
13-yr	0-10	0.47 ±0.01	0.49 ±0.00	0.51 ±0.01	0.50 ±0.00	0.49	0.47 ±0.01	0.49 ±0.00	0.50 ±0.01	0.51 ±0.02	0.49
	10-20	0.34 ±0.00	0.37 ±0.00	0.39 ±0.01	0.39 ±0.02	0.37	0.33 ±0.02	0.36 ±0.02	0.36 ±0.01	0.39 ±0.01	0.36
16-yr	0-10	0.56 ±0.01	0.61 ±0.01	0.61 ±0.01	0.59 ±0.00	0.59	0.57 ±0.01	0.60 ±0.03	0.60 ±0.02	0.60 ±0.01	0.59
	10-20	0.52 ±0.00	0.53 ±0.03	0.54 ±0.01	0.52 ±0.03	0.53	0.52 ±0.01	0.52 ±0.00	0.51 ±0.00	0.51 ±0.01	0.52

S-spring, W-winter, R-rainy, A-autumn

± SEM (n=3)

Concentration of available soil-P was maximum during rainy season and minimum during winter in all three regrowths. The concentration of available-P in soil increased gradually from the 7-year old to the 16-year old stand, however, it showed a declining trend with the increase in soil depth (Table 4.5).

The carbon to nitrogen (C/N) ratio in soil varied significantly ($P<0.01$) due to season, soil depth and stand age. The C/N ratio was higher in the surface soil layer of the 7- and 13-year old regrowths, while in the case of 16-year old regrowth it was greater in the subsurface layer. Though a lot of inconsistency was there, generally, the ratio was lower during rainy season.

BIOLOGICAL PROPERTIES OF SOIL

Seasonal and spatial variations in bacterial and fungal populations

In all the three regrowths bacterial population was maximum during rainy season ($6.08-13.82 \times 10^4$ g^{-1}) and minimum during winter ($5.92-11.28 \times 10^4$ g^{-1}). The population of bacteria was greater in the surface soil layer than the subsurface layer and increased significantly with the progression of vegetation recovery (Figure 4.3). Seasonality in fungal population was similar to that of bacteria, except that the maximum values were recorded during autumn (Figure 4.4). Fungal population also increased with the regrowth age, however, it showed a negative trend with the soil depth. The population of bacteria was higher than fungi in all the three forest regrowths (Table 4.6). The microbial population in general recorded greater values during the second year of the study.

Fungal species composition

Altogether 29 species of fungi were isolated in the three forest regrowths. In the 7-year old stand, *Trichoderma harzianum* and

Table 4.5. Seasonal variation in soil available phosphorus (µg g-1) in three forest regrowths.

Age of the forest regrowth	Soil depth (cm)	1993-94					1994-95				
		S	R	A	W	Mean	S	R	A	W	Mean
7-yr	0-10	2.66 ±0.03	7.64 ±0.59	8.15 ±0.51	5.15 ±0.49	5.90	4.88 ±0.08	6.15 ±0.15	10.17 ±0.11	10.89 ±0.09	8.02
	10-20	1.76 ±0.07	7.06 ±0.17	7.24 ±0.05	3.06 ±0.04	4.78	3.44 ±0.14	6.41 ±0.18	9.72 ±0.02	7.34 ±0.04	6.73
13-yr	0-10	10.30 ±0.06	11.94 ±0.35	12.11 ±0.53	6.61 ±0.03	10.24	14.09 ±0.41	13.44 ±1.37	14.70 ±0.12	12.78 ±0.21	13.75
	10-20	4.96 ±0.02	7.74 ±0.50	8.24 ±0.14	3.74 ±0.37	6.17	12.08 ±0.12	12.02 ±0.42	10.72 ±0.31	11.45 ±0.18	11.57
16-yr	0-10	10.13 ±0.03	11.23 ±0.09	11.28 ±0.14	7.01 ±0.06	9.91	15.36 ±0.84	18.98 ±0.50	19.90 ±0.03	19.60 ±0.20	18.46
	10-20	6.90 ±0.02	9.88 ±0.42	10.22 ±0.43	4.73 ±0.11	7.93	13.02 ±0.15	18.06 ±0.34	16.20 ±0.06	10.32 ±0.13	14.40

S-spring, W-winter, R-rainy, A-autumn

± SEM (n=3)

Table 4.6 Mean bacterial and fungal populations in soils of the forest regrowths of three different ages.

Microbial population	Soil depth (cm)	Age of the forest regrowth		
		7-year old	13-year old	16-year old
Bacteria*	0-10	8.31±0.60	11.29±0.35	12.62±0.28
	10-20	6.05±0.29	9.45±0.33	10.68±0.53
Fungi**	0-10	2.97±0.46	4.65±0.59	5.62±0.61
	10-20	1.99±0.39	2.58±0.55	3.23±0.56

*number of colonies x 10^4 g^{-1} dry weight of soil

**number of colonies x 10^3 g^{-1} dry weight of soil

±SEM (n=12)

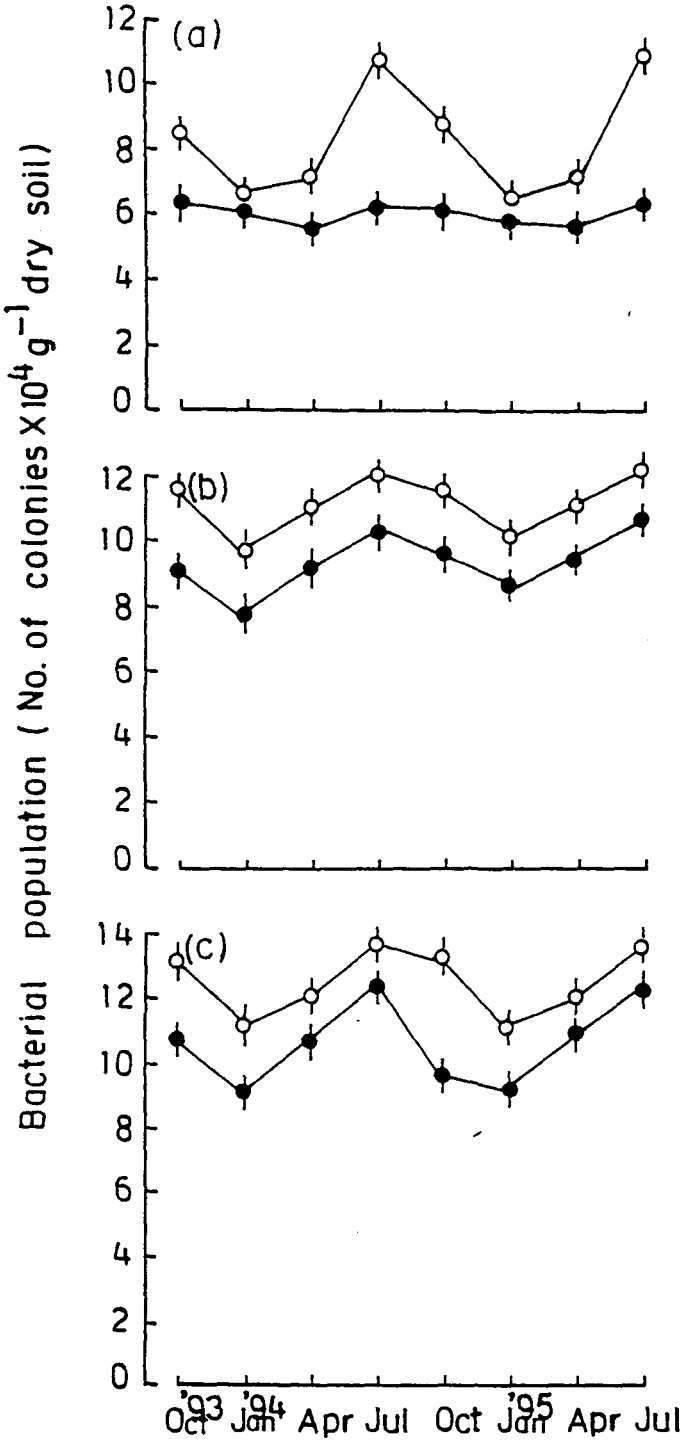

Figure 4.3: Seasonal variation in bacterial population in two soil depths (o 0-10 and • 10-20 cm) in (a) 7, (b) 13 and (c) 16-year old forest regrowths. Vertical bars represent standard error.

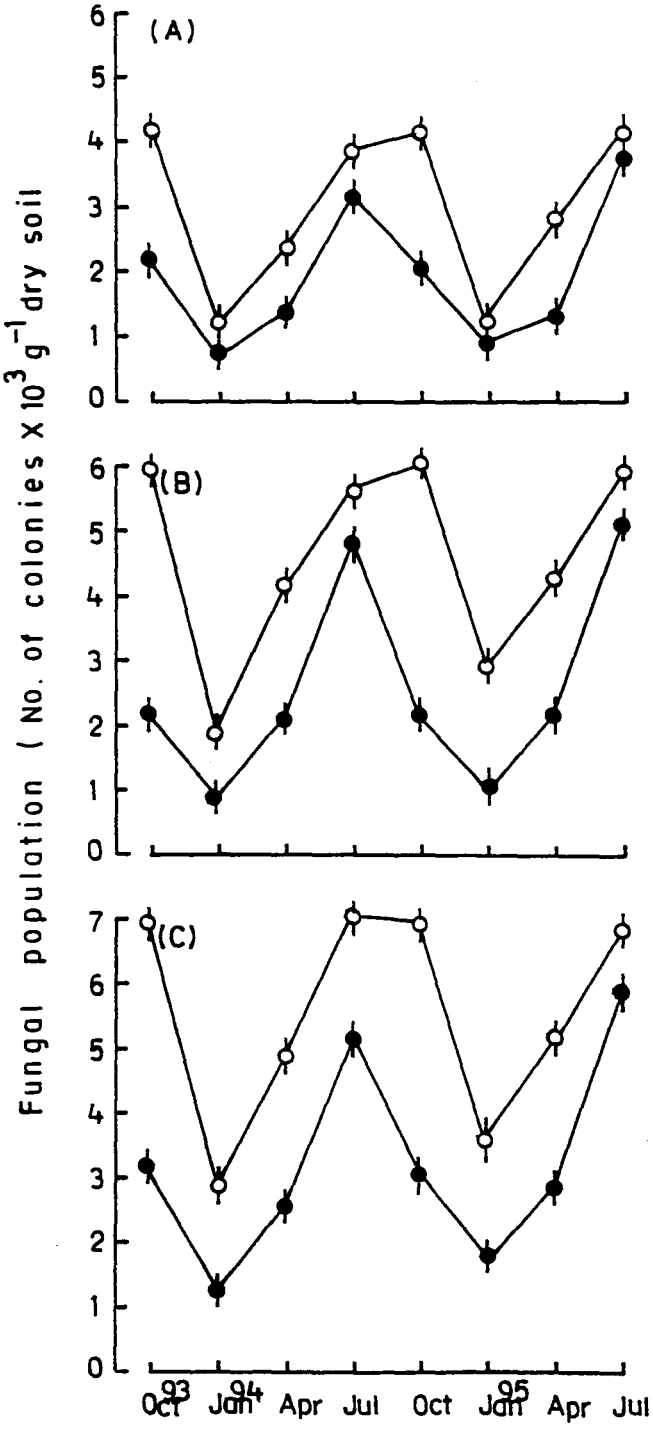

Figure 4.4: Seasonal variation in fungal population in two soil depths (o 0-10 and • 10-20 cm) in (a) 7, (b) 13 and (c) 16-year old forest regrowths. Vertical bars represent standard error.

Aspergillus flavus were dominant in the surface soil layer and in the subsurface soil layer *Penicillium chrysogenum* was dominant. *P. chrysogenum* had the highest relative abundance (9.19) value, followed by *Trichoderma viride* and *A. flavus* in the surface soil layer of the 13-year old regrowth, however, in the subsurface layer *Cladosporium* sp. was dominant. *T. viride* was the dominant fungus in both the soil depths of the 16-year old stand, the co-dominant species was *Rhizopus stolonifer* in the surface soil layer and *Mucor candida* in the subsurface layer (Table 4.7). In general, most of the fungal species isolated were found in all the three forest stands. However, some species were exclusively present in a particular stand. For example, *Aspergillus niger, Bromella* sp., *Penicillium vermiculatum, R. stolonifer, Humicola fuscoatra* and *Trichoderma* spp. were found only in the 16-year old stand, *Fusarium solani* was present exclusively in the 13-year old stand, and *Mucor heimalis, M. racemosus* and *Penicillum claviforme* were confined only to the 7-year old stand. Total number of fungal species increased from 17 in the 7-year old regrowth to 29 in the 16-year old regrowth.

The Shanon-Wiener diversity index increased with the age of the stand, 1.18-1.37 in the surface soil layer and 0.81-1.05 in the subsurface layer. Sorensen"s similarity index was less between 7- and 13-year old regrowths (63.4) than between 13- and 16-year old regrowths (86.8) (Table 4.8). Correlation of the diversity index by linear regression analysis with SOM yielded significant relationship in both 0-10 ($r=0.965$, $P<0.01$) and 10-20 ($r=0.820$, $P<0.01$) cm soil depth.

Table 4.1 Species composition and relative abundance of soil microfungi in forest regrowths of three different ages.

Species	Age of the forest regrowth/Soil depth (cm)					
	7-yr		13-yr		16-yr	
	0-10	10-20	0-10	10-20	0-10	10-20
Absidia glauca	-	-	1.15	-	1.82	-
Aspergillus flavus	10.41	19.05	8.05	12.90	5.45	8.51
Aspergillus fumigatus	8.51	-	3.45	-	1.82	-
Aspergillus compestris	2.13	-	3.45	6.45	1.82	-
Aspergillus niger	-	-	-	-	2.73	6.38
Aspergillus nedulans	-	-	4.59	-	0.91	-
Bromella sp.	-	-	-	-	3.63	-
Cladosporium sp.	-	-	5.75	19.35	2.73	-
Cylindrospora sp.	8.38	-	1.15	-	1.82	-
Fusarium moniliforme	-	-	1.15	-	1.82	-
Fusarium oxysporum	4.25	-	5.75	-	5.45	4.26
Fusarium solani	-	-	2.29	-	-	-
Humicola fuscoatra	-	-	-	-	1.82	-
Mucor candida	-	9.52	6.89	9.67	5.45	12.76
Mucor heimalis	4.25	-	-	-	-	-
Mucor mucodea	-	-	4.59	-	2.73	-
Mucor recemosus	8.38	9.52	-	-	-	-
Penicillium chrysogenum	8.38	23.80	9.19	16.12	7.27	10.63
Penicillium citrinum	8.38	19.05	4.59	9.67	1.92	10.63
Penicillium claviforme	8.51	-	-	-	-	-
Penicillium fellutatum	-	-	4.59	-	3.63	6.38
Penicillium luteum	-	-	2.29	-	1.82	
Penicillium notatum	4.25	-	4.59	-	6.36	
Penicillium rubrum	-	-	2.29	-	1.82	-
Penicillium vermiculatum	-	-	-	-	2.73	-
Pythium debaryanum	8.38	-	2.29	-	3.63	-
Rhizopus stolonifer	-	-	-	-	9.01	-
Rhizopus sp.	4.25	9.52	4.59	12.90	-	8.51
Trichoderma harzianum	10.41	9.52	5.75	-	5.45	6.38
Trichoderma koningii	8.51	-	2.29	-	3.63	-
Trichoderma viride	-	-	8.05	12.90	10.90	14.89
Trichoderma sp.	-	-	-	-	1.82	-
Verticillium sp.	4.25	-	1.15	-	1.82	4.26

Table4. Species content and diversity ($\bar{H}$) of soil microfungi in the three forest regrowths.

Variable	Soil depth (cm)	Age of the forest regrowth		
		7-yr	13-yr	16-yr
Species number	0-10	16	24	28
	10-20	7	8	12
$\bar{H}$	0-10	1.18	1.32	1.37
	10-20	0.81	0.88	1.05

RELATIONSHIP OF MICROBIAL POPULATION WITH MACROCLIMATE AND SOIL CHARACTERISTICS

Populations of bacteria and fungi at both soil depths showed positive correlation with rainfall ($P<0.05$). However, the relationship with air temperature was not significant. Both bacterial and fungal populations showed strong positive ($P<0.01$) correlations with density and basal area of the woody vegetation. Among the physical and chemical properties of the soil, clay content, bulk density, WHC, SMC, SOC, TKN and available-P showed significant positive correlations with bacterial and fungal populations, whereas the percentage sand particles showed negative correlation. Soil pH and temperature did not show significant relationship with the microbial population (Tables 4.9, 4.10).

DISCUSSION

CHANGES IN SOIL PROPERTIES

Canopy harvesting in forests results in, erosion of the top soil due to extreme rainfall events (Scholes *et al.* 1994). Loss of finer soil particles, especially the clay component increases the proportion of sand in the soil during the early developmental stages after disturbance (Eyre 1968). This could be one of the reasons for lower proportion of finer soil particles in the 7-year old stand than the 13- and 16-year old stands. A strong positive correlation ($r=0.665$, $P<0.05$) was observed between the clay percentage and bulk density. Similar finding has been reported by Scholes *et al.* (1994). Maximum retention of moisture in the surface soil layer might be due to greater accumulation of litter on the forest floor (Maithani *et al.* 1996b) and higher soil organic matter content in this layer.

Table 4.9. Correlation coefficients (r) for the relationship between mean (n=6) microbial population and physical properties of the soil.

Microbial population	Soil depth (cm)	Sand (%)	Clay (%)	BD (g cm^3)	WHC (%)	SMC (%)
Bacteria[1]	0-10	-0.978*	0.994**	0.986*	0.943*	0.992**
	10-20	-0.998**	0.999**	0.996*	0.896ns	0.999**
Fungi[2]	0-10	-0.989*	0.996**	0.963*	0.973*	0.999**
	10-20	-0.997**	0.984*	0.976*	0.824ns	0.994**

[1]number of colonies x 10^4 g^{-1} dry weight of soil

[2]number of colonies x 10^3 g^{-1} dry weight of soil

*, P<0.05; **, P<0.01; ns, not significant

BD-bulk density, WHC-water holding capacity, SMC-soil moisture content,

Table 4.10. Correlation coefficients (r) indicating the relationship between mean seasonal (n=8) microbial population and chemical properties of soil.

Microbial population	Soil depth (cm)	CEC (me $100g^{-1}$)	pH	SOM (%)	TKN (%)	Available-P ($\mu g\ g^{-1}$)
Bacteria[1]	0-10	0.995**	-0.260ns	0.670***	0.771***	0.635***
	10-20	0.896ns	-0.282ns	0.681***	0.725***	0.609**
Fungi[2]	0-10	0.973*	-0.176ns	0.699***	0.793***	0.595**
	10-20	0.824ns	-0.225ns	0.603**	0.626***	0.495*

[1]number of colonies x 10^4 g^{-1} dry weight of soil

[2]number of colonies x 10^3 g^{-1} dry weight of soil

*, P<0.05; **, P<0.005; ***, P<0.001; ns, not significant

CEC-cation exchange capacity, SOM-soil organic matter, TKN-total Kjeldahl nitrogen

CEC was negatively correlated (r=-0.0.831, P<0.01) with the percentage of sand particles and positively correlated (r=0.822, P<0.01) with the clay content of the soil. This explains the low CEC in the 7-year old stand and high CEC in the older stands. This corroborates the findings of Scholes *et al.* (1994) who found a linear relationship between clay particles and CEC. The declining trend in soil pH with stand age is understandable as the organic matter content and nutrient availability increased with the progression of forest regrowth.

According to Odum (1960) and Aweto (1981), the organic matter content in the top soil approaches to the level of mature forest by the end of the tenth year of secondary succession in the forest ecosystems. In the present study, SOM, TKN and available-P in the 16-year stand is comparable to the values reported by Das *et al.* (Unpublished data) from a mature virgin forest of this region.

MICROBIAL POPULATION DYNAMICS

Soil microbial population varied both seasonally and annually in all forest regrowths. The low microbial population during winter could be due to prevailing low temperature and greater physiological water stress which are conducive to microbial growth and activity. The peak in bacterial population during rainy season in both soil depths could be due to favourable soil moisture and temperature conditions during those periods. In this respect, the present results are different from Jha *et al.* (1992b) who recorded higher bacterial population during spring and autumn. However, fungi recorded their highest population during autumn due to prevailing favourable moisture and temperature conditions. The relatively low population of fungi during rainy season could be linked to run-off losses of fungal propagules along with the

plant materials from the hill slope due to heavy rainfall in the region. The higher population of bacteria and fungi in the surface soil layer than the subsurface layer may be ascribed to many factors such as variation in nutrient status at different depths (Balasubramanium *et al.* 1972) and moisture regime (Selvaraj & Rangaswamy 1978). A strong positive correlation between SOM, TKN and available-P with microbial population (Table 4.9) could well explain the depthwise variation in bacterial and fungal population in the regrowing forest communities. The higher counts of bacteria and fungi in the 13- and 16-year old regrowths compared to the 7-year old regrowth may be attributed to dense growth of plants and to greater availability of nutrients on account of greater accumulation of litter (Maithani *et al.* 1996b). The greater microbial biomass in the older regrowths (Chapter 5) may also have contributed to the higher microbial population in the 13- and 16-year old regrowths. The extent of change in fungal species composition was more from the 7-year old to the 13-year old regrowth, than from the 13-year old to the 16-year old stand. This may be attributed to the more rapid changes in canopy cover and soil nutrient status in the early stages of succession.

FUNGAL SPECIES DIVERSITY

The rate of change in the fungal species composition was more from the 7-year old to the 13-year old forest regrowth after which, the rate slowed down. This may be attributed to the changes in vegetation cover at the sites and to variation in soil nutrient status (Mishra & Sharma 1977). Jha *et al.* (1992b) pointed out that for a given community one or few species are numerically dominant. The results obtained fully corroborate this point, as three species of fungi, *P. chrysogenum, P. citrinum, A.flavus* and *T. viride* were

dominant in the regrowing forest soils. Nevertheless, majority of the fungi were common to all the three regrowths indicating their widal ecological amplitude. In the three forest regrowths, the genus *Penicillium* was represented by 8 species, *Aspergillus* by 15 species and *Mucor* and *Trichoderma* by 4 species each. The greater species diversity of the genus *Penicillium* and *Aspergillus* could be due in part to their greater spore production and dispersion, and partly, due to the resistance over extreme environmental conditions (Schimel 1995).

The study of soil properties revealed that clay content, WHC, SMC and the level of SOC, TKN and available-P increased with the increase in the age of the stand. The study also reveals that the population of bacteria and fungi showed a gradual increase with the progression of vegetation recovery following disturbance. Further, higher species diversity of fungi in the soil of the 16-year old regrowth than the other two younger regrowths signals a relatively stable environment in the former.

CHAPTER 5

MICROBIAL BIOMASS C, N AND P DYNAMICS

INTRODUCTION

Microbial biomass, which represents an important labile pool of nutrients in soil (Jenkinson & Ladd 1981, Singh *et al.* 1989, Henrot & Robertson 1994) plays a significant role in nutrient transformation and conservation processes in grassland, forest and cropland ecosystems (Sarathchandra *et al.* 1984, Alef *et al.* 1988, Srivastava & Singh 1989, Luizao *et al.* 1992, Bolton *et al.* 1993) in both tropical and temperate climates. Many factors such as temperature, moisture content, clay content and pH are known to affect microbial biomass in soil (Carter 1986, Kaiser *et al.* 1992, Gestel *et al.* 1993, Nicojardot *et al.* 1994). Changes in the size of the microbial biomass pool may also indicate changes in soil organic matter pool that are not otherwise easily detectable.

Although studies emphasizing the role of microbial biomass in soil organic matter and nutrient dynamics during secondary succession have been carried out in degraded forest ecosystems on the Piedmont of North Carolina (Christensen & MacAller 1985) and in San Diego County, Southern California (Fenn *et al.* 1993), the changes that occur during regrowth of disturbed forest are not fully understood.

A marked seasonal cycle of microbial biomass has been reported

for both tropical and temperate forest soils (Singh *et al.* 1989, Diaz-Ravina *et al.* 1995). Whereas, Ross *et al.* (1981) reported large annual fluctuations in soil microbial biomass, Patra *et al.* (1990) observed only small annual changes. A few recent studies (Srivastava & Singh 1991, Diaz-Ravina *et al.* 1995) have highlighted the influence of land use and soil physico-chemical properties on microbial biomass.

The high altitude (1000-2000 m asl) subtropical humid forests of north-east India are often disturbed by clear cutting or selective felling of trees to meet the fuel and timber requirements of the local inhabitants or for purposes of shifting cultivation. In many instances, the disturbed areas are allowed to undergo natural recovery of vegetation for 5 to 20 years depending on population pressure and land availability. Study of microbial biomass in soil along a chronosequence of vegetation regrowth in these disturbed sites may give insights into the role of microbes in restoring soil fertility during secondary succession. The present chapter analyses temporal and spatial variations in microbial biomass C, N and P and their contributions to soil nutrient pools in the three forest regrowths.

METHODS

The study period covered two annual cycles starting from the spring season during 1993 in the 7-, 13- and 16-year old forest regrowths. Soil was sampled from two depths (0-10 and 10-20 cm) during spring (April), rainy (July), autumn (October) and winter (January) seasons. Ten randomly collected soil cores (6.5 cm diameter) were obtained from each stand and bulked to form composite sample. The soil was sieved through 2 mm mesh screen and was used in field moist

condition for the determination of microbial biomass C, N and P.

MICROBIAL BIOMASS C

Microbial biomass C was estimated by chloroform fumigation incubaton (FI) method of Jenkinson & Powlson (1976) with a minor modification as suggested by Srivastava & Singh (1988). The soils were pre-incubated for 7 days at room temperature to settle down the microbial activity. 100 g of field moist soil samples were fumigated with alcohol-free liquid chloroform for 24 h and then evacuated to remove the chloroform vapours. The soil samples were then adjusted to 60% of water holding capacity and transferred to rectangular glass jars along with two beakers, one containing 20 ml. deionized water to prevent soil drying during incubation and another containing 50 ml 1 N NaOH 1 g of unfumigated soils were then added and jars were incubated at 25±1°C for 10 days. After 10 days, the residual alkali was titrated against 1 N HCL to find out the amount of CO_2 evolved in the fumigated soil samples (F_c). The same soils were further incubated for the next 10-20 days. The titrated values for the next 10-20 days of incubation were treated as control (UF_c) as suggested by Merckx *et al.* (1985). Flush of CO_2 during fumigation was calculated by subtracting the UF_c from F_c, and microbial biomass C was calculated by dividing the flush of CO_2 by a K_c factor of 0.45 (Jenkinson & Ladd 1981).

Microbial biomass C = $F_c - UF_c/K_c$, where, K_c is the fraction of carbon mineralized during first 10 days.

MICROBIAL BIOMASS N

Microbial biomass N was estimated by chloroform fumigation-extraction method proposed by Brookes *et al.* (1985). 50 g of field moist unfumigated soil samples were extracted for 30 minutes at 150

r.p.m. with 200 ml of 0.5 M K_2SO_4 (soil and solution in the ratio of 1:4). Simultaneously, 50 g of field moist soils were fumigated with alcohol-free liquid chloroform for 24 h at 25± 1° C. After removing alcohol vapours completely the soil samples were extracted with 200 ml of 0.5 M K_2SO_4 in the manner described above. The extracts were filtered through Whatman No. 1 and aliquots were analysed for total N using 1030 Kjeltec autoanalyzer. The flush of total N (K_2SO_4-extractable N in unfumigated soil subtracted from that in the fumigated soil) was divided by K_N value of 0.54 as proposed by Brookes *et al.* (1985).

Microbial biomass N = Flush of decomposition of total N/K_N,

where, K_N is the fraction of biomass N mineralized and extracted after chloroform fumigation.

MICROBIAL BIOMASS P

Microbial biomass P was determined through chloroform fumigation extraction method of Brookes *et al.* (1982). Field moist samples of 10 g each were taken in the conical flask (250 ml) and extracted for 20 minutes at 120 r.p.m. with 200 ml 0.5 M $NaHCO_3$ adjusted to 8.5 pH. Simultaneously, the 10 g of soil samples were fumigated with alcohol-free liquid chloroform and incubated for 24 h at 25°C . After removal of chloroform vapours the soil samples were extracted similarly as the unfumigated soils. The resultant solution was filtered through Whatnman NO. 42. The neutralised aliquots of soil extracts were analyzed for inorganic P using ammonium-molybdenum blue method (Allen *et al.* 1974).

The microbial biomass P was calculated as b-a/0.40, where, "a" is the amount of inorganic P (µg g^{-1}) extracted from unfumigated soil

and "b" is the inorganic P (μg g^{-1}) extracted from fumigated soil, 0.40 is the fraction of biomass P mineralised and extracted in 0.5 M $NaHCO_3$. correction for P fixation wwas made by adding a known quantity of Pi (as KH_2PO) during the $NaHCO_3$ extraction stage and measuring the recovery of added P.

The data presented are the means of three replicate determinations and have been expressed on an oven-dry weight basis (24 h at 105°C).

STATISTICAL ANALYSIS

The data collected from each of the three stand were analysed using three-way ANOVA (fixed effects model) to test whether the variations due to soil depth, season and year were statistically significant or not. Tukey's test was carried out to compare the mean values of microbial biomass C, N and P between stands. The percentage of microbial C, N and P were transformed using Arcsine transformation (Zar, 1974). Correlation analysis was used following Zar (1974) to study the relationships between microbial biomass C, N and P and some edaphic variables.

RESULTS

TEMPORAL AND SPATIAL VARIATION IN MICROBIAL BIOMASS C

The microbial biomass C in soil differed significantly ($P<0.01$) by season, year, soil depth and regrowth age. The concentration of microbial biomass C ranged from 165-507, 425-835 and 489-1682 ug g^{-1} for 7-, 13- and 16-year old regrowths, respectively. In the surface soil layer (0-10 cm) microbial biomass C values peaked during the winter and gradually declined through the spring to its minimum level in the rainy and autumn in the three regrowths. The seasonal trend in

the subsurface soil layer was not consistent in the 7- and 13-year old regrowths. However, in the 16-year old regrowth the subsurface soil layer showed the similar seasonality as the surface layer (Figure 5.1).

In general microbial biomass C was higher during the second year of the study. The microbial biomass C increased by two fold from 7- to 13-year old regrowth, and it again showed a significant increase from 13- to 16-year old regrowth (Table 5.1).

TEMPORAL AND SPATIAL VARIATION IN MICROBIAL BIOMASS N

The microbial biomass N values ranged between 38-86, 52-144 and 66-206 $\mu g\ g^{-1}$ in the surface soil layer of 7-, 13- and 16-year old regrowths, respectively (Figure 5.2). In the subsurface layer concentration of microbial biomass N was significantly ($P<0.01$) lower than the surface layer (16-50, 24-104 and 40-126 $\mu g\ g^{-1}$) in the three forest regrowths.. The seasonal trend observed in microbial biomass N was similar to that of carbon with its peak during winter and trough during rainy season. In the subsurface soil layer also the similar seasonality was recorded in both 7- and 13-year old regrowths. However, in the subsurface layer of 16-year old stand the lowest biomass N during the second year of the study was recorded in the spring season. In all the three forest regrowths the microbial biomass N was significantly higher during the second year of the study than the first year.

There was about 35% increase in the concentration of microbial biomass N from 7- to 13-year old forest regrowth and about 28% from 13- to 16-year old regrowth (Table 5.1).

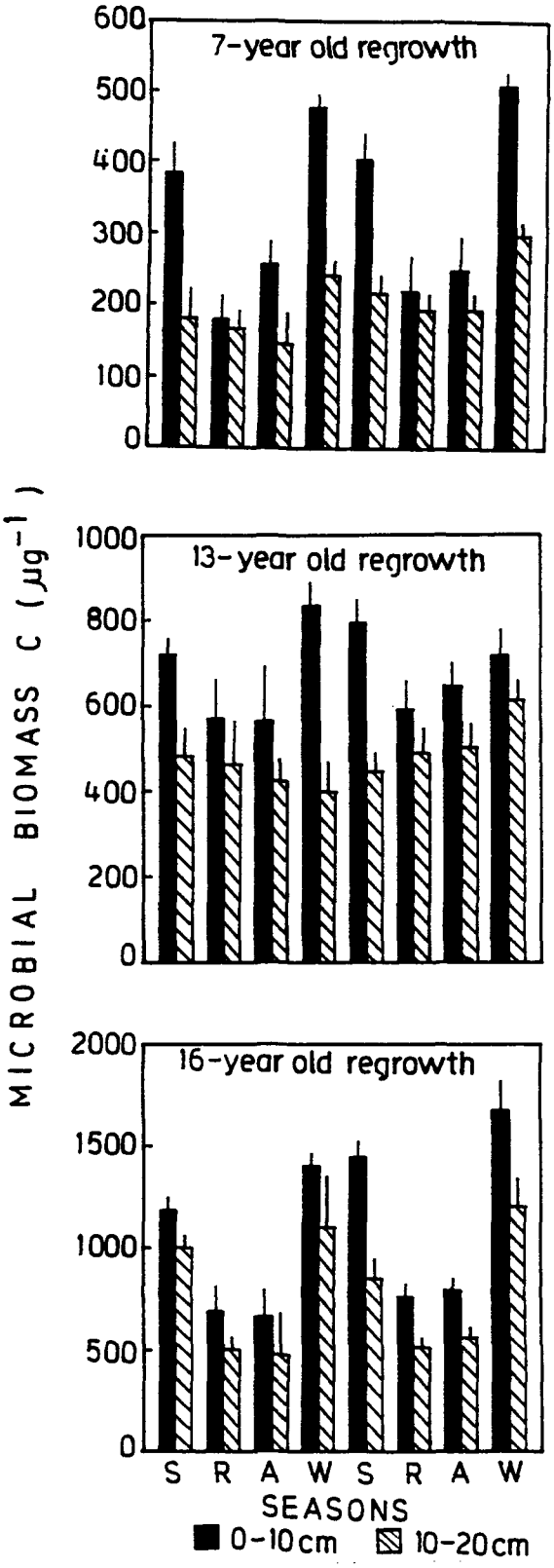

Figure 5.1: Seasonal variation in microbial biomass C ($\mu g\ g^{-1}$) in the three forest regrowths. S-spring; R-rainy; A-autumn; W-winter. Vertical bars represent standard error.

Table 5.1. Microbial biomass C, N and P ($\mu g\ g^{-1}$) in forest regrowths of three different ages and their contribution (%) to the soil organic-C, soil-N and soil-P. (Values are the means of four seasons across two years, n=24)

Age of the forest regrowth (years)	Soil depth (cm)	Microbial-C		Microbial-N		Microbial-P		Microbial C/N	Microbial C/P
		$\mu g\ g^{-1}$	% of Organic-C	$\mu g\ g^{-1}$	% of total N	$\mu g\ g^{-1}$	% of total P		
7	0-10	333.74^{a}	0.92 $(5.50)^{a}$	57.70^{a}	1.60 $(7.26)^{a}$	18.35^{a}	4.78 $(12.62)^{a}$	5.7^{a}	$20.0^{a,c}$
	10-20	203.62^{d}	0.73 $(4.90)^{d}$	37.79^{d}	1.13 $(6.10)^{d}$	11.59^{c}	2.76 $(9.56)^{d}$	6.4^{d}	20.7^{d}
13	0-10	680.69^{b}	1.23 $(6.36)^{a,c}$	88.63^{b}	1.81 $(7.73)^{a}$	25.19^{a}	5.03 $(12.96)^{a}$	8.2^{b}	31.5^{b}
	10-20	478.59^{e}	1.33 $(6.62)^{e}$	47.19^{d}	1.31 $(6.57)^{d}$	16.94^{c}	4.62 $(12.41)^{d}$	12.1^{e}	35.7^{e}
16	0-10	1087.70^{c}	1.74 $(7.57)^{b,c}$	123.85^{c}	2.10 $(8.33)^{a}$	42.19^{b}	7.90 $(16.32)^{a}$	9.0^{b}	$26.4^{b,c}$
	10-20	785.64^{f}	1.29 $(6.52)^{e}$	70.07^{e}	1.33 $(6.62)^{d}$	24.85^{d}	6.65 $(14.94)^{d}$	11.6^{e}	35.5^{e}

Note: Values with similar superscripts for a particular soil depth are not significant at $P<0.05$ between stands. Transformed values ('Y') for percentages are given in parentheses.

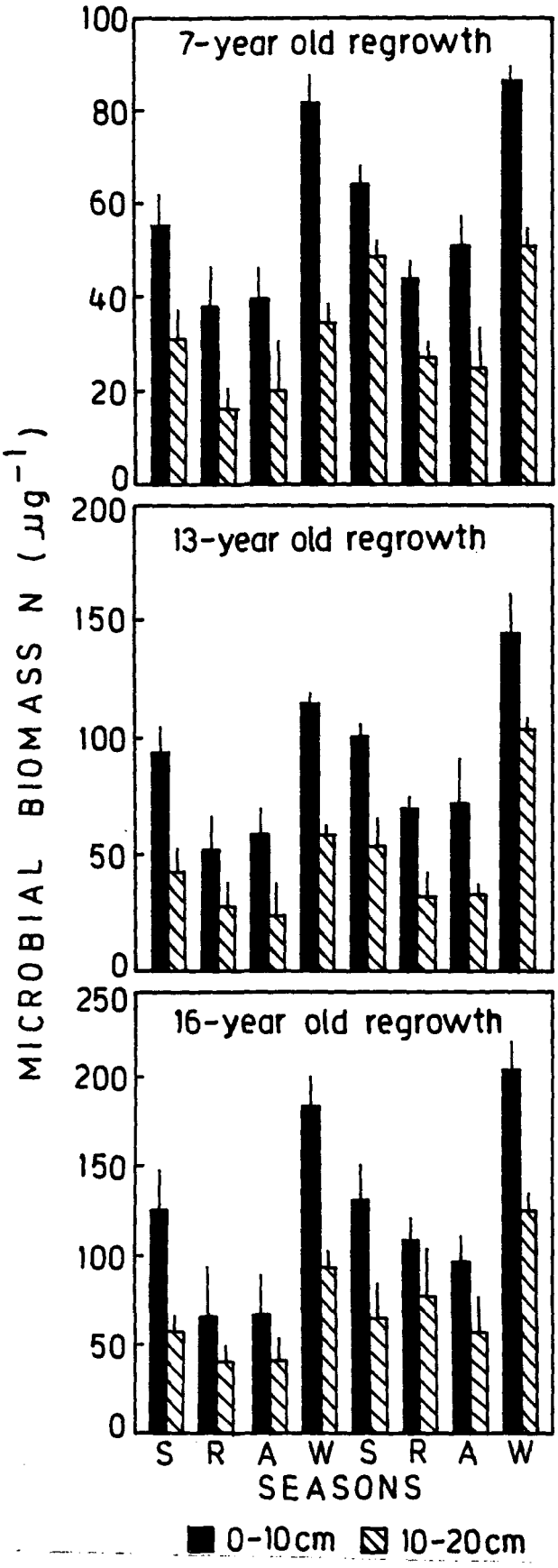

Figure 5.2: Seasonal variation in microbial biomass N (μg g^{-1}) in the three forest regrowths. S-spring; R-rainy; A-autumn; W-winter. Vertical bars represent standard error.

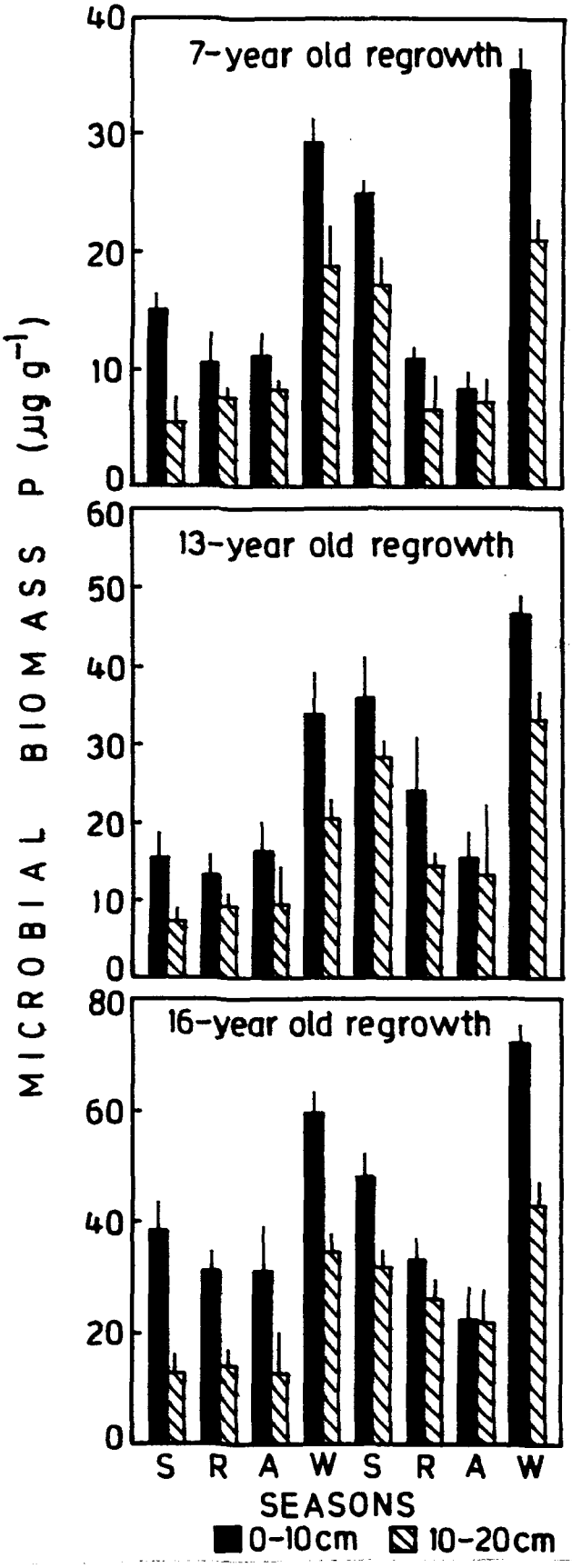

Figure 5.3: Seasonal variation in microbial biomass P (µg g^{-1}) in the three forest regrowths. S-spring; R-rainy; A-autumn; W-winter. Vertical bars represent standard error.

TEMPORAL AND SPATIAL VARIATION IN MICROBIAL BIOMASS P

The concentration of microbial biomass P in the present study ranged from 5-35, 7-46 and 13-72 $\mu g\ g^{-1}$ in the 7-, 13- and 16-year old forest regrowths, respectively. No significant differences in the microbial biomass P values were observed between 7- and 13-year old regrowth, however, the biomass P values showed a significant increase from 13- to 16-year old forest regrowth. The seasonality recorded in the microbial biomass P in the surface soil layer was similar to that of Microbial biomass C and N. In the subsurface layer the peak was observed during winter and the values recorded during spring, autumn and rainy season generally did not show significant variations (Figure 5.3). The concentrations of microbial biomass P recorded during the second year of the study were significantly ($P<0.05$) higher than the previous year.

TEMPORAL AND SPATIAL VARIATIONS IN MICROBIAL C/N and C/P

Microbial C/N ratios were lower in the 7-year old regrowth than 13- and 16-year old forest regrowths. The ratio ranged 4.5-10.1, 4.9-17.7, 6.8-12.7 for 7-, 13- and 16-year old regrowths, respectively (Table 5.2). The ratios were generally lower in the upper soil depth than in the lower depth in all the regrowths. Seasonality in microbial C/N was not consistent. The surface soil layer of 7-year old regrowth though had its minimum C/N ratio during rainy season, the seasonal variation was not significant. In the subsurface layer minimum C/N ratios were recorded during spring and maximum during rainy season. In the 13- and 16-year old regrowths minimum C/N ratios were recorded during winter, while the maximum was observed during rainy season in

Table 5.2. Seasonal variation in microbial C/N in the three forest regrowths during 1993 and 1994.

Age of the forest regrowth	Soil depth (cm)	Spring 1993	Spring 1994	Rainy 1993	Rainy 1994	Autumn 1993	Autumn 1994	Winter 1993	Winter 1994
7-yr old	0-10	6.90 ±0.83	6.25 ±1.01	4.65 ±0.32	4.95 ±0.83	6.43 ±0.61	4.87 ±0.26	5.81 ±1.09	5.84 ±0.83
	10-20	5.72 ±0.91	4.45 ±1.08	10.08 ±1.16	7.09 ±1.03	7.19 ±1.01	7.67 ±0.92	6.91 ±0.32	5.83 ±0.27
13-yr old	0-10	7.66 ±1.31	7.85 ±0.97	10.91 ±1.87	8.43 ±0.86	9.56 ±1.13	8.92 ±0.78	7.27 ±0.83	4.95 ±0.26
	10-20	11.32 ±1.21	8.34 ±1.76	16.49 ±0.93	14.90 ±1.03	17.68 ±1.32	15.15 ±1.14	6.85 ±0.76	5.88 ±1.08
16-yr old	0-10	9.44 ±0.56	11.00 ±1.71	10.66 ±0.43	7.08 ±0.32	9.96 ±0.61	8.27 ±0.39	7.61 ±1.12	8.16 ±1.03
	10-20	17.47 ±1.38	13.50 ±1.16	12.72 ±0.83	6.79 ±0.81	11.76 ±0.32	9.87 ±1.01	11.87 ±0.39	9.56 ±0.06

± Standard error
n=3

13-year old regrowth and during spring in 16-year old regrowth (Table 5.2).

The microbial C/P ratios also showed more or less similar spatial and temporal trends as microbial C/N. The C/P ratios were lower in the 7-year old stand which showed significant increase ($P<0.05$) in the 13-year old stand and again declined in the 16-year old stand. Generally, the higher microbial C/P ratios were observed in the subsurface soil layer, except during winter in the 7- and 13-year old regrowths when the ratios were lower in the subsurface layer than the surface soil layer (Table 5.3).

CONTRIBUTION OF MICROBIAL C, N AND P TO SOIL NUTRIENT POOL

The percent contribution of microbial biomass C to total soil organic-C (SOC) ranged from 0.49-1.65. 0.81-2.02 and 0.74-2.68 in the 7-, 13- and 16-year old regrowths, respectively. Generally, the contribution of microbial biomass C to total soil organic-C was higher in the top soil layer (0-10 cm), except during the rainy and autumn seasons when no variation was observed between the two depths (Table 5.4).

The percentage contribution of microbial biomass N to total Kjeldahl nitrogen (TKN) and microbial biomass P to total soil P was 1.1-2.1 and 2.8-7.9, respectively (Table 5.5, 5.6). The proportion of these nutrients was higher in the surface soil layer than the subsurface layer. In general, the proportion of microbial biomass C, N and P in soil nutrient pool increased with the age of the regrowth (Table 5.1).

Table 5.3. Seasonal variation in microbial C/P ratios in the forest regrowths during 1993 and 1994.

Age of the forest regrowth	Soil depth (cm)	Spring		Rainy		Autumn		Winter	
		1993	1994	1993	1994	1993	1994	1993	1994
7-yr old	0-10	25.23 ±1.32	16.06 ±1.01	16.71 ±1.12	19.79 ±2.31	22.59 ±1.86	29.43 ±2.08	16.17 ±1.70	14.20 ±0.86
	10-20	32.53 ±2.01	12.59 ±1.12	21.46 ±1.08	28.66 ±2.36	17.53 ±1.17	26.06 ±3.36	12.77 ±1.18	14.10 ±0.92
13-yr old	0-10	46.22 ±3.81	21.99 ±1.01	43.05 ±4.86	24.61 ±2.83	34.40 ±2.39	41.57 ±2.94	24.69 ±1.86	15.37 ±1.32
	10-20	65.56 ±4.72	15.79 ±0.87	49.29 ±2.67	34.04 ±2.42	44.77 ±2.87	37.97 ±3.92	19.59 ±2.96	18.49 ±1.32
16 -yr old	0-10	30.94 ±2.01	30.13 ±1.19	22.32 ±0.76	23.29 ±1.39	21.42 ±0.68	35.88 ±3.16	23.61 ±0.92	23.25 ±2.37
	10-20	78.11 ±2.96	26.84 ±3.21	35.53 ±4.87	20.09 ±1.46	37.74 ±2.31	25.66 ±1.53	31.94 ±1.77	28.02 ±2.67

± Standard error
n=3

Table 5.4. Seasonal variation in percentage contribution of microbial biomass C to total soil organic C in the three forest regrowths.

Age of the forest regrowth	Soil depth (cm)	Spring 1993	Spring 1994	Rainy 1993	Rainy 1994	Autumn 1993	Autumn 1994	Winter 1993	Winter 1994
7-yr old	0-10	1.00 ±0.02	0.59 ±0.08	0.49 ±0.11	0.59 ±0.16	0.64 ±0.08	0.69 ±0.09	1.30 ±0.11	1.65 ±0.12
	10-20	0.65 ±0.06	0.73 ±0.01	0.61 ±0.11	0.73 ±0.18	0.49 ±0.18	0.66 ±0.12	0.81 ±0.11	1.18 ±0.12
13-yr old	0-10	1.42 ±0.16	1.46 ±0.18	1.19 ±0.18	1.15 ±0.08	0.81 ±0.16	1.14 ±0.16	1.57 ±0.12	1.41 ±0.12
	10-20	1.34 ±0.87	1.15 ±0.16	1.38 ±0.12	1.28 ±0.16	1.13 ±0.09	1.29 ±0.06	1.08 ±0.09	2.02 ±0.16
16-yr old	0-10	1.88 ±0.21	2.31 ±0.21	1.26 ±0.19	1.31 ±0.16	0.92 ±0.10	1.33 ±0.15	2.28 ±0.23	2.68 ±0.13
	10-20	1.61 ±0.08	1.39 ±0.11	0.96 ±0.88	0.89 ±0.09	0.74 ±0.08	0.94 ±0.03	1.82 ±0.12	2.02 ±0.15

± Standard error.
n=3

Table 5.5. Seasonal variation in percentage contribution of microbial biomass N to total total Kjeldahl nitrogen in the three forest regrowths.

Age of the forest regrowth	Soil depth (cm)	Spring 1993	Spring 1994	Rainy 1993	Rainy 1994	Autumn 1993	Autumn 1994	Winter 1993	Winter 1994
7-yr old	0-10	1.57 ±1.02	1.78 ±0.06	0.99 ±0.02	1.19 ±0.11	1.09 ±0.12	1.31 ±0.08	2.33 ±0.13	2.55 ±0.16
	10-20	1.11 ±0.03	1.61 ±0.02	0.54 ±0.11	1.12 ±0.13	0.67 ±0.09	0.86 ±0.01	1.13 ±0.21	1.82 ±0.20
13-yr old	0-10	1.93 ±0.18	2.06 ±0.19	1.02 ±0.08	1.40 ±0.01	1.18 ±0.13	1.42 ±0.16	2.43 ±0.09	3.09 ±0.28
	10-20	1.16 ±0.09	1.48 ±0.09	0.71 ±0.03	0.91 ±0.08	0.61 ±0.03	0.84 ±0.07	1.76 ±0.25	3.04 ±0.29
16-yr old	0-10	2.07 ±0.16	2.20 ±0.17	1.07 ±0.13	1.82 ±0.26	1.12 ±0.11	1.63 ±0.17	3.23 ±0.18	3.69 ±0.31
	10-20	1.08 ±0.18	1.26 ±0.18	0.74 ±0.09	1.51 ±0.03	0.80 ±0.03	1.13 ±0.12	1.80 ±0.11	2.41 ±0.19

± Standard error.
n=3

Table 5.6. Seasonal variation in percent contribution of microbial biomass P to total soil phosphorus in the three forest regrowths.

Age of the forest regrowth	Soil depth (cm)	Spring 1993	Spring 1994	Rainy 1993	Rainy 1994	Autumn 1993	Autumn 1994	Winter 1993	Winter 1994
7-yr old	0-10	6.08 ±0.13	7.59 ±0.38	2.13 ±0.31	2.70 ±0.42	2.68 ±0.11	2.65 ±0.36	6.81 ±1.03	7.70 ±0.86
	10-20	1.51 ±0.38	5.50 ±0.61	1.18 ±0.81	1.63 ±0.39	1.61 ±0.41	1.80 ±0.32	4.51 ±0.33	4.40 ±0.28
13-yr old	0-10	4.28 ±0.70	7.68 ±0.52	2.10 ±0.83	4.70 ±0.72	2.23 ±0.33	3.73 ±0.18	7.11 ±0.16	8.41 ±0.13
	10-20	2.24 ±0.36	7.66 ±0.83	1.45 ±0.13	2.30 ±0.09	2.00 ±0.18	3.39 ±0.38	6.39 ±0.82	11.11 ±1.21
16-yr old	0-10	8.98 ±1.16	12.15 ±1.01	3.18 ±0.08	8.07 ±0.85	3.54 ±0.29	5.66 ±0.77	8.97 ±0.83	12.72 ±1.16
	10-20	3.39 ±0.46	7.76 ±0.77	1.34 ±0.06	6.67 ±0.57	2.19 ±0.18	5.68 ±0.84	9.62 ±1.32	16.60 ±1.15

± Standard error.
n=3

RELATIONSHIP BETWEEN MICROBIAL C, N AND P WITH SOIL PROPERTIES

All the three variables *viz.* microbial biomass C, N and P showed significant ($P<0.01$) negative correlation with soil temperature and pH (Table 5.7), and a strong positive correlation with the clay content in both soil depths. As for the relationship between water holding capacity (WHC) and microbial biomass, only microbial biomass C showed a significant ($P<0.01$) positive correlation (Table 5.7). Microbial biomass C, N and P in the upper soil depth were positively correlated with SOM, however, in the lower soil depth only biomass C showed significant ($P<0.05$) correlation (Figure 5.4). Total Kjeldahl-N and available-P also influenced strongly the biomass C, N and P, respectively (Figure 5.5).

Microbial biomass N and P were positively correlated with the microbial biomass C in both the soil depth. Besides, microbial biomass P and N were also positively ($P<0.01$) correlated with each other (Figure 5.6).

DISCUSSION

Seasonal variation in microbial biomass C, N and P observed in the present study is slightly different from those reported for tropical deciduous forests, savanna and temperate pastures where peak values for microbial nutrients were observed during early spring and summer (Sarathchandra *et al.* 1984, Singh *et al.* 1989, Srivastava 1992b, Diaz-Ravina *et al.* 1995). Peak microbial biomass obtained during winter in the present study coincides with low air and soil temperatures (12.3° and 11.5° C, respectively) which indicates the

Table 5.7. Correlation coefficients (r) between mean microbial biomass C, N and P concentrations and soil characteristics.

Microbial biomass	Soil depth (cm)	Soil temperature (°C)	Clay content (%)	WHC (%)	pH	SOC (%)	TKN (%)	Available-P ($\mu g\ g^{-1}$)
		n=6				n=12		
C	0-10	-0.951*	0.987**	0.998**	-0.935*	0.798*	0.860*	0.913**
	10-20	-0.496NS	0.982**	0.977**	-0.860NS	0.866**	0.871*	0.907**
N	0-10	-0.991**	0.999**	0.969*	-0.958*	0.891**	0.891**	0.817*
	10-20	-0.993**	0.978*	0.805NS	-0.991**	0.859*	0.886**	0.682NS
P	0-10	-0.998**	0.994**	0.945NS	-0.979*	0.819*	0.882**	0.815*
	10-20	-0.971*	0.996**	0.873NS	-0.966*	0.864*	0.877**	0.699NS

**-P<0.01, *-P<0.05, NS-Not Significant

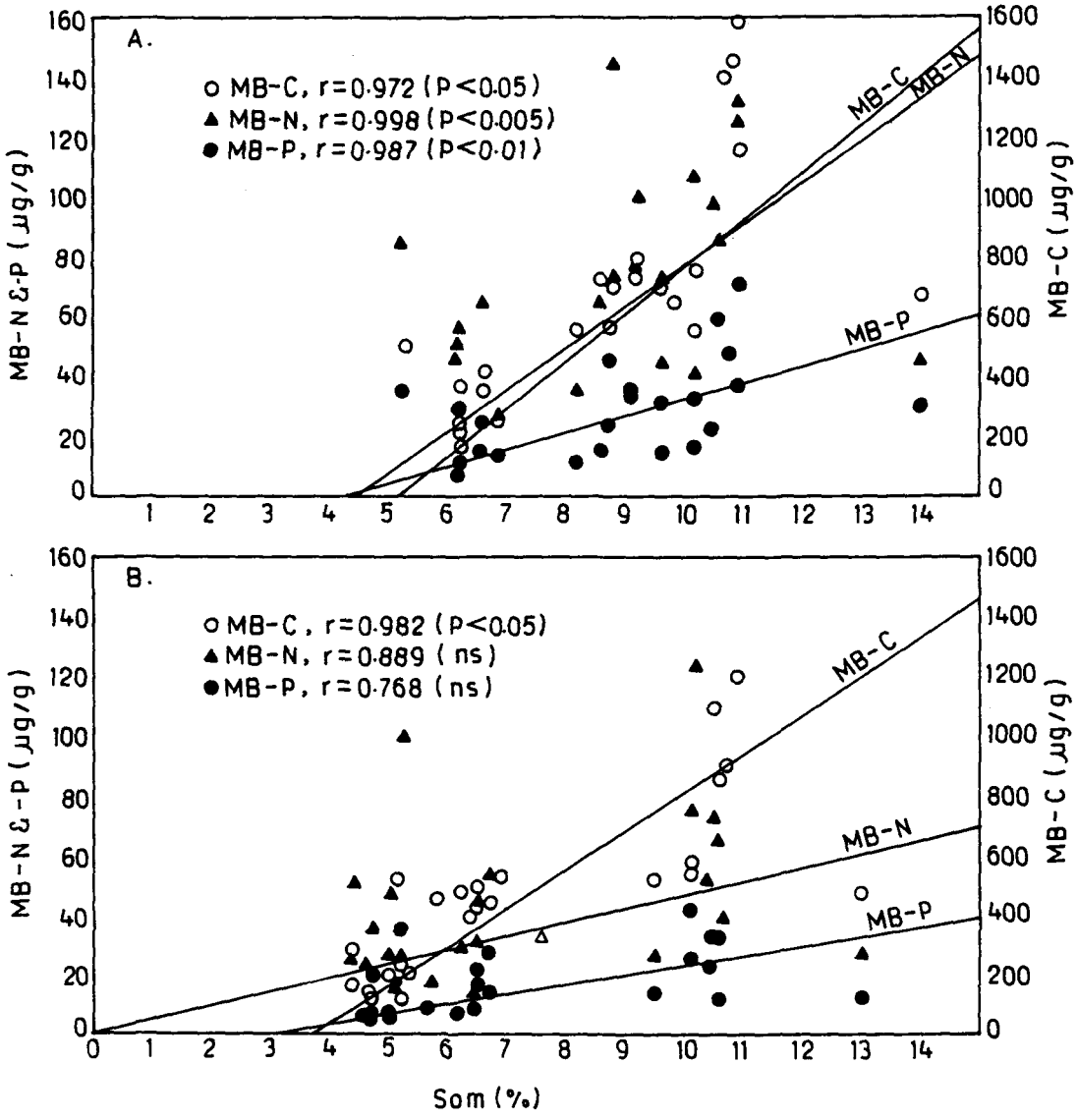

Figure 5.4: Linear regression plot between microbial biomass C, N and p with soil organic matter (a) 0-10 cm (b) 10-20 cm depth.

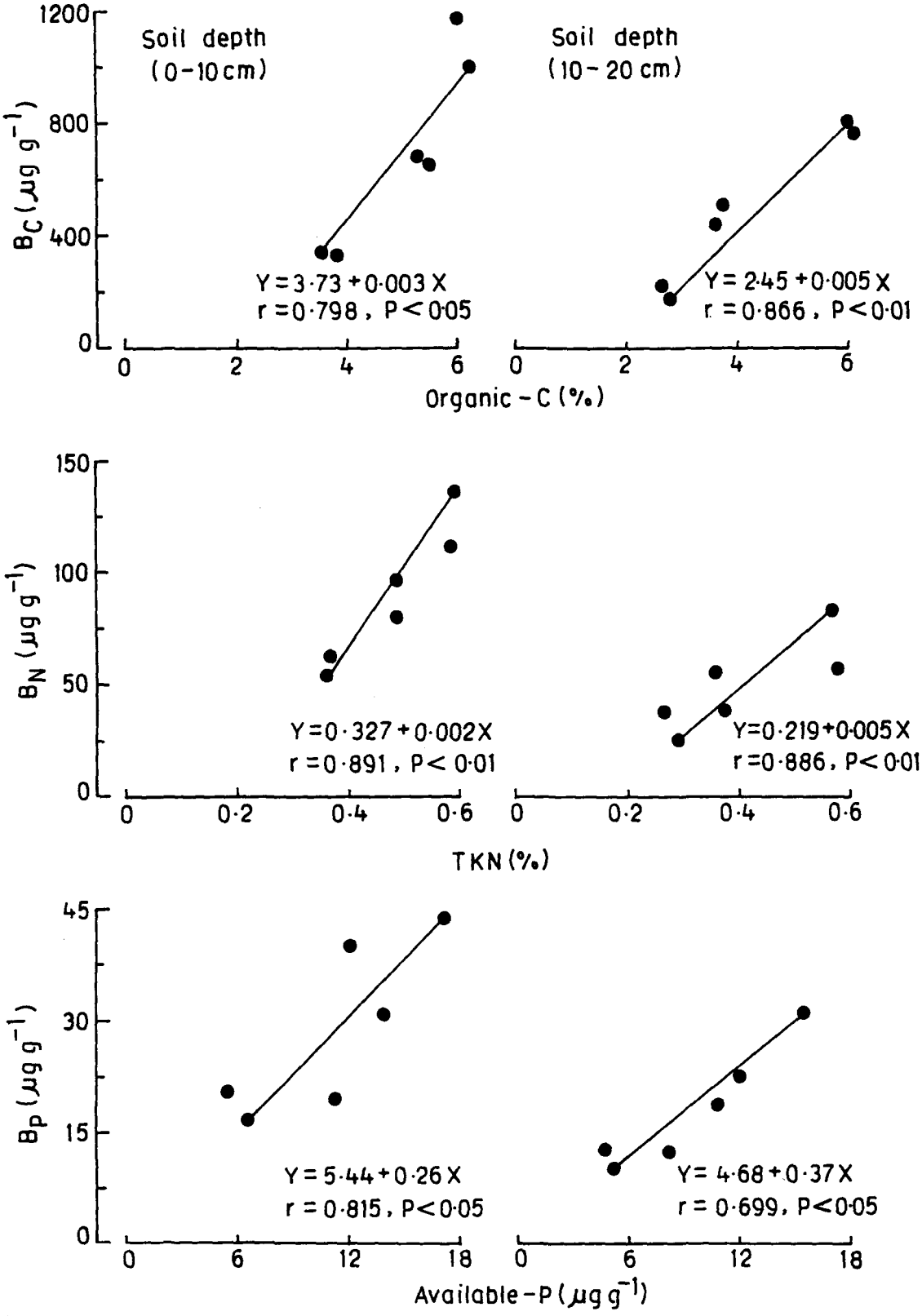

Figure 5.5: Relationship of microbial biomass C with soil organic-C, microbial N with total Kjeldahl N and microbial P with available-P at two soil depths.

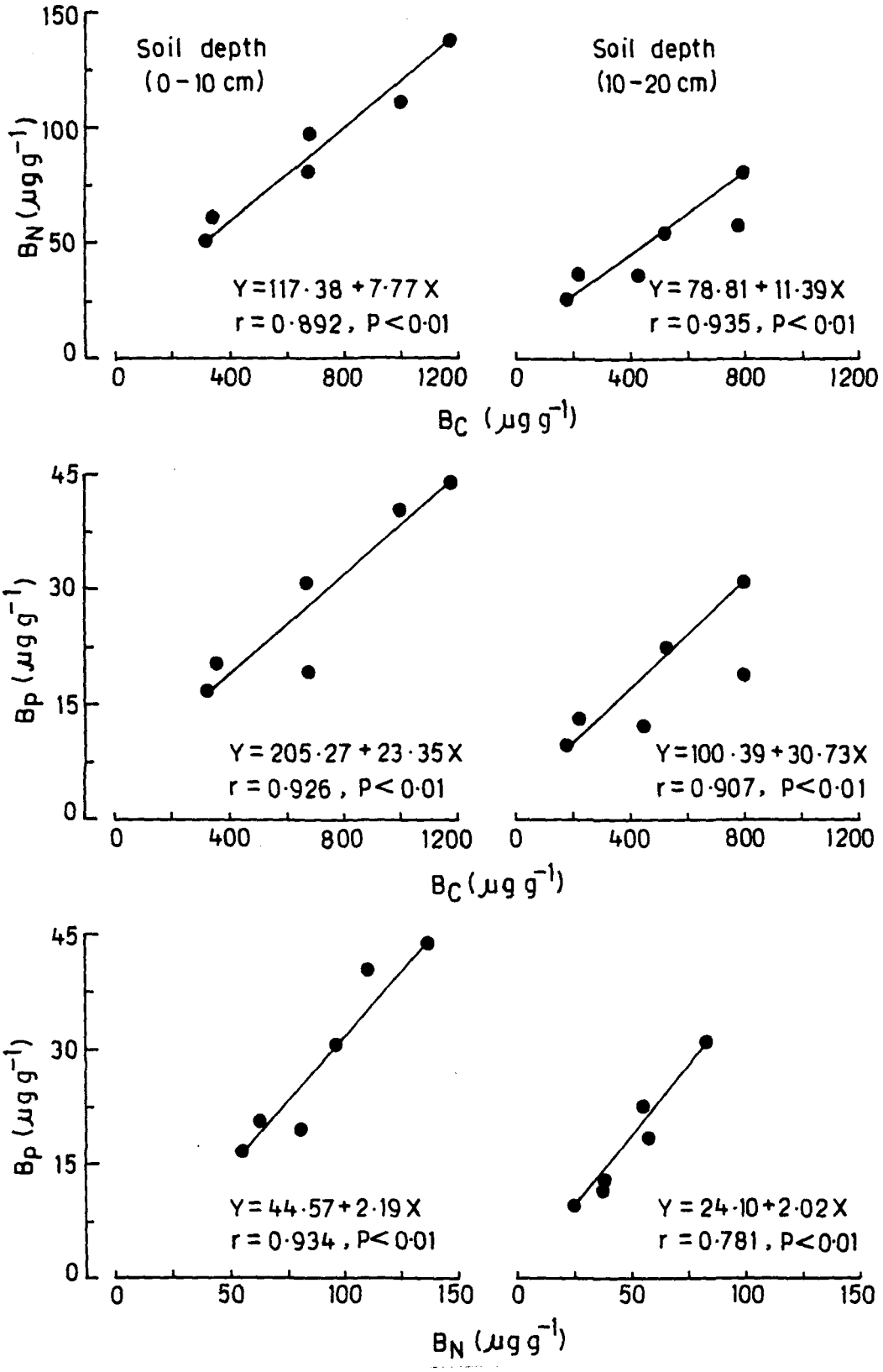

Figure 5.6: Relationship among microbial nutrients at two soil depth.

period of low microbial activity and greater nutrient retention in the soil microbial biomass. In contrast, lower values of microbial biomass C, N and P observed during rainy or autumn seasons when temperature and soil moisture conditions were favourable for the growth of microbes indicated the period of rapid mineralization in soil. Relatively greater demand for nutrients by plants during rainy season when majority of them are at their peak vegetative growth, further limits the availability of nutrients to soil microbes, thereby reducing their immoblization in microbial biomass (Sarathchandra *et al.* 1989, Singh *et al.* 1989).

In the spruce forest soil, Von Lutzao *et al.* (1992) recorded the highest microbial biomass N in autumn and lowest in spring. Seasonal variations observed in microbial biomass C, N and P in the present study is contradictory to the above-mentioned seasonality, but agree with Ding Ming Mao *et al.* (1992) who reported maximum biomass during winter and minimum during rainy season in tropical forest soils, and with Soderstorm (1979) who reported similar seasonal fluctuation in fungal biomass in the Swedish pine forest soil.

The concentration of microbial biomass C obtained in the present study is well within the reported range (61-2000 $\mu g\ g^{-1}$) for various temperate and tropical forest soils (Vance *et al.* 1987, Henrot & Robertson 1994, Diaz-Ravina *et al.* 1995). However, the values reported for microbial biomass C, N and P in our study are comparatively higher than those reported for tropical dry deciduous sal forest and dry deciduous mixed forest of Indian sub-continent (Srivastava & Singh 1991).

The relatively greater accumulation of litter and fine roots in the 13- and 16-year old regrowrh compared to the 7-year old regrowth

(Arunachalam *et al.* 1996a) seems to have favoured the growth of microbial population and accumulation of microbial biomass in the older regrowths. This is in agreement with Marion & Black (1988) who reported an increase in organic matter content in the older forest stands owing to the greater accumulation of plant-derived organic matter and microbial products. Likewise, relatively higher microbial biomass C, N and P in the surface layer than the subsurface layer could also be attributed to the same reasons. The microbial biomass N values (52-125 $\mu g\ g^{-1}$) obtained in the present study are higher than those reported by Martikainen & Palojarvi (1990) for coniferous forest soils, but are comparable to soils of broadleaved deciduous (132-240 $\mu g\ g^{-1}$), evergreen (42-242 $\mu g\ g^{-1}$) and a 100-year old *Quercus robur* forest (58-83 $\mu g\ g^{-1}$) located in Spain (Diaz-Ravina *et al.* 1988, 1995). On the other hand, the values obtained for microbial biomass P (5.3-67 $\mu g\ g^{-1}$) are comparable to those reported by Brookes *et al.* (1984) for the arable land, grassland and woodland soils. However, the values for microbial biomass P in the present study are lower than those reported by Diaz-Ravina *et al.* (1995) for 30-40 year old *Pinus pinaster* forest (33-148 $\mu g\ g^{-1}$) but comparable to 130-year old *Q. robur* forest (45-64 $\mu g\ g^{-1}$).

A significant positive correlation between microbial biomass C and N (Figure 5.6) suggests that the dynamics of these two nutrients in forest soils are closely interrelated. As a result, both microbial biomass C and N showed a gradual build-up in the soil during the course of ecosystem recovery after tree cutting. This is, however contradictory to findings of Fenn *et al.* (1993), who reported an insignificant increase in soil microbial biomass along a fire-induced chaparral chronosequence in San Diego County, California. Significant

positive correlation of microbial biomass C, N and P with SOM (Figure 5.4) indicates a close relationship between microbial biomass and status of soil fertility.

As the vegetation regrows following disturbance, there occurs a greater detrital input and gradual accumulation of organic matter and humic substances. This might have indirectly contributed to the enrichment of clay particles in the older stands. Since clay contains *ca.* 65% of total organic-C (Christensen & Sorensen 1985), microbial biomass is expected to be positively correlated with the amount of clay particles, and the present study fully corroborates this point (Table 5.7).

The mean C/N ratio (6-12) in soil microbial biomass of the three forest regrowths is close to the range reported by Martikainen & Palojarvi (1990) for various soil types (6-9) and by Fenn *et al.* (1993) for chaparral soils (7-13). Higher C/N ratios in the subsurface layer than the surface layer is mainly due to the greater depletion of microbial biomass N in the former. Apart from greater N loss from the subsurface soil layer, decreased microbial activity might have also contributed to lower N immobilization in this depth (Bolton *et al.* 1993). The C/P ratios obtained in the present study are very low (1.05-35.9) compared to the range reported by Brookes *et al.* (1984).

The contribution of microbial biomass C to SOC in the three forest regrowths (0.73-1.74%) is very low compared to other tropical forests (1.5-5.3%, Theng *et al.* 1989, Luizao *et al.* 1992) and temperate forest soils (1.8-2.9%, Vance *et al.* 1987). Similarly, as a percentage of TKN, microbial biomass N is very low (1.1-2.1%) compared to the values reported for agricultural soils (2.8-9.8%, Brookes *et al.* 1985), acid-organic soils (2.8-9.8%, Williams &

Sparling 1984) and forest soils (3.4-5.9%, Martikainen & Palojarvi 1990). The percentage contribution of microbial biomass P to total soil-P was (2.4-7.9%), which is comparable to the values reported by Brookes *et al.* (1984) from deciduous woodlands (4.7%), grasslands (2.4-3%) and arable lands (1.4-3.5%). Minimum microbial biomass in the surface soil layer might have resulted in its relatively lower percentage contribution by this component to the total soil nutrient pool and *vice-versa*.

The results discussed in this chapter clearly indicate that the microbial biomass C, N and P were dependent on the clay content, WHC, SOC, TKN and available-P, all of which gradually increased with the input of litter and detrital root mass during the course of forest regrowth. Likewise, the contribution of microbial biomass to total soil nutrient pool also increased significantly from the 7-year old to the 16-year old regrowth.

CHAPTER 6

N MINERALIZATION PATTERN

INTRODUCTION

Nitrogen mineralization *i.e.* its release from the organically bound materials into inorganic and/or plant-available forms, is of crucial importance in natural forest ecosystems where nitrogen has been reported to be a limiting nutrient for plant growth. Therefore, several studies have been carried out on N cycling in tropical and temperate forests (Nadelhoffer *et al.* 1984, Adams *et al.* 1989, Singh *et al.* 1991, Clein & Schimel 1995). The initial product of heterotrophic mineralization of organic-N is ammonium which is further oxidized by autotrophic microbes to form nitrate through nitrification. Level of ammonium and nitrate in soil and their uptake by plants have important implications for the growth and survival of forest trees (Vitousek *et al.* 1982). In fact, N mineralization is an important mechanism which regulates the biological productivity and nitrogen cycling in non-fertilized forest ecosystems.

Vitousek *et al.* (1982) have reported wide variation in N-mineralization among soils of different vegetation types, even when they are in close proximity and develop from similar parent material. It has also been suggested by Rice & Pancholy (1972) that the ionic form of mineral-N in soil may indicate the stage of secondary

succession. But no consistent relationship between pattern of N mineralization and stage of vegetational succession has been demonstrated as yet.

Many studies have reported increased N loss from forest ecosystems following tree cutting. It has been suggested that these losses are caused either by increased N mineralization rates or reduced N uptake by plants after disturbance (Vitousek & Melillo 1979). Increased N mineralization following disturbance could be due to changes in microenvironment and/or substrate quality. Matson and Vitousek (1981) found soil temperature and soil moisture to be more important in controlling mineral-N flux in a 1-yr old clear-cut site, while substrate quality was more important in 4-yr old site.

This part of the present study analyses the N mineralization pattern in soils of the forest regrowths of three different ages. In order to understand the dynamics of mineral-N in the soil, seasonal and depthwise variations in ammonium and nitrate-N were determined periodically across two annual cycles.

METHODS

N MINERALIZATION

In situ N mineralization in soil was measured on monthly basis using buried-bag technique (Eno 1960). During each sampling period, paired soil cores were collected randomly from five points in each site and from two soil depths (0-10 and 10-20 cm) using a steel corer (6.5 cm diameter). One of the cores from each pair was sealed in sterilized polyethylene bags after removing the coarse roots and larger organic debris, if any, and reinserted to its respective depth. The other soil cores were brought to the laboratory, and composite

samples were made for each depth in respect of each stand and sieved through 2 mm mesh screen. Initial moisture content (SMC) and ammonium and nitrate concentrations were determined within 24 h after soil sampling, following standard procedures outlined by Anderson & Ingram (1993). After 1 month, the buried bags were retrieved from each stand, pooled according to depth and were analysed for final ammonium and nitrate concentrations. The changes in ammonium and nitrate concentration were obtained by subtracting the initial concentration from the respective final concentration, and the resultant values have been referred to in this paper as ammonification and nitrification rates, respectively for that period. Net N mineralization was calculated as the sum of changes in extractable ammonium-N and nitrate-N over one month. All the analyses were done in triplicates and the results are expressed on oven-dry weight basis (24 h at 105°C).

STATISTICAL ANALYSIS

The monthly and seasonal data were analysed using ANOVA (fixed effects model) to find out whether the variations due to soil depth, months and sites are statistically significant or not. Tukey's test was carried out to compare the mean values across stands and soil depths. Linear regressions were worked out following Zar (1974) to study the relationship between N mineralization and climatic, vegetation and edaphic variables.

RESULTS

SEASONAL VARIATION IN AMMONIUM- AND NITRATE-N POOL

In all the three forest regrowths, ammonium-N was maximum during winter and minimum during rainy season in both soil depths, except in

the case of surface soil layer (0-10 cm) of the 7-year old regrowth where the maximum value was observed during spring (Figure 6.1). The seasonality in case of nitrate-N was not consistent. In the 7-year old regrowth, its seasonality in the surface soil layer was similar to that of ammonium-N, but the subsurface layer (10-20 cm) had highest concentration of nitrate-N during late-spring and lowest during autumn. In the 13-year old regrowth, the seasonal variation was similar to the 7-year old regrowth, except that in the surface soil layer nitrate-N concentration was maximum during spring. In the 16-year old regrowth, the nitrate concentration was maximum during winter and minimum during late or post-rainy season in both soil depths (Figure. 6.2). Two-way ANOVA revealed significant ($P<0.01$) differences in ammonium and nitrate concentrations due to the season and regrowth age. The mean concentration of ammonium and nitrate in both the soil depths increased with the development of vegetation (Table 6.1).

SPATIAL DISTRIBUTION OF INORGANIC-N

Temporal variation in inorganic-N was significant ($P<0.05$) due to the soil depth and regrowth age. In the 7-year old regrowth, ammonium-N averaged 13.3 and 9.5 $\mu g\ g^{-1}$ in the surface and subsurface soil layers, respectively (Table 6.1). The corresponding values for the 13-year old regrowth were 13.9 and 11.7 ug g^{-1} and for the 16-year old regrowth 17.2 and 13.5 $\mu g\ g^{-1}$. The level of nitrate-N did not vary significantly between the two soil depths. Over- all, it averaged 29, 36 and 37% of the total inorganic-N in the 7-, 13- and 16-year old regrowths, respectively.

N MINERALIZATION

Monthly and seasonal variation in ammonification rate was similar in all the three forest regrowths (Figure. 6.3). In both years, peak

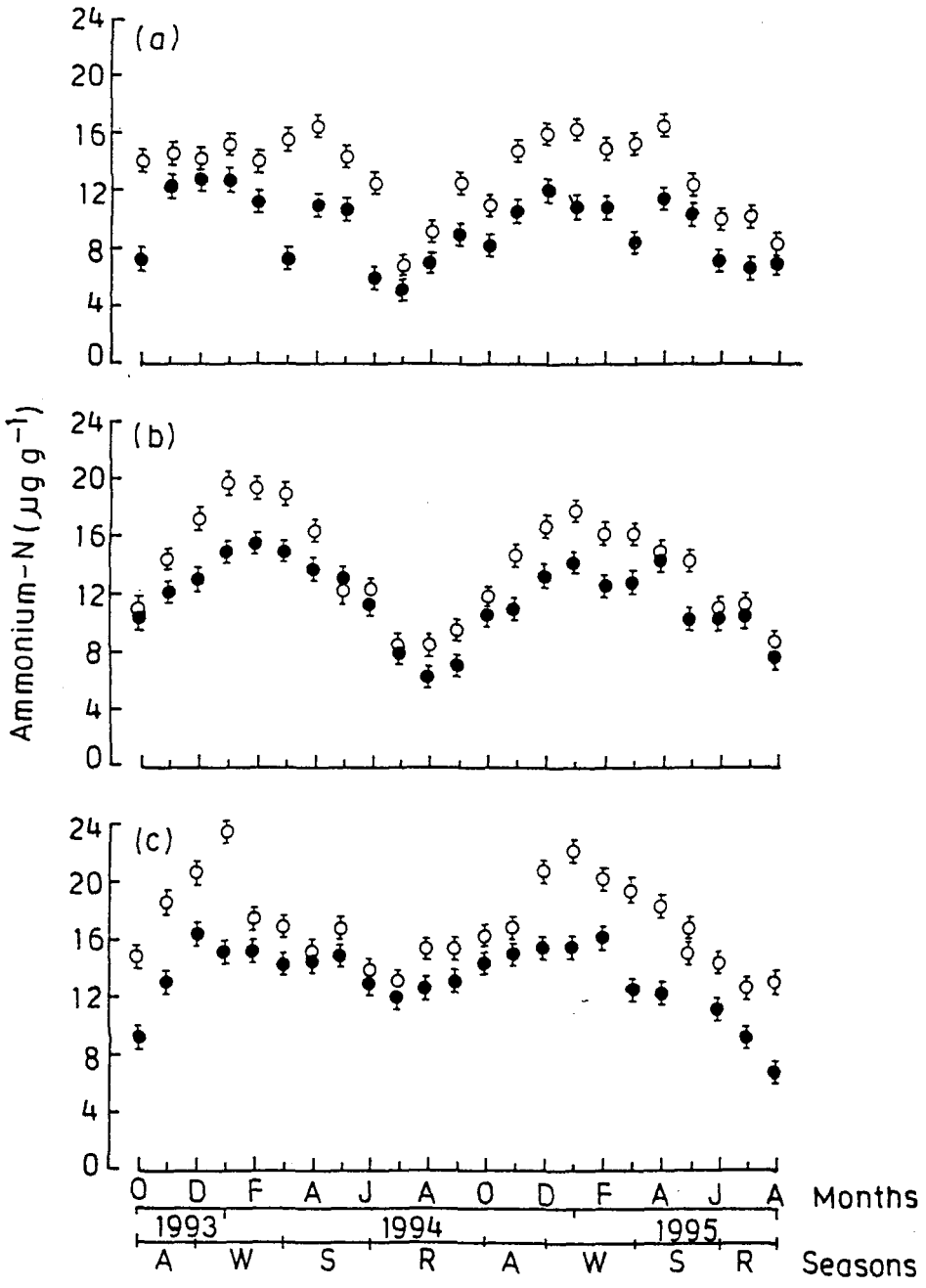

Figure 6.1: Monthly variation in ammonium concentration (μg u^{-1}) in two soil depths (o 0-10 and ● 10-20 cm) in (a) 7-, (b) 13- and (c) 16-year old regrowths. A-autumn; W-winter; S-spring; R-rainy season. Vertical bars represent standard error.

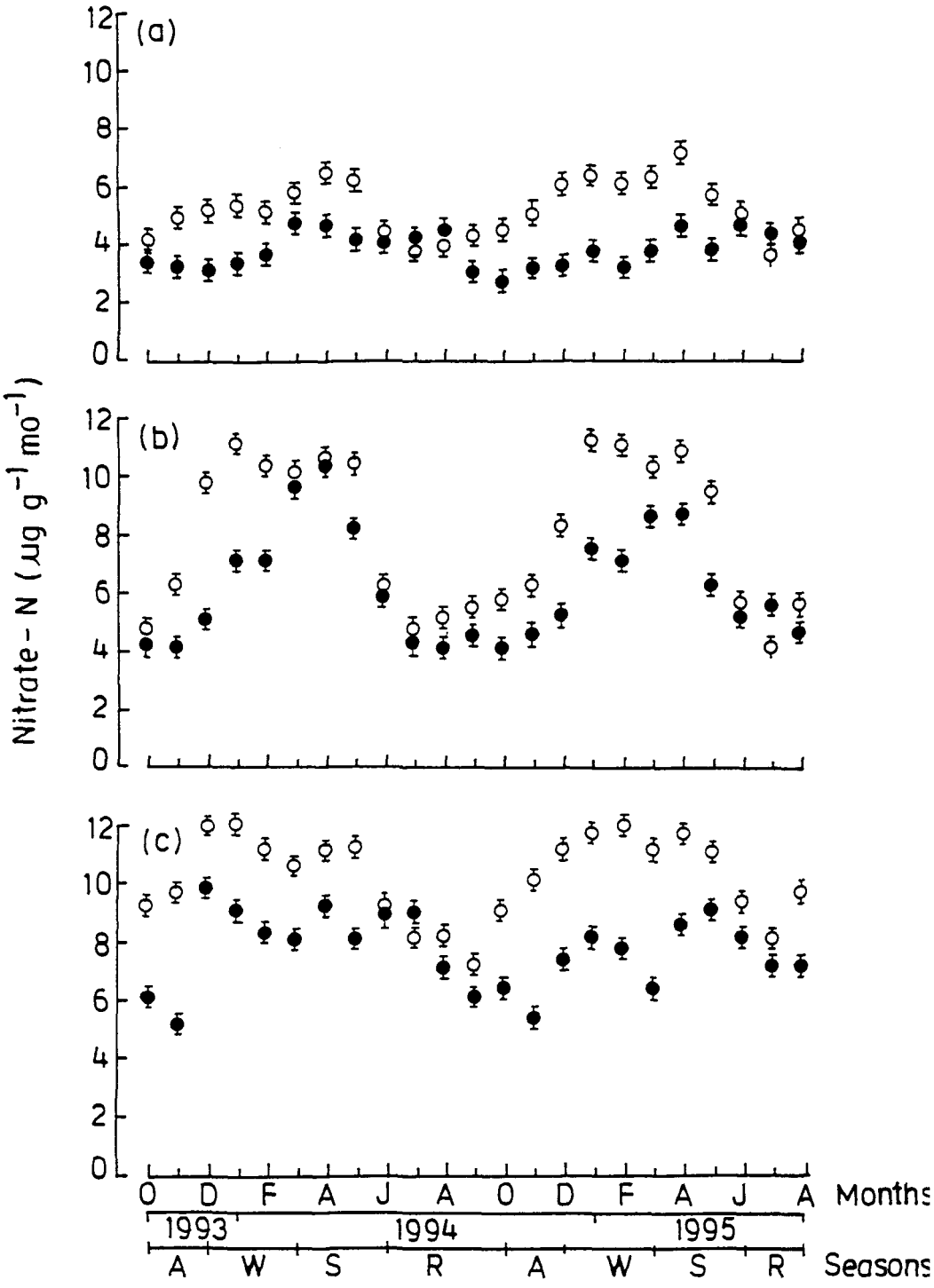

Figure 6.2: Monthly variation in nitrate concentration (µg g^{-1}) in twp soil depths (o 0-10 and • 10-20 cm) in (a) 7-, (b) 13- and (c) 16-year old regrowths. A-autumn; W-winter; S-spring; R-rainy season. Vertical bars represent standard error.

Table 6.1. Average inorganic-N pool ($\mu g\ g^{-1}$) and net N-mineralization rate ($\mu g\ g^{-1}\ mo^{-1}$) in the three forest regrowths.

Age of the forest regrowth	Soil depth (cm)	Concentration ($\mu g\ g^{-1}$)			Net Rate ($\mu g\ g^{-1}\ mo^{-1}$)		
		Ammonium	Nitrate	Inorganic-N*	Ammonification	Nitrification	N-Mineralizatior
7-yr	0-10	13.34^{a}	5.31^{a}	18.65^{a}	4.16^{a}	4.64^{a}	8.80^{a}
	10-20	9.49^{a}	3.90^{a}	13.39^{a}	2.27^{a}	2.94^{a}	5.21^{a}
13-yr	0-10	13.87^{a}	8.08^{b}	21.95^{b}	5.50^{b}	3.52^{b}	9.02^{a}
	10-20	11.72^{a}	6.28^{b}	18.00^{b}	3.51^{b}	2.63^{a}	6.14^{b}
16-yr	0-10	17.15^{b}	10.40^{c}	27.55^{c}	$6.38^{b,c}$	3.42^{b}	9.80^{a}
	10-20	13.66^{b}	7.71^{b}	21.37^{c}	$4.02^{b,c}$	2.55^{a}	$6.57^{b,c}$

Note: The values are the means of 23 monthly samplings

*Ammonium+Nitrate

Note: Values with similar superscripts in the columns are not significant at $P<0.05$ between stand in that particular soil depth.

ammonification was obtained during July (rainy season) and minimum during January (winter). The seasonal variation between winter and autumn was significant only in the 13- and 16-year old regrowths. Three-way ANOVA of the ammonification data revealed significant ($P<0.01$) differences due to season, soil depth and regrowth age. Ammonification rate was 0.6-8.3 $\mu g\ g^{-1}\ mo^{-1}$ in the 7-year old regrowth, 0.6-9.9 $\mu g\ g^{-1}\ mo^{-1}$ in the 13-year old regrowth and -1.1-13.8 $\mu g\ g^{-1}\ mo^{-1}$ in the 16-year old regrowth. During winter the surface soil layer recorded low rate of ammonification and/or immobilization compared to the subsurface layer. The percentage contribution of ammonification rate to net N-mineralization increased from 40-48% in the 7-year old regrowth to 59-68% in the 16-year old regrowth (Table 6.2).

Nitrification rates did not vary significantly due to year in both soil depths in all the three forest regrowths. However, three-way ANOVA revealed significant ($P<0.01$) variations due to soil depth, seasons and regrowth age within the two annual cycles. The seasonal behaviour in nitrification rate in the two soil depths was similar to that of ammonification, except that in case of the 16-year old regrowth the peak was obtained during autumn (October) in the first year (Figure 6.4). Nitrification rate was significantly ($P<0.01$) higher in the 7-year old regrowth (0-10 cm depth - 4.7 $\mu g\ g^{-1}\ mo^{-1}$ and 10-20 cm depth - 2.9 $\mu g\ g^{-1}\ mo^{-1}$) than the two older regrowths. No significant differences were observed between nitrification rates in the 13- and 16-year old regrowths. Net nitrification ranged 52-74 kg $ha^{-1}\ yr^{-1}$ in the surface soil layer and 43-50 kg $ha^{-1}\ yr^{-1}$ in the subsurface layer (Table 6.2).

The net N mineralization also peaked during rainy season in both years in all the three regrowths (Figure. 6.5). Overall, the N mineralization (5.2-9.8 $\mu g\ g^{-1}\ mo^{-1}$) increased with the age of the

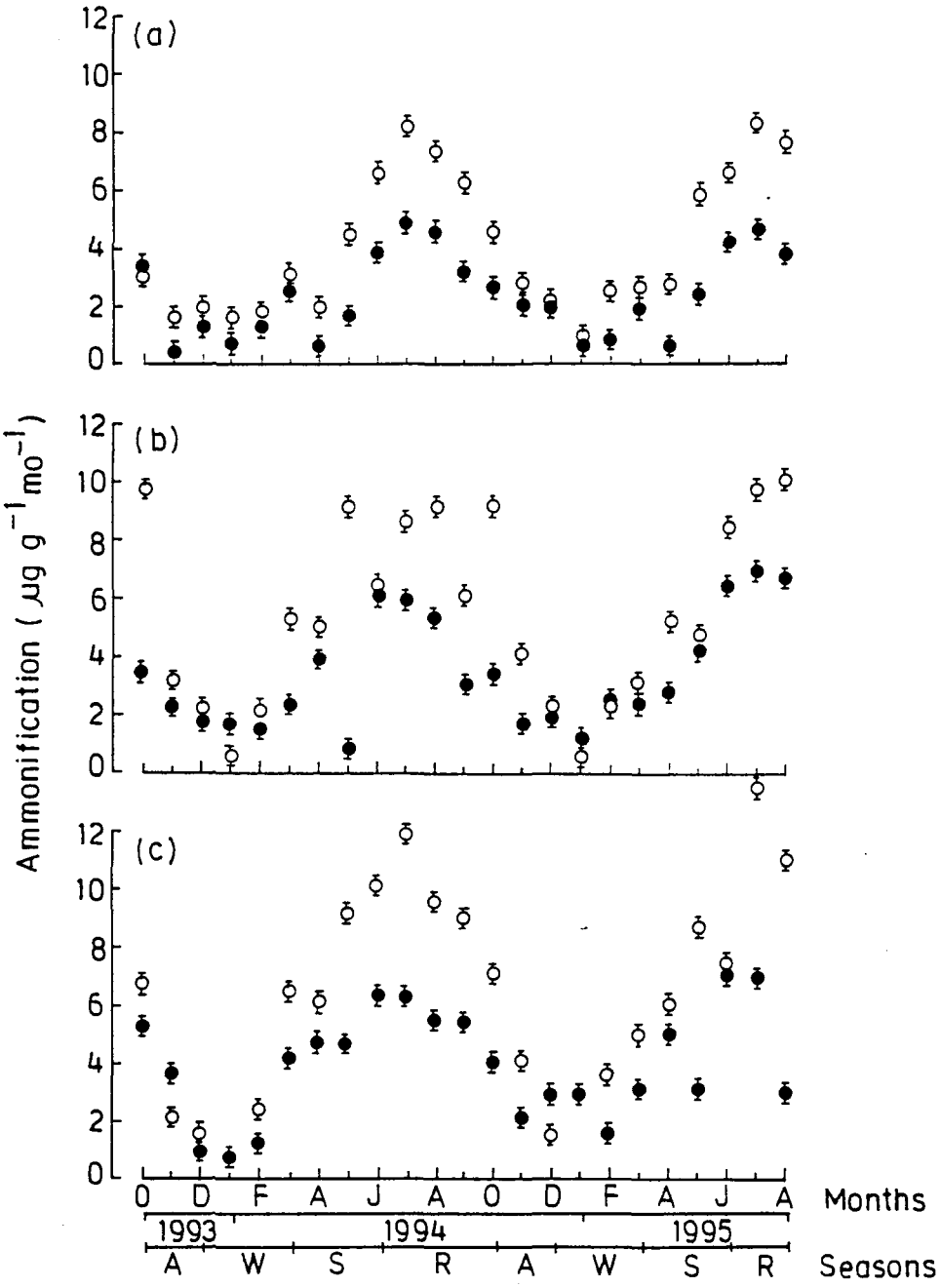

Figure 6.3: Monthly variation in ammonification rate (µg g^{-1} mo^{-1}) in two soil depths (o 0-10 and • 10-20 cm) in (a) 7-, (b) 13- and (c) 16-year old regrowths. A-autumn; W-winter; S-spring; R-rainy season. Vertical bars represent standard error.

Table 6.2. Net ammonification, nitrification and N mineralization rates (kg ha^{-1} yr^{-1}) in forest regrowths of three different ages.

Age of the forest regrowth	Soil depth (cm)	Ammonification		Nitrification		N mineralizatior	
		I-yr	II-yr	I-yr	II-yr	I-yr	II-yr
7-yr	0-10	63.60 (46.2)	68.28 (48.1)	74.16 (53.8)	73.80 (52.0)	137.76	142.08
	10-20	39.48 (44.4)	33.12 (39.5)	47.88 (53.9)	50.28 (60.0)	87.36	83.40
13-yr	0-10	93.12 (61.0)	93.24 (61.0)	59.52 (37.8)	59.52 (38.9)	152.64	152.76
	10-20	57.36 (55.8)	63.48 (58.5)	45.36 (44.2)	45.12 (41.6)	102.72	108.60
16-yr	0-10	109.56 (67.6)	108.00 (62.4)	51.96 (32.0)	65.04 (37.6)	162.00	173.04
	10-20	72.84 (62.6)	67.80 (59.5)	43.44 (37.3)	46.08 (40.4)	116.28	113.88

I-yr represents the period from October, 1993 to September, 1994

II-yr represents the period from October, 1994 to August, 1995

Note: Values in parentheses are percentages of net N mineralization.

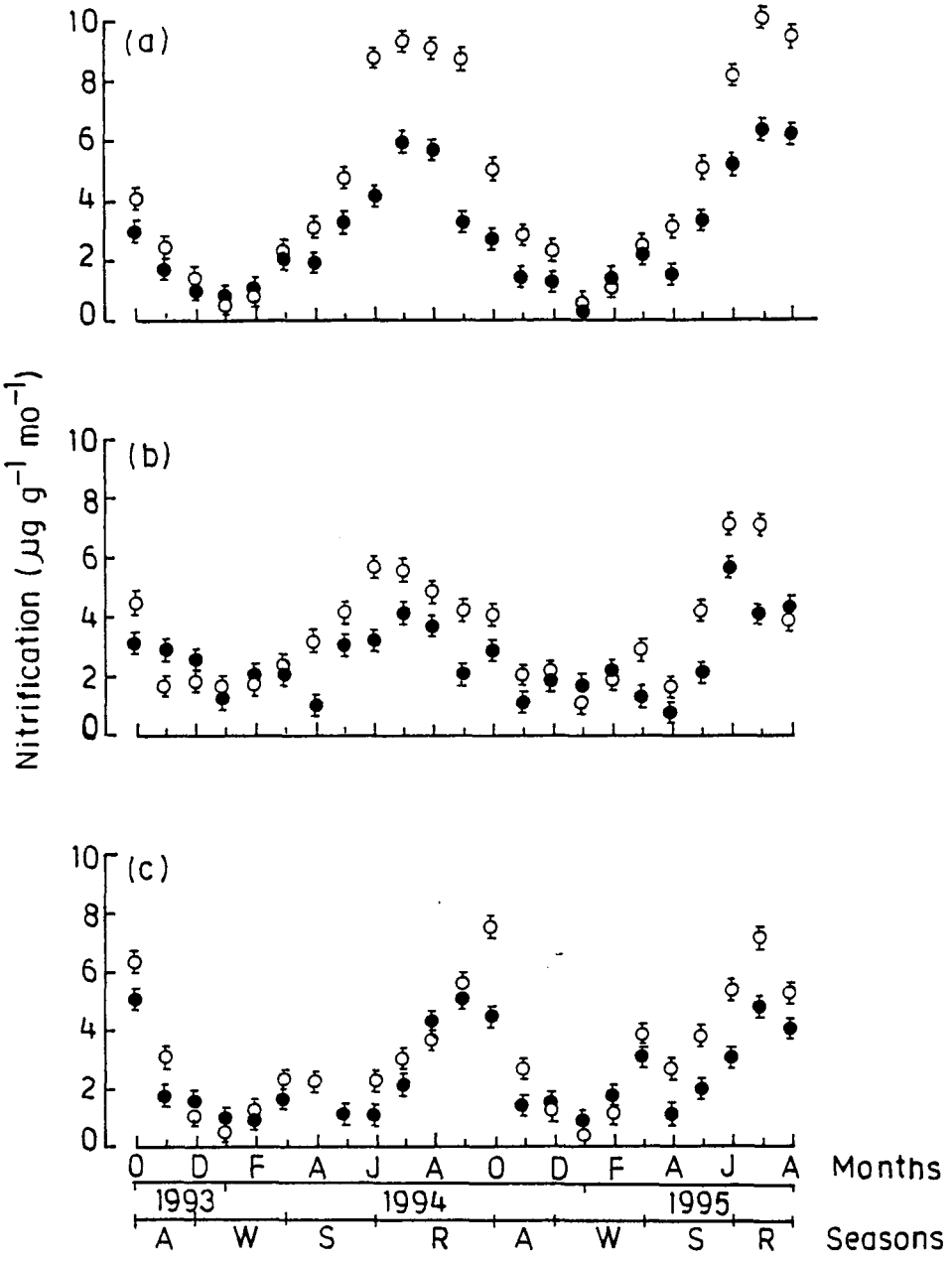

Figure 6.4: Monthly variation in nitrification rate (µg g^{-1} mo^{-1}) in two soil depths (o 0-10 and • 10-20 cm) in (a) 7-, (b) 13- and (c) 16-year old regrowths. A-autumn; W-winter; S-spring; R-rainy season. Vertical bars represent standard error.

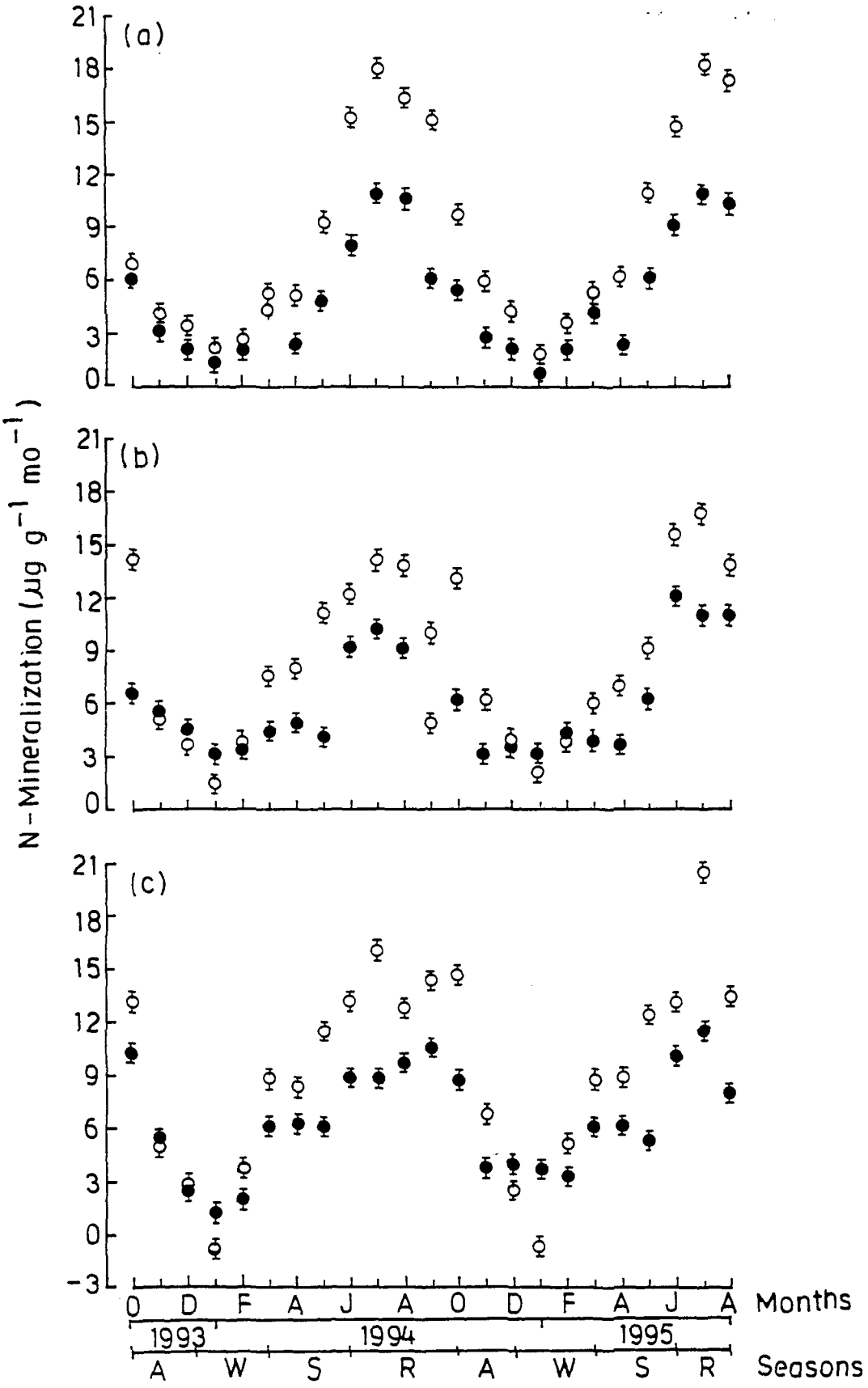

Figure 6.5: Monthly variation in N mineralization rate (µg g^{-1} mo^{-1}) in two soil depths (o 0-10 and • 10-20 cm) in (a) 7-, (b) 13- and (c) 16-year old regrowths. A-autumn; W-winter; S-spring; R-rainy season. vertical bars represent standard error.

forest regrowth (Table 6.1), but the increase was not appreciable.

RELATIONSHIP OF N MINERALIZATION WITH FOREST CLIMATE, SOIL AND VEGETATION PROPERTIES

While testing the relationship of net N mineralization with 2 microclimatic and 4 edaphic variables, and 3 microbiological characteristics in the two soil depths, it was found that N mineralization bore significant positive correlations only with rainfall, soil temperature, soil moisture, and fungal and bacterial populations (Table 6.3). Apart from these variables, density and basal area of the woody vegetation also had favourable effect on the rates of ammonification and net N mineralization in both soil depths (Table 6.4). Nitrification, however, was negatively correlated with the density and basal area of woody vegetation.

DISCUSSION

Inorganic-N pool varied widely in time and space which could be mainly attributed to three factors *viz.* variation in mineralization rates, uptake by plants and microbes, and losses through leaching, run-off and denitrification. The decrease in ammonium and nitrate-N during rainy season is due mainly to the greater demand for these nutrients by higher plants which grow vigorously during this period (Arunachalam *et al.* 1996a). However, the significance of leaching and denitrification which are paramount factors responsible for N loss during rainy season cannot be ruled out completely. Conversely, an increase in ammonium and nitrate concentrations during winter may be partly associated with their decreased demand by plants owing to slow growth. Birch (1958) suggested that during dry spring season the soil starts drying due to increased evaporation, thereby enhancing the upward movement of nitrate and release of free ammonium and amino

Table 6.3. Correlation coefficients (r) for the relationships between mean seasonal N-mineralization rate (ug g^{-1} mo^{-1}) in two soil depths and macroclimate, soil and microbiological characteristics during forest regrowth (n=24).

Soil depth (cm)	Climatic variables		Soil properties				Microbiological characteristics		
	Rainfall (mm)	Air temp. (°C)	Temp. (°C)	Moisture (%)	SOM (%)	TKN (%)	Microbial biomass N ($\mu g\ g^{-1}$)	Bacterial[a] population (Colony Nos. g^{-1} soil)	Fungal[b] population
0-10	0.472*	0.287	0.507*	0.548*	0.317	0.262	-0.222	0.601**	0.398
10-20	0.474*	0.122	0.346	0.510*	0.377	0.345	-0.082	0.813**	0.592**

[a]Values x 10^4,

[b]Values x 10^3

*Significant at $P<0.05$

**Significant at $P<0.005$

Table 6.4. Relationship of ammonification, nitrification and N mineralization rates ($\mu g\ g^{-1}\ mo^{-1}$) with density (plants ha^{-1}) and basal area ($m^2\ ha^{-1}$) of woody vegetation in the three forest regrowths. (n=6).

Variate	Soil depth (cm)	Regression equation	r	P
Relationship with density				
Ammonification	0-10	Y=-776.15+356.33X	0.986	0.001
	10-20	Y=-234.75+420.62X	0.999	0.001
Nitrification	0-10	Y=3251.24-550.88X	-0.977	0.001
	10-20	Y=6331.61-1917.10X	-0.999	0.001
N mineralization	0-10	Y=-3933.79+547.27X	0.789	0.05
	10-20	Y=-2113.01+541.75X	0.996	0.001
Relationship with basal area				
Ammonification	0-10	Y=-75.29+18.38X	0.975	0.001
	10-20	Y=-44.11+20.69X	0.942	0.005
Nitrification	0-10	Y=115.73-24.17X	-0.836	0.05
	10-20	Y=265.84-89.56X	-0.899	0.05
N-mineralization	0-10	Y=-299.71+34.88X	0.965	0.005
	10-20	Y=-139.17+27.09X	0.955	0.005

acids from the drying soil. This might be true in the case of 7-year old regrowth where the over-head canopy is completely absent and the soil is directly exposed to solar insolation, thus encountering the peak inorganic-N (ammonium+nitrate) during spring in both soil depths; the values were *ca.*39% higher in the surface soil layer than the subsurface layer.

Nadelhoffer *et al.* (1984) reported that the variation in ammonium concentration in soil during different months is related to net ammonification. A comparison of the mean values across the regrowth age fully corroborates this point. However, a strong negative correlation ($Y=11.70+5.21X$, $r=-0.605$, $df=46$, $P<0.001$) between ammonium-N pool and ammonification rate was obtained when mean seasonal values were used for regression analysis irrespective of regrowth age. This suggests that in the regenerating forest communities, the ammonium concentration in soil may be more related to the pace of plant uptake and microbial immobilization than the ammonification rate. Jackson *et al.* (1989) reported that the uptake of ammonium-N by microbes is about 5-times that by the forest trees. In a recent study (Maithani *et al.* 1996a) peak microbial biomass N was reported during winter. The lower ammonification during winter in all three forest regrowths observed in the present study supports the possible immobilization of the inorganic-N by the micro-organisms. Heavy rainfall during June-September seems to be responsible for leaching of nitrate from the surface soil layer to the subsurface layer, as indicated by a decreased nitrate concentration in the upper soil layer and increased concentration in the lower soil depth.

The higher concentration of ammonium-N than nitrate-N in the present study may be attributed to slightly acidic nature of the soil which might have inhibited the growth and activity of autotrophic nitrifiers in soil as also reported by Chao *et al.* (1993). The level

of inorganic-N was very low as compared to total soil nitrogen and its contribution to the latter varied with the soil depth and regrowth age. Inorganic-N in 0-10 and 10-20 cm depths was 0.24 and 0.19% of the total soil-N, respectively in the 7-year old regrowth, 0.18 and 0.17% in the 13-year old regrowth, and 0.17 and 0.13% in the 16-year old regrowth. This shows that in both soil depths most of the N is organically bound. This is in agreement with Singh *et al.* (1981) who reported that organic-N is the major constituent of total soil-N. The increased level of inorganic-N in the older regrowths is due to increased mineralization of nutrients from soil organic matter during forest regrowth.

Net N mineralization rates in the three forest regrowths ranged from 84 to 175 kg ha^{-1} yr^{-1} which are well within the range (40-200 kg ha^{-1} yr^{-1}) reported by Vitousek *et al.* (1982) for forest sites. However, the range for nitrification rates (0.56-10.13 μg g^{-1} mo^{-1}) are narrower than that (0.2-23 μg g-*1* mo-*1*) reported by Singh *et al.* (1991) for a disturbed dry tropical savanna. Comparatively low nitrification in the present study might be due primarily to two reasons: (1) nitrification might be taking place at a slower rate, and (2) mineralization may be proceeding at normal rates but denitrification in localized anaerobic conditions might have influenced the measurement of nitrification which was based on nitrate-N determination. Firestone (1982) reported that the rate of N loss from soil due to denitrification is increased by factors that increase the extent of anaerobic microsites in soil. Increasing soil moisture on account of heavy rainfall in this part of India, could act as one such factor which may lead to the development of anaerobic microsites by decreasing the rate of oxygen diffusion through the soil matrix. However, in absence of any data on denitrification response to increasing soil moisture it is not possible to substantiate the

role of soil moisture in N mineralization during forest regrowth.

Nitrification varied between 0 and 100% of N mineralization in temperate forest ecosystems (Aber *et al.* 1985). In the present study, nitrification accounts for *ca.* 53% in the 7-year old regrowth and *ca.* 35% in the 16-year old regrowth in the upper soil depth. The corresponding values for the lower depth were 56 to 39%. Chao *et al.* (1993) attributed rapid nitrification to low clay content. They argued that low clay in soil may create better aeration for the autotrophic nitrifiers. This could well explain the declining trend in nitrification rate during forest regrowth, as the clay content in the soil increased significantly with the regrowth age (Chapter 4).

Moisture-limited seasonality in N mineralization have been reported by Singh *et al.* (1991) in dry tropical savanna, Morecroft *et al.* (1992) in Scotish highlands and Clein & Schimel (1995) in taiga forests. However, in the north-east India soil moisture is not a limiting factor except during dry winter season. During dry periods, plant uptake is reduced and the mineralized-N is either immobilized in microbial biomass and fine roots or it accumulates in soil as inorganic-N (Singh *et al.* 1991) resulting in greater pool of inorganic-N during these periods compared to wet periods. It has been emphasized by several workers (Birch 1958, Sorensen 1974) that rewetting of a dry soil increases N mineralization. In the present study, following dry winter and spring seasons, there was a peak N-mineralization during rainy season when the soil moisture conditions were optimum for microbial activity. This finds support from the significant positive correlations of N mineralization with rainfall and soil moisture (Table 6.3). Further, significant positive correlations between N mineralization and bacterial and fungal populations, and soil temperature (Table 6.3) could explain well the gradual increase of N mineralization rate during forest regrowth after

disturbance.

In the present study, the ammonification rate showed an increasing trend, while nitrification rate declined during forest regrowth. The mechanism controlling the decline in nitrification rate has been attributed by Montagnini *et al.* (1986) to allelopathic inhibition by organic compounds in soil or plant extracts, and to a reduction in N-availability. However, in the present study the nitrification was greater in the 7-year old regrowth than in the 16-year old regrowth, although the younger regrowth had a profuse growth of *Eupatorium adenophorum* which has been reported to produce allelopathic effects (Tripathi *et al.* 1981). This suggests that differences in nitrification were due to factors other than allelopathic inhibition. It is expected that the soil organic matter in the older regrowth would supply readily available C sources to soil microbes resulting in increased rate of internal-N cycling, but due to excessive heterotrophic immobilization, the N available to nitrifiers and roots is reduced, which may lead to low activity of nitrifier population (Adams & Attiwill 1986). Consequently, the 16-year old regrowth had low net nitrification rate. These results suggest that with the development of vegetation in the disturbed forest N turnover is stimulated.

Despite a reduction in nitrification rates with the increase in vegetation growth, the inorganic-N pool showed a steady increase during forest succession. This indicates the importance of microbe-mediated plant detrital decomposition sub-system. For example, peak N-mineralization during rainy season coincided with the period of rapid litter and fine-root decomposition and decreased accumulation of litter, fine roots and microbial biomass (Arunachalam *et al.* 1996a, Maithani *et al.* 1996a) on the forest floor. Conversely, during cold and dry winter slow rate of decomposition attributable to low

microbial activity might have resulted in the immobilization of inorganic-N. This phenomenon coincided with the peak root mass and microbial biomass, suggesting an intimate association between N mineralization and detrital accumulation on the forest floor.

The results discussed above clearly depict that N mineralization increases with the age of the regrowth and decreases with soil depth. Ammonium-N is the dominant form of inorganic-N in the forest regrowths. Though nitrification rate declined with regrowth age, nitrate concentration in soil was higher in the 16-year old regrowth than in the 7-year old regrowth. Ammonification and net N mineralization rates, however, were greater in the older regrowth indicating greater N turnover with the development of vegetation. These findings suggest that the state of N cycling in the forest regrowths is close to the steady-state conditions. N-mineralization was closely associated with fine roots and microbial biomass indicating the role of microbe-mediated decomposition sub-system which is mainly controlled by soil moisture regimes in the forest regrowths. The density and basal area of the woody vegetation which increased during forest regrowth promoted N mineralization. The percentage increase in net N mineralization was, however, low during 7 to 13 years of forest regrowth (1.5% yr^{-1}) and got doubled to about 3% yr^{-1} during the next 3 years of vegetation development.

CHAPTER 7

INFLUENCE OF LEAF LITTER ON N MINERALIZATION

INTRODUCTION

Residue decomposition is the primary mechanism for organic matter and nutrient release in non-fertilized forest ecosystems. It has been reported that about 70-90% of nutrients needed annually for the forest growth comes through decomposition of detritus matter (Vogt *et al.* 1986). Litter decomposition is a complex microbe-mediated process which is accelerated by the conditions that enhance microbial growth or activity (Singh & Gupta 1977, Swift *et al.* 1979). Apart from the climatic factors such as temperature, moisture and relative humidity, substrate quality which is defined as the chemical composition of decomposing material has been considered a critical factor in determining rate of decay. Chemical indices of substrate quality are element concentrations such as C, N and P and concentrations of various classes of organic compounds *viz* cellulose, lignin, polyphenol etc.

Harmon *et al.* (1990) and Taylor *et al.* (1991) reported that nitrogen content of plant material is an important factor which controls the rate of decomposition. Other studies indicated that lignin content of litter exerts greater control over the rate of decomposition than N (Fogel & Cromack 1977, Melillo *et al.* 1982,

Muller *et al.* 1988). C/N and lignin/N ratios are also used as a measure of both litter quality and decomposition. Recent studies (Palm & Sanchezz 1991, Tian *et al.* 1992) suggest that polyphenol also plays a significant role in determining the decay rates of litter. Arianoutsou (1993) pointed out that litter decay rate and magnitude of nutrient release in forest ecosystem depends on the nutrient status of the soil and age of the stand. In forest ecosystems losses of nutrients are minimal and whatever loss occurs is balanced by input from various sources. However, this dynamic equilibrium is often disturbed by various anthropogenic activities, thus resulting in the nutrient loss. These losses can be minimised to a great extent by synchronizing the supply of nutrients with plant growth demand. One way of achieving this synchrony is through manipulating the quality and quantity of organic inputs on the forest floor. The quality of organic matter depends on its chemical composition. In view of the above facts, the chemical composition of tree species from the three forest regrowth was determined and the effects of various combinations of different types of leaf litter were studied on N mineralization under the laboratory conditions.

METHODS

Surface soil (0-10 cm) from the three forest regrowths was collected during April, 1994. The soil was sieved through 2 mm sieve, homogenized separately for each stand and stored at 4°C until use. Fresh leaf litter samples of dominant tree species were collected from 7-, 13- and 16-year old regrowths. The leaf samples were air-dried and chopped into approximately 1 mm size. Plant materials were analyzed for the concentrations of C, N, P and lignin by following the standard

techniques. The ash content of plant material was determined by igniting the sample in muffle furnace at 550°C for 6 h. The carbon content was taken as 50% of the ash-free weight (Allen *et al.* 1974). N was determined as total kjeldahl N using TECATOR 1030 auto analyzer (Anderson & Ingram 1993). Total P was estimated colorimetrically (Allen *et al.* 1974). Lignin content of the samples was determined by the standard technique given by Peach and Tracey (1955). The physico-chemical properties of the soils collected from the three forest regrowths used for the incubation study were determined by standard procedures given by Allen *et al.* (1974; see Chapter 4).

INCUBATION PROCEDURE

100 g of field moist soil from each forest regrowth was taken into 250 ml polyethylene beakers. Before putting the soil into beakers the soil was adjusted to 60% of water holding capacity. Soil was incubated for 7 days at room temperature to allow microbial activity to settle down. The leaf litter collected from each forest regrowth was then added to their respective soils at a loading rate of 0.01 g plant material per gram of oven-dried soil and was mixed thoroughly with soil. The beakers were sealed with polythene sheets and incubated for 90 days at 25±1°C in a BOD incubator.

In the case of 7-year old regrowth following treatments were maintained:

(i) Soil + *Pinus kesiya* leaf litter

(ii) Soil + *Quercus dealbata* leaf litter

(iii) Soil only (control)

For the 13-year old regrowth also three treatments were used:

(i) Control

(II) Soil + *Pinus kesiya* leaf litter

(III) Soil + *Quercus dealbata* leaf litter.

However, in the case of 16-year old regrowth, the six treatments were maintained and they are as follows :

(i) Soil + *Pinus kesiya* leaf litter

(ii) Soil + *Q. dealbata* leaf litter

(iii) Soil + *Q. griffithii* leaf litter

(iv) Soil + *Schima khasiana* leaf litter

(v) Soil + *Rhododendron arboreum* leaf litter and

(vi) Soil only, serving as control.

Leaf litter of only those tree spcies were mixed with the soil of a given forest regrowth which were dominant in that particular regrowth. About 27 replicates of each treatment from each stand were incubated for 90 days. The moisture content of the incubated soil samples were kept constant throughout the experiment by the periodic addition of deionised water using a syringe. The soil pH and ammonium and nitrate-N in soils were measured at time intervals of 2, 4, 8 and 12 weeks.

CALCULATION METHODS

N mineralization rate was calculated by subtracting the initial inorganic-N present in soil from the N accumulated in the soil after the respective incubation period.

$$\text{N mineralization rate } (\mu g\ g^{-1} day^{-1}) = \frac{\text{Final N conc. - Initial N conc.}}{\text{Number of incubation days}}$$

By subtracting the total extractable-N in the control ('Soil only') from that in leaf litter+soil treatment, accumulation or

depletion of inorganic-N attributable to the presence of leaf litter was calculated. The difference in concentration was then divided by the initial leaf-N added to each incubation and the fraction thus obtained was multiplied by 100 to express the result as percent of the initial leaf-N.

STATISTICAL ANALYSIS

Tukey's test was used to determine the statistical significance of variations among initial chemical properties of leaf litter. Linear regressions were worked out following Zar (1974), wherever necessary.

RESULTS

CHEMICAL COMPOSITIOIN OF LEAF LITTER

Concentrations of C and lignin and C/N and lignin/N ratios gradually increased with the regrowth age. On the contrary, nitrogen and phosphorus concentrations showed the reverse trend with the age of the forest regrowth. *P.kesiya* and *Q. dealbata* were the common species present in all the three regrowths. *P. kesiya* generally had greater concentrations of carbon and lignin than the *Q.dealbata*. C/N and lignin/N ratios were also significantly ($P<0.05$) greater for *P.kesiya* than *Q. dealbata*. However, N and P concentration showed a reverse trend. In the 16-year old regrowth *S. khasiana* had the highest concentration of N and P followed by *Q. griffithii*, *Q. dealbata* and *R. arboreum* in the descending order. Lignin content was minimum in *Q. dealbata* and *S. khasiana* litter and maximum in *P. kesiya* and *R. arboreum* litter (Table 7.1). C/N and lignin/N ratios were lowest for *S. khasiana*, whereas the maximum was recorded for *R. arboreum* litter.

Table 7.1. Initial chemical composition of the leaf litter from the three forest regrowths.

Sources of litter in different regrowths	Chemical properties (%) C	N	P	Lignin	C/N	Lignin/N
7-year old regrowth						
P. kesiya litter	40.80 ±0.16	0.80 ±0.31	0.098 ±0.005	36.21 ±1.32	51.0	45.26
Q. dealbata litter	36.08 ±0.29	0.84 ±0.16	0.121 ±0.08	18.32 ±0.19	42.95	21.80
13-yr old regrowth						
P. kesiya	47.60 ±3.21	0.73 ±0.06	0.068 ±0.03	43.34 ±2.87	65.20	59.36
Q. dealbata	37.30 ±1.09	0.87 ±0.12	0.089 ±0.01	24.40 ±1.32	42.87	28.04
16-year old regrowth						
P. kesiya litter	47.90 ±2.87	0.70 ±0.12	0.054 ±0.08	45.10 ±2.87	68.42	64.42
Q. dealbata litter	39.30 ±1.62	0.78 ±0.16	0.079 ±0.06	24.00 ±1.09	50.38	30.76
Q. griffithii litter	36.40 ±0.81	0.83 ±0.16	0.058 ±0.06	36.80 ±1.31	43.85	44.33
S. khasiana litter	32.10 ±0.87	1.09 ±0.031	0.087 ±0.06	23.20 ±0.88	29.45	21.28
R. arboreum litter	42.30 ±1.87	0.60 ±0.81	0.044 ±0.02	41.30 ±1.68	70.5	68.83

CHANGES IN pH DURING SOIL INCUBATION

Initially, pH of the soil was 5.21, 5.26 and 5.12 in the 7-, 13- and 16-year old regrowths, respectively (Table 7.2). Soil pH in the control as well as in other treatments increased after 15 days of incubation. There was a gradual increase in soil pH in the litter treated soils with respect to the controls during first 15 days of incubation, however, *P.kesiya* added soil showed a decline. There was not much variation in the pH during next 30, 60 and 90 days of incubation in the non-amended soils of 7- and 13-year old stands. On the contrary, the control treatment representing 16-year old stand showed a marked decline in the pH during 30, 60 and 90 days of incubation when compared to the pH after 15 days incubation. *Q. dealbata*-added soil usually had higher soil pH than *P. kesiya*-treated soil irrespective of the regrowth age. In the soil of 16-year old regrowth there was not much variation in pH between the different treatments.

CHANGES IN THE CONCENTRATION OF AMMONIUM AND NITRATE N DURING INCUBATION

Initially, the concentration of total inorganic-N was 8.25 13.49 and 16.54 $\mu g\ g^{-1}$ in the soils of 7-, 13- and 16 year old forest stands, respectively. Ammonium and nitrate-N concentration did not show much variation among them when the experiment was started. The level of nitrate in the control (i.e. soil only treatment) remained almost constant for the initial 30 days, after which there was a two-fold increase in nitrate level in the soil of 13-year old regrowth and about three-fold increase in the soils of 7- and 16-year old regrowths

Table 7.2. Changes in pH of soil amended with leaf litter of dominant tree species from the three forest regrowths during 90 days of incubation

Treatment	Incubation period (days)			
	15	30	60	90
7-year old stand				
control **5.21***	5.88	5.73	5.85	5.88
Soil + *P.kesiya* litter	5.13	5.50	5.03	5.12
Soil + *Q.dealbata* litter	5.78	5.64	5.55	5.52
13-year old stand				
control **5.26***	5.46	5.20	5.29	5.31
Soil + *P.kesiya* litter	5.20	4.98	4.96	5.01
Soil + *Q. dealbata* litter	5.35	5.19	5.24	5.41
16-year old stand				
control **5.12***	5.86	5.27	5.39	5.41
Soil + *P. kesiya* litter	4.96	5.13	5.10	5.03
Soil + *Q. dealbata* litter	5.50	5.12	5.36	5.47
Soil + *Q. griffithii* litter	5.29	5.27	5.38	5.40
Soil + *S. khasiana* litter	5.62	5.29	5.40	5.48
Soil + *R. arboreum* litter	5.69	5.26	5.35	5.36

*Values in bold are initial soil pH values.

at 60 days. The nitrate level remained more or less same for next 30 days (i.e. from 60-90 days) but in the soil of 13-year old regrowth an increase in nitrate level was recorded during this period (Table 7.2).

Concentration of ammonium-N in the control as well as litter amended soils showed a gradual increase during initial 15 days of incubation. After 15 days a sharp decline was recorded in the level of ammonium-N in 7- and 13-year old regrowths. The concentration of ammonium-N in the soil of 7- and 16-year old regrowth again showed a steady increase after 60 days of incubation which continued to increase till 90 days in the 7-year old regrowth. Two peaks were observed in the concentration of ammonium-N in the 16-year old regrowth; one after 15 days and another after 60 days of incubation. In the soils of 13-year old regrowth the level of ammonium-N showed a steady increase up to 60 days of incubation after which it dropped significantly in both control as well as litter-amended soils (Table 7.2).

N MINERALIZATION OR IMMOBILIZATION

The net N mineralization rate ($\mu g\ g^{-1}\ day^{-1}$) showed altogether different trend. Mineralization rate was lower in the litter-amended soils when compared to control after 15 days incubation. In control as well as litter-added soils from the 7- and 16- year old regrowths, generally, immobilization was recorded after 15 days incubation, except for the *S. khasiana*-amended soil from the latter regrowth. The peak mineralization rate was observed after 60 days of incubation in control as well as litter-amended soils of all the three forest regrowths (Figure 7.1). Mineralization rate in the control was singnificantly greater in the soils of 13- and 16-year old regrowths than that of 7-year old regrowth. The soil from the 13-year old

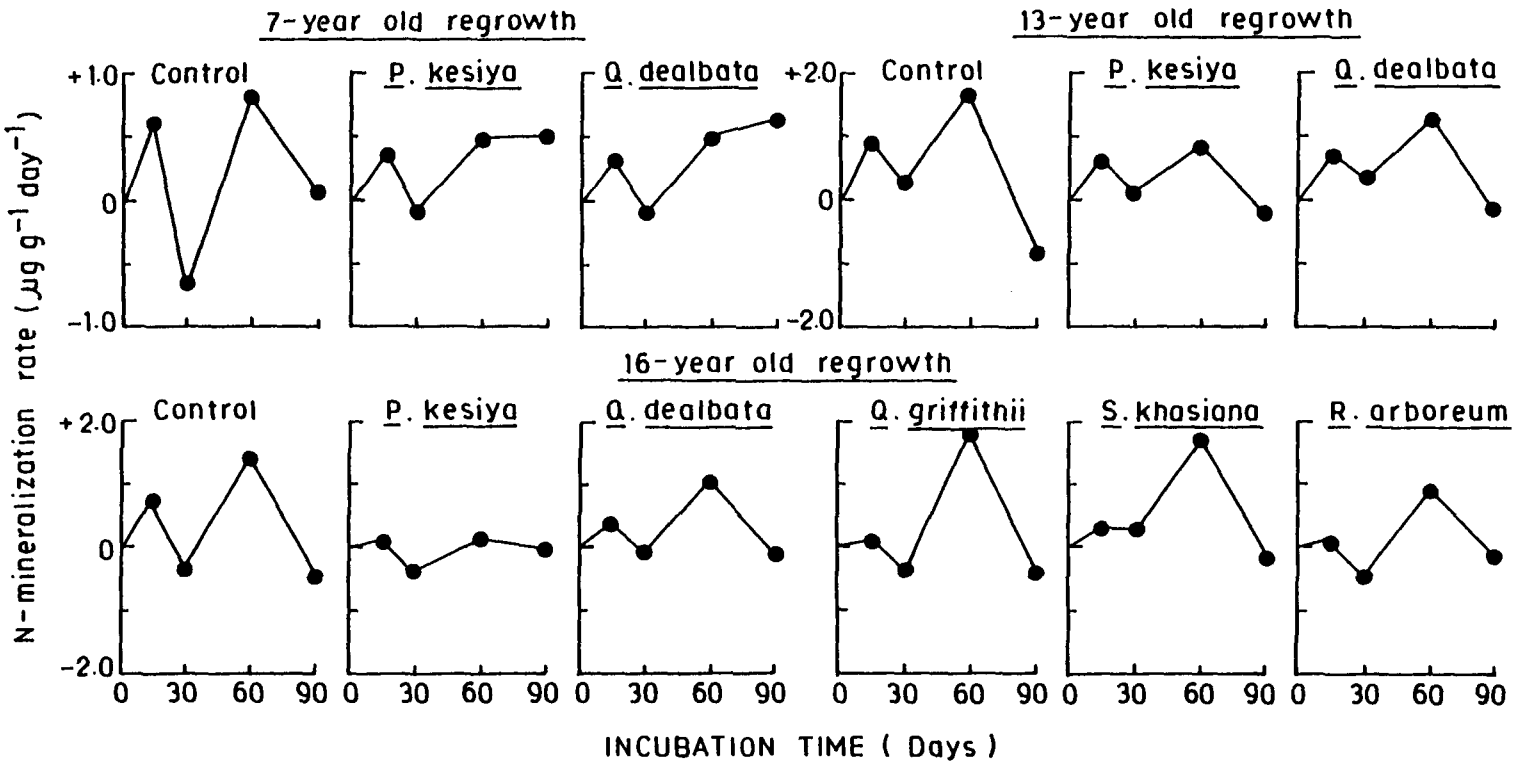

Figure 7.1: N mineralization rate during 0-90 days of incubation from soils of the three forest regrowths.

Table 7.3. Changes in the concentration of ammonium and nitrate-N (µg g^{-1}) during 90 days of incubation.

Treatments	INCUBATION PERIOD														
	0 day			15 days			30 days			60 days			90 days		
	Amm.	Nit.	Tot.	Amm.	Nit.	Tot.	Amm.	Nit.	Tot.	Amm.	Nit.	Tot.	Amm.	Nit.	Tot.
7-yr old															
Control	4.81	3.44	8.25	12.62	4.13	16.75	3.50	4.14	7.64	18.54	12.49	31.03	20.31	12.51	32.70
						(0.57)			(-0.61)			(0.78)			(0.06)
S+*P. kesiya* litter				6.34	7.16	13.50	0.05	3.04	3.09	11.05	7.56	18.60	18.31	15.81	34.10
						(0.35)			(-0.70)			(0.52)			(0.52)
S+*Q. dealbata* litter				6.96	6.80	13.76	2.74	1.25	3.99	10.26	8.79	19.05	21.31	17.80	39.11
						(0.37)			(-0.65)			(0.50)			(0.67)
13-yr old regrowth															
Control	8.32	5.17	13.49	18.93	8.05	26.98	25.00	6.25	31.25	66.90	13.38	80.28	31.21	21.08	52.29
						(0.90)			(0.28)			(1.63)			(-0.90)
S+*P. kesiya* litter				14.66	7.53	22.19	19.73	3.81	23.54	37.70	10.88	48.58	26.31	16.91	43.20
						(0.58)			(0.09)			(0.83)			(-0.18)
S+*Q. dealbata* litter				18.40	5.29	23.69	25.00	9.14	29.14	55.30	11.73	67.03	39.12	21.98	61.10
						(0.68)			(0.36)			(1.26)			(-0.20)
16-yr old regrowth															
Control	9.46	7.08	16.54	20.45	7.21	27.66	15.32	6.45	21.77	46.64	18.53	64.17	29.81	18.11	47.92
						(0.74)			(-0.38)			(1.41)			(-0.54)
S+*P. kesiya* litter				9.85	5.35	17.58	8.62	3.70	12.32	25.32	14.19	39.50	22.31	11.12	33.43
						(0.07)			(-0.35)			(0.91)			(-0.20)
S+*Q. dealbata* litter				14.10	8.03	22.13	10.86	4.71	15.57	35.80	11.69	35.80	28.32	15.71	44.03
						(0.37)			(-0.43)			(1.06)			(-0.12)
S+*Q. griffithii* litter				12.00	6.69	18.69	11.86	6.18	12.04	47.32	19.87	67.19	32.81	21.71	54.52
						(0.14)			(-0.44)			(1.83)			(-0.42)
S+*S. khasiana* litter				14.28	6.51	20.79	16.93	8.03	24.96	52.78	22.01	74.79	42.81	26.81	69.22
						(0.28)			(0.28)			(1.70)			(-0.18)
S+*R. arboreum* litter				11.75	5.83	17.58	6.29	4.03	10.32	25.80	11.28	37.08	20.12	10.11	30.23
						(0.07)			(-0.48)			(0.88)			(-0.23)

Note: Values in parentheses are the mineralization rates (µg g^{-1} d^{-1}); S-Soil, Amm.-Ammonium, Nit.-Nitrate, Tot.-Ammonium+Nitrate

regrowth showed net N mineralization up to 60 days after which immobilization was recorded in control as well as litter-amended soils.

Cumulative N mineralization, ammonification and nitrification rates calculated at the end of 90 days of incubation showed different trends. The control from the 13-year old regrowth exhibited highest rates of ammonification, nitrification and N mineralization followed by 16- and 7-year old regrowths, respectively (Table 7.5). *Q.dealbata*-amended soils exhibited greater rates of N mineralization than the *P.kesiya*-amended soils in all the three forest regrowths. *S.khasiana*-amended soils from the 16-year old regrowth recorded highest rates of ammonification, nitrification and N mineralization, whereas the least values were recorded for the *R.arboreum*-added soils (Table 7.4).

ACCUMULATION/DEPLETION OF N IN THE LEAF LITTER

The release of nitrogen from the leaf litter followed similar trend during first 60 days of incubation in the soils of all the three forest regrowths. The leaf litter of *Q.dealbata* and *P.kesiya* generally showed net depletion of N or immobilization during first 60 days of incubation. There was a net release of N after 30 days of incubation from the soils of 16-year old regrowth when amended with leaf litter of *S.khasiana*. This gradually increased with the incubation time. *Q.griffithii* litter also showed net release of N after 60 days of incubation. However, *P.kesiya* litter exhibited net immobilization of N throughout the 90 days of incubation in the soils from 13- and 16-year old forest regrowths. However, in the case of soil from the 7-year old regrowth slight mineralization was recorded for *P.kesiya* leaf litter after 60 days of incubation (Figure 7.2). Leaf litter of *S.khasiana*

Table 7.4 : Ammonification, nitrification and N-mineralization rates of soils from the three forest regrowths amended with leaf litter over 90 days of incubation.

Treatment	Ammonification ($\mu g\ g^{-1}\ day^{-1}$)	Nitrification ($\mu g\ g^{-1}\ day^{-1}$)	N-mineralization ($\mu g\ g^{-1}\ day^{-1}$)
7-year old stand			
Control	0.172	0.100	0.271
Soil + *P. kesiya* litter	0.156 (10.25)	0.137 (27.0)	0.287 (5.57)
Soil + *Q. dealbata* litter	0.183(6.01)	0.159 (37.10)	0.342 (20.76)
13-year old stand			
Control	0.254	0.176	0.431
Soil + *P. kesiya* litter	0.199 (27.63)	0.130 (35.3)	0.330 (30.60)
Soil + *Q. dealbata* litter	0.342 (25.73)	0.187 (5.88)	0.529 (18.52)
16-year old stand			
Control	0.226	0.122	0.348
Soil + *P. kesiya* litter	0.142 (59.15)	0.045 (171.1)	0.187 (87.09)
Soil + *Q. dealbata* litter	0.209 (8.13)	0.095 (28.42)	0.305 (14.09)
Soil + *Q. griffithii* litter	0.259 (12.74)	0.162 (24.69)	0.585 (40.51)
Soil + *S. khasiana* litter	0.370 (38.91)	0.219 (44.29)	0.585 (40.51)
Soil + *R. arboreum* litter	0.118 (91.52)	0.033 (26.98)	0.152 (128.9)

Values in parentheses are % increase or decrease in rates with respect to control.

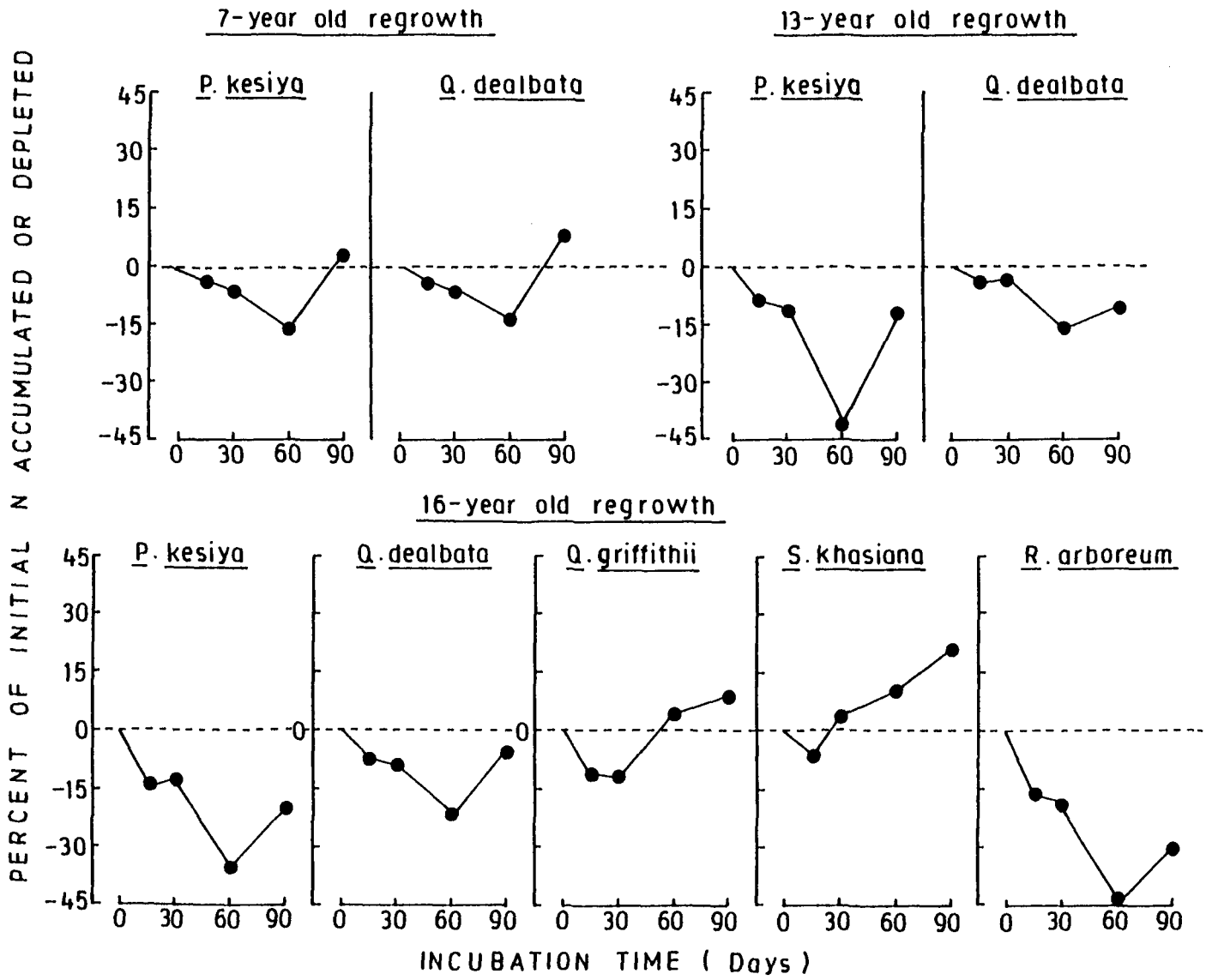

Figure 7.2: Net N accumulated or depleted in different treatments during 90 days of incubation.

showed maximum release of nitrogen followed by *Q.griffithii*, and the maximum immobilization was recorded in the leaf litter of *R.arboreum* followed by *P.kesiya*.

RELATIONSHIP AMONG THE INITIAL LITTER CHEMICAL PROPERTIES

Across all the litter types initial leaf-N was positively correlated with the initial P and negatively with C, lignin, C/N and lignin/N ratios (Table 7.5). Initial lignin content was negatively correlated with the initial N and P and positively with initial C, C/N and lignin/N ratios. The C/N and lignin/N ratios showed a strong negative correlation with initial leaf-N (Table 7.5).

RELATIONSHIP OF N ACCUMULATION/DEPLETION WITH INITIAL N CONCENTRATION OF THE LEAF LITTER

The percent nitrogen accumulated in or depleted from the leaf litter was positively correlated with initial N concentration (Table 7.6, 7.7). P concentration of litter also showed a positive correlation with percent original N accumulated or depleted but the relationship was not significant. Carbon, lignin, C/N and C/P ratios showed a significant ($P<0.001$) negative correlation with N accumulation or depletion.

DISCUSSION

Chemical composition of litter determines the turnover rate of organically bound nutrients. Initial N (0.60-1.09%) and lignin (23-45%) concentrations of leaf litter of dominant tree species are well within the range (0.36-3.90%) and 4.5-46%) reported by Vogt *et al.*

Table 7.5. Correlation matrix for the relationship among initial chemical composition of leaf litter from the three forest regrowths (n = 9).

Variates	C	N	P	Lignin	C/N ratio	Lignin/N ratio
C	1.000	-0.786**	-0.512	0.811**	0.922***	0.840***
N		1.000	0.549	-0.670*	-0.935***	-0.822***
P			1.000	-0.776**	-0.638*	-0.794***
Lignin				1.000	0.808***	0.958***
C/N					1.000	0.921***
Lignin/N						1.000

*P<0.05; ** P <0.01; *** P <0.005; **** P <0.001

Table 7.6. Correlation coefficients (r) of initial chemical composition of litter with fraction of leaf litter N mineralized/immobilized during different time intervals. n=9.

Incubation time (days)	Chemical properties					
	carbon	nitrogen	phosphorus	lignin	C/N	lignin/N
15	-0.438	0.604	0.887**	-0.678*	-0.654*	-0.766**
30	-0.680*	0.922***	0.766**	-0.760**	-0.872**	-0.877**
60	-0.871**	0.872**	0.441	-0.604	-0.937***	-0.759**
90	-0.831**	0.928***	0.673*	-0.728**	-0.973***	-0.868**

*P<0.05; **P<0.01; ***P<0.001

Table 7.7. Relationship of percent initial N accumulated/depleted with initial chemical composition of litter. n = 36.

Variates	Regression equation	r^2
C (%)	$Y = 59.71 - 1.731\ x^{***}$	-0.385
N (%)	$Y = -69.978 + 75.169\ x^{***}$	0.473
P (%)	$Y = -10.345 + 8.092\ x^{NS}$	0.0079
Lignin	$Y = 16.015 - 0.784\ x^{***}$	-0.285
C/N	$Y = 30.233 - 0.769\ x^{***}$	-0.518
Lignin/N	$Y = 12.766 - 0.521\ x^{***}$	-0.419

$^{***}P < 0.001$; ^{NS} = Not significant

(1986, 1991, VanVauren *et al.* 1992, Myers *et al.* 1994) for various tropical and temperate tree species. Species having more sclerenchymatous cells *e.g. P.kesiya* and *R.arboreum* had greater lignin concentration and low nutrient level. The C/N ratio of the resource varied between 29.5-70.0 thus indicating that the organic material used in the present study is of low quality. The low quality material releases nutrients slowly, and/or immobilizes nutrients during the early stages of decomposition The material that has a high C/N ratio (>25) and which immobilizes N as it decomposes, is considered to be of low quality.

The changes in the soil pH during the incubation was not consistent. The lower soil pH in *P.kesiya*-amended soil as compared to *Q.dealbata* in all the three regrowths is attributable to the chemical composition. Pine litter usually lowers the soil pH. Ammonium was the dominant form of inorganic N in the soils of all the three regrowths. At 60 days of incubation about 2 to 6 fold increase was observed in the ammonium concentration *vis-a-vis* the nitrate level. The cumulative ammonification rate recorded at 90 days of incubation ranged between 0.12-0.37 $\mu g\ g^{-1}\ day^{-1}$ which are comparable to the rates obtained under *in situ* conditions from the same soils (0.14-0.21 $\mu g\ g^{-1}\ day^{-1}$). The nitrification rates were, however, lower (0.045-0.219 $\mu g\ g^{-1}\ day^{-1}$) than those obtained under the field conditions (0.12-0.15 $\mu g\ g^{-1}\ day^{-1}$). *P.kesiya* addition resulted in 10-59% decrease in the ammonification rate when compared to the control. The immobilization caused by *P.kesiya* needle was highest in the soil from 16-year old regrowth. *Q.dealbata* addition resulted in *ca.* 6 and 25% increase in the ammonification rates in the 7- and 13-year old regrowths, respectively. However, in the 16-year old forest soils the addition of

Q.dealbata leaf litter resulted in 8% decrease in the cumulative ammonification rate measured after 90 days of incubation. In the soils from the 16-year old regrowth the addition of *S.khasiana* litter resulted in about 38% increase in ammonification rate and *Q.griffithii* registered about 12% increase. The maximum decrease (91%) in the ammonification rate was observed due to addition of *R. arboreum*-amended soil followed by *P. kesiya* which caused 59% decrease in ammonification rate. The net N mineralization rate showed about 5 and 20% increase due to the addition of *P. kesiya* needle and *Q. dealbata* leaf litter in the 7-year old stand. However, in the 13- and 16-year old forest soils about 30 and 86% decrease was recoreded due to the addition of *P. kesiya* needles (Table 7.4).

The immobilization or depletion of nitrogen observed in the present study may be attributed to the low N content of the litter. The N content of leaf litter was less than 2% which might have resulted in the initial immobilization observed in most of the cases. However, the fast release or mineralization of N from the leaf litter of *S. khasiana* in the soil of 16-year old regrowth may be attributed to its low lignin content. Though the initial N content was less than 2%, the low lignin and low C/N ratio might have contributed to the release of N after 15 days (Fig 7.2). A strong positive relationship was observed between initial nitrogen and percent of leaf-N accumulated or depleted during incubation (Table 7.6, 7.7). This is in agreement with the reports of Singh & Gupta (1977); Vogt *et. al* (1991) and Bloomfield *et. al* (1993).

The significant negative ($P<0.001$) correlation observed between the initial lignin content and percent N accumulated or depleted suggests that apart from N concentration lignin content had also

influenced N release from the litter. Therefore, *R. arboreum* leaves which had high lignin and low N content decomposed at a slow rate, while *S. khasiana* and *Q. griffithii* which had high N content and low lignin content decomposed at a faster rate.

In the present study C/N and lignin/N ratios showed a significant ($P<0.001$) negative correlation with percent nitrogen accumulated or depleted. These findings are in agreement with Melillo *et. al* (1982) who reported a significant inverse relationship between initial lignin/N ratio and net decay rate. Relatively slow rate of N release from the leaf litter in the present study is attributed to their sclerenchymatous nature. These substances are known to control decay rate by showing resistance to enzymatic attack and by physically interfering with the degradation of other chemical features of the cell wall (Bloomfield *et al.* 1993).

It is quite evident from the foregoing discussion that the release of N from the leaf litter is regulated by its initial N and lignin content. The rates of N accumulation from various plant species and soil can be used in the modelling studies to evaluate the long-term effects of organic inputs on soil fertility. However, in order to reach any conclusion, long-term studies are essential as the plant material initially immobilizes N and subsequently releases it slowly.

CHAPTER 8

INFLUENCE OF FINE AND COARSE ROOTS ON N MINERALIZATION

INTRODUCTION

Fine roots play a major role in nutrient absorption in forest ecosystems and contribute substantially to the soil organic matter (Vogt *et al.* 1991). McClaugherty *et al.* (1982) suggested that fine roots together with the aboveground litter provide the main bulk of organic material for the complex decomposition cycle in the soil system. In spite of their importance, only a few reports are available on the decomposition dynamics and nutrient mineralization patterns of the roots in the forest ecosystem (McClaugherty *et al.* 1984, Bloomfield *et al.* 1993). Swift *et al.* (1979) reported that decomposition of detritus on the forest floor is influenced by the climatic and edaphic conditions prevailing there. Apart from these environmental variables, initial N, lignin, and polyphenol content and C/N as well as lignin/N ratios are also quite important in the decomposition dynamics of roots in the forest ecosystems (cfs. Vogt *et al.* 1991).

Recent studies on detrital organic matter dynamics in forest ecosystems have shown marked variation between foliage and root litter with respect to their chemical composition (Bloomfield *et al.* 1993, Arunachalam *et al.* 1996c). Vogt *et al.* (1991) in their significant

review paper reported that the fine roots (< 2 mm diameter) have higher nutrient concentration compared to the leaf litter. Although N mineralization of leaf litter has been studied in detail, the decomposition and N mineralization of fine and coarse roots are not well understood.

The present chapter deals with *in vivo* effects of fine and coarse root addition on N mineralization in soils collected from forest regrowths of three different ages.

METHODS

Soil samples down to 10 cm depth together with the roots present in them were collected during August 1994 from the degraded subtropical forest stands representing three different stages *viz.*, 7, 13 and 16 years of vegetation regrowth after tree cutting. Roots from the mineral soils were retrieved by dry-sieving method (Bohm 1979). The soils were seived through 2 mm mesh screen and homogenized separately for each of the three stands. pH, moisture and initial nitrate and ammonium concentrations of the field moist soils were determined. The separated roots were washed under a gentle flow of tap water to remove the extraneous matter. The roots were grouped into fine (< 2 mm diameter) and coarse (> 2 mm diameter) fractions, and were air-dried. Roots beyond 15 mm diameter were discarded. The dried materials were choped into approximately 1 mm size and then used for the incubation study. A portion of the roots was oven-dried at 80 °C, ground in a cyclotec (TECATOR) and used for the chemical analyses. Initial chemical properties of root were determined following the methods given in Chapter 7. The physico-chemical properties of the soil collected from the three forest regrowths used for incubation are

given in Chapter 4.

LABORATORY INCUBATION EXPERIMENT

100 g of field moist soil was collected from each of the three forest regrowths, seived (2 mm mesh size) and adjusted separately to 60% of the water holding capacity by adding deionized water, and was then taken in a 250 ml polyethylene beaker and incubated at room temperature for 1 week to allow the microbial activity to settle down. 1 g of fine and/or coarse root sample was added to the respective forest soils (100 g), and one treatment of fine plus coarse root in the ratio 1:1 (0.5+0.5 g) was also maintained for all the three forest soils. The 'soil only' treatment served as control. Twelve beakers were set up for each treatment in each of the forest soil and the replicated samples were incubated at 25±1°C in a BOD incubator. The soil moisture content was kept constant throughout the experiment by periodic addition of deionized water. Three beakers from each treatment were randomly selected after 15, 30, 60 and 90 days of incubation for the determination of soil pH and extraction of nitrate and ammonium.

CALCULATION METHODS

N mineralization rate was calculated by subtracting the initial inorganic-N present in soil from the N accumulated in the soil after the respective incubation period.

$$\text{N mineralization rate } (\mu g\ g^{-1}\ day^{-1}) = \frac{\text{Final N conc. - Initial N conc.}}{\text{Number of incubation days}}$$

By subtracting the total extractable-N in the control ('soil only') from that in 'root+soil' treatments, the accumulation or

depletion of inorganic-N attributable to the presence of roots was calculated. This difference was divided by the initial root-N added to each incubation and the fraction thus obtained was multiplied by 100 to express the result as percent of the initial root-N.

STATISTICAL ANALYSIS

Tukey's test was used to determine the statistical significance of variations between sampling time, type of treatment, etc. Linear regressions were worked out following Zar (1974), wherever necessary.

RESULTS

INITIAL CHEMISTRY OF FINE AND COARSE ROOTS

Concentrations of C, P and lignin and C/N as well as lignin/N ratios in fine and coarse roots were significantly higher ($P<0.05$) in the 16-year old regrowth compared to the root materials from the 7-year old regrowth. However, N concentration was maximum in the latter stand followed by the 13- and 16-year old regrowths (Table 8.1).

Fine and coarse roots also showed wide variation with respect to their initial chemical composition. The concentrations of C and lignin were significantly higher ($P<0.05$) in the coarse roots than the fine roots of all the three forest stands. On the contrary, N and P concentrations were significantly greater in the fine roots than the coarse ones. C/N and lignin/N ratios also followed the similar trend (Table 8.1).

CHANGES IN SOIL pH DURING INCUBATION

The soil pH showed a gradual change with incubation time. During

Table 8.1. Initial chemical composition of fine and coarse roots collected from the three forest regrowths.

Component	Chemical composition					
	C (%)	N (%)	P (%)	Lignin	C/N	Lignin/N
7-year old regrowth						
Fine roots	37.10 ±0.13	1.26 ±0.03	0.036 ±0.008	16.91 ±1.36	29.44 ±3.21	13.42 ±1.82
Coarse roots	56.31 ±0.28	0.98 ±0.06	0.039 ±0.006	26.32 ±1.14	57.45 ±3.87	26.85 ±2.13
13-year old regrowth						
Fine roots	44.40 ±0.86	1.18 ±0.31	0.058 ±0.011	22.93 ±1.36	37.62 ±1.68	19.43 ±2.08
Coarse roots	58.14 ±0.82	0.95 ±0.01	0.047 ±0.002	34.18 ±3.16	61.2 ±4.08	35.97 ±3.17
16-year old regrowth						
Fine roots	44.60 ±1.61	1.02 ±0.61	0.088 ±0.016	22.32 ±0.82	43.72 ±1.87	21.88 ±2.16
Coarse roots	59.12 ±0.62	0.78 ±0.31	0.078 ±0.001	39.82 ±0.96	75.79 ±1.32	51.05 ±0.83

± SEM (n=3)

15-30 days of incubation, soil pH did not vary much, except the control in case of 7-year old regrowth which registered a 12% increase from 15 to 30 days of incubation. After 30 days, there was a significant increase in soil pH in all the three forest soils. After 60 days of incubation, however, a gradual decline was observed in soil pH.

During initial 15 days of incubation the fine and coarse root addition caused an increase in the pH of soil from the 7-year old regrowth, however, in the case of soils from the older regrowths there was a decrease in pH due to fine and coarse root addition. At 30 days of incubation fine and coarse root loading lowered the pH of soils from all the three regrowths with respect to control. But again, there was an increase in the soil pH at 60th and 90th day of incubation in all treatments. This increase was, however significant only in the case of 7-year old regrowth (Table 8.2).

CHANGES IN AMMONIMUM AND NITRATE CONCENTRATIONS DURING INCUBATION

Concentration of nitrate-N generally showed a gradual increase with the incubation time in soils from all the three stands. However, no definite trend was observed in case of ammonium-N concentration. Initially (at 15 days of incubation), the concentration of ammonium-N registered an increase due to treatments in the soils from the three forest stands, but later on no consistent trend was noticed. Despite these fluctuations, a significant increase ($P<0.05$) in ammonium-N concentration was observed at 90th day of incubation. The concentration of total inorganic-N (ammonium+nitrate) generally increased with the incubation time, except in the case of soil from the 16-year old regrowth where a decline was observed at 60 days of

Table 8.2. Changes in pH during 90 days of incubation of the soil amended with fine and coarse roots from the forest regrowths of three different ages.

	Incubation time (days)			
	15	30	60	90
7-year old regrowth				
Treatments				
control	4.59	5.21	6.29	6.21
soil+FR	5.02	5.0	7.21	7.05
soil+CR	5.17	5.12	6.56	6.32
soil+FCR	5.21	5.06	6.82	6.39
13-year old regrowth				
Treatments				
control	5.20	5.25	5.98	5.81
soil+FR	4.89	4.92	6.28	6.10
soil+CR	4.92	5.12	6.13	5.78
soil+FCR	4.86	5.09	6.28	5.86
16-year old regrowth				
Treatments				
control	4.99	5.01	5.84	5.76
soil+FR	4.90	5.12	5.97	5.83
soil+CR	5.02	5.06	5.86	5.79
soil+FCR	4.93	5.35	5.72	5.59

FR-fine roots, CR-coarse roots, FCR-fine+coarse roots (1:1)

incubation (Table 8.3).

N MINERALIZATION AND IMMOBILIZATION

N mineralization rate showed a sharp increase during the initial 15 days of incubation except in the coarse-root treated soils of 13- and 16-year old regrowths. At 30th day of incubation there was a sharp decline in N mineralization rate in the control and 'soil+fine root' treatment in all the three forest soils. However, in the 'soil+coarse root' and 'soil+coarse & fine root' treatments a slightly different pattern was observed. In the 16-year old regrowth, during 60 days of incubation immobilization was the dominant process in all the treatments, after which there was a sharp increase in N mineralization rate until 90 days of incubation (Figure 8.1). Thus, two peaks, one during initial 15 days of incubation and another at the end of the experiment (at 90th day of incubation) were observed in the soil of the 16-year old regrowth, whereas in the soils of the 7- and 13-year old regrowths, N mineralization rates ramained more or less the same throughout the experimental period (Figure 8.1).

Net ammonification, nitrification and N mineralization rates calculated for 90 days of incubation showed different trends. Generally, ammonification rates were greater than the nitrification rates except for the soil of the 7-year old regrowth in control where a reverse trend was observed.

Ammonification, nitrification and N mineralization rates were higher in the soil of the 7-year old stand, the rates declined in the 13-year old stand and again showed a significant ($P<0.05$) increase in the soil of the 16-year old forest stand (Table 8.4).

Table 8.3. Changes in the concentration of ammonium and nitrate-N (μg g^{-1}) during 90 days of incubation.

	Incubation time (days)														
	0			15			30			60			90		
	Amm.	Nit.	Total	Amm.	Nit.	Total	Amm.	Nit.	Total	Amm.	Nit.	Total	Amm.	Nit.	Total
7-year old regrowth															
Treatments															
control	10.94	4.76	15.70	22.35	7.06	29.41	18.15	12.11	30.26	16.47	14.41	30.88	24.31	19.84	47.15
soil+FR				20.47	6.47	26.94	23.85	10.21	33.06	27.30	19.53	46.83	39.01	27.31	66.32
soil+CR				15.53	6.00	21.53	16.14	9.87	26.01	20.35	12.18	32.53	32.01	20.42	52.43
soil+FCR				17.32	6.41	23.73	22.87	10.11	32.98	20.87	14.86	35.73	38.88	26.89	65.77
13-year old regrowth															
Treatments															
control	14.79	8.32	23.11	26.53	16.60	43.13	21.01	9.12	30.43	24.76	9.83	34.69	31.32	18.76	50.08
soil+FR				18.38	10.97	29.35	18.32	16.81	35.13	29.47	16.29	45.76	36.72	23.47	62.19
soil+CR				14.58	7.60	22.13	16.32	7.98	24.30	28.70	10.12	38.82	30.76	21.03	51.79
soil+FCR				16.21	7.83	22.41	18.88	14.86	33.74	26.87	14.86	41.73	35.70	21.84	57.54
16-year old regrowth															
Treatments															
control	15.81	9.40	25.27	29.62	16.00	45.62	28.41	19.85	48.23	25.91	11.11	37.03	48.47	31.24	79.19
soil+FR				27.40	11.38	38.79	26.21	21.37	47.58	28.60	12.05	40.65	56.83	31.89	88.72
soil+CR				20.00	8.31	28.31	24.01	12.81	36.82	21.68	11.21	32.89	48.36	27.37	75.73
soil+FCR				22.84	8.88	31.72	24.39	16.66	41.05	22.86	10.87	33.75	46.66	29.33	75.99

FR-fine roots, CR-coarse roots, FCR-fine+coarse roots (1:1)
Amm. - Ammonium, Nit. - Nitrate

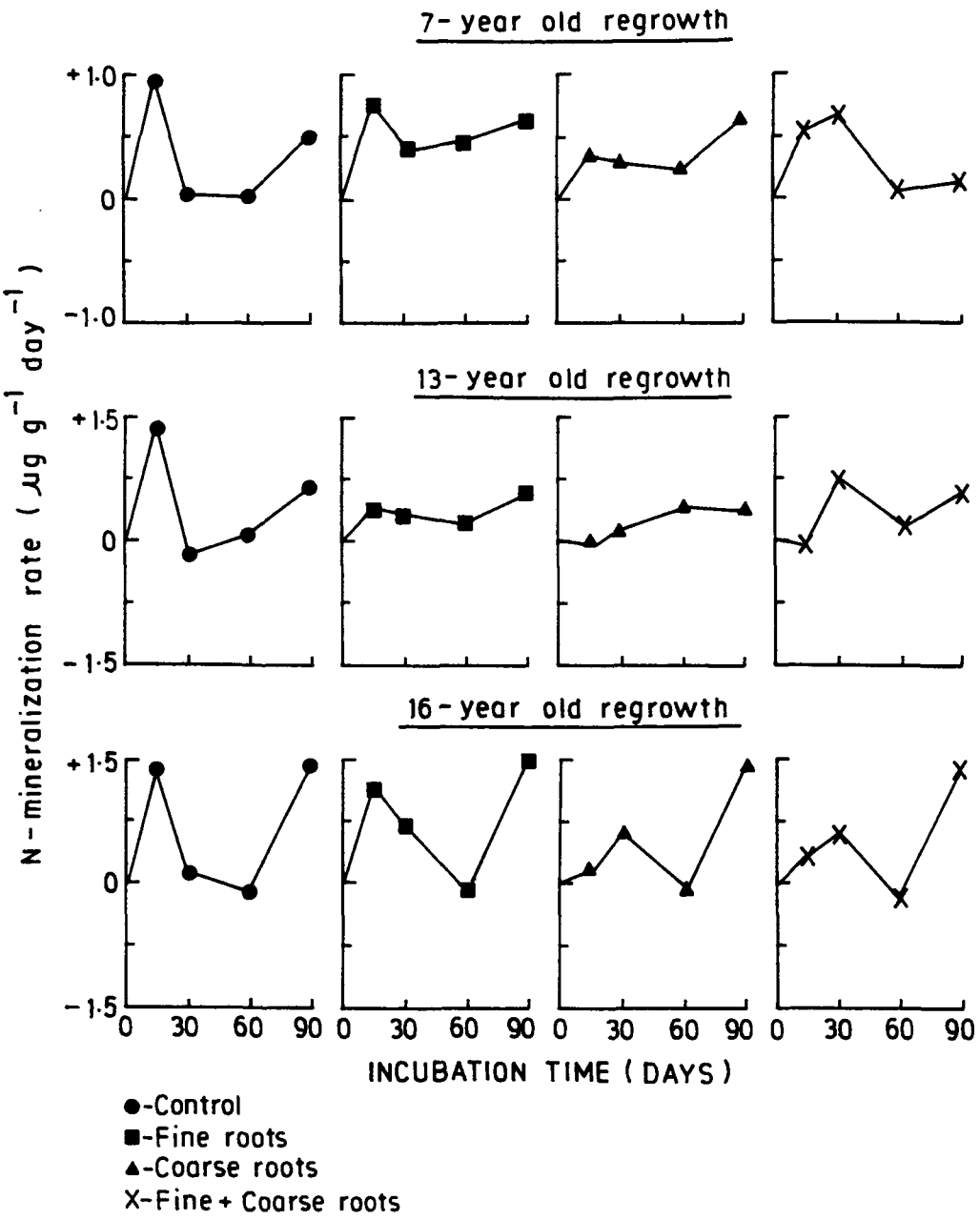

Figure 8.1: N mineralization rate during 0-90 days of incubation from soils of the three forest regrowths.

Table 8.4. Net ammonification, nitrification and N mineralization rates ($\mu g\ g^{-1}\ day^{-1}$) in different treatments during 0-90 days of incubation.

	Ammonification	Nitrification	N mineralization
7-year old regrowth			
Treatments			
control	0.148	0.167	0.349
soil+FR	0.311 (52.41)	0.250 (33.2)	0.562 (37.90)
soil+CR	0.234 (36.75)	0.174 (4.0)	0.410 (14.8)
soil+FCR	0.310 (52.2)	0.246 (32.1)	0.556 (36.65)
13-year old regrowth			
Treatments			
control	0.183	0.116	0.299
soil+FR	0.266 (31.20)	0.168 (30.92)	0.434 (31.10)
soil+CR	0.177 (-3.3)	0.141(17.73	0.318 (5.97)
soil+FCR	0.232 (21.12)	0.150 (22.66)	0.382 (21.72)
16-year old regrowth			
Treatments			
control	0.362	0.242	0.607
soil+FR	0.455 (20.0)	0.249 (2.8)	0.705 (13.90)
soil+CR	0.361 (-0.28)	0.199 (-21.80)	0.560 (-8.39)
soil+FCR	0.342 (-5.8)	0.277 (-6.60)	0.563 (-7.81)

FR-fine roots, CR-coarse roots, FCR-fine+coarse roots (1:1)

Values in parentheses are the percent increase or decrease in ammonification, nitrification and N mineralization rates with respect to control.

N RELEASE FROM THE ROOTS

Release of N from the roots followed altogether different trend with incubation time. During first 15 days of incubation there was N immobilization/accumulation in the fine and coarse roots loaded soils of all the three forest regrowths. However, during next 15 days of incubation (*i.e.* at 30th day) N depletion was observed in case of 'soil+fine root' treatment, whereas 'soil+coarse root' treatment continued to accumulate N. Addition of fine and coarse root in 1:1 ratio caused a gradual release of N in the soils of 7- and 13-year old regrowths, however, in the 16-year old regrowth there was N accumulation. During 60-90 days of incubation the fine roots continued to release N in the soil and the release was significantly ($P<0.05$) greater in the soils of the younger regrowth than those of the 13- and 16-year old regrowths. Similar trend was observed in case of coarse root-treated soils of the 13- and 7-year old regrowths. However, in the soil of the 16-year old stand there was no release of N from the coarse roots throughout 90 days of incubation (Figure 8.2).

RELATIONSHIP AMONG INITIAL CHEMICAL PROPERTIES OF ROOTS

Initial root N was positively correlated with the initial P and negatively with C, lignin, C/N and lignin/N ratios (Table 8.5), whereas initial lignin content of roots was negatively correlated with initial N and P and positively with initial C, C/N and lignin/N ratios.

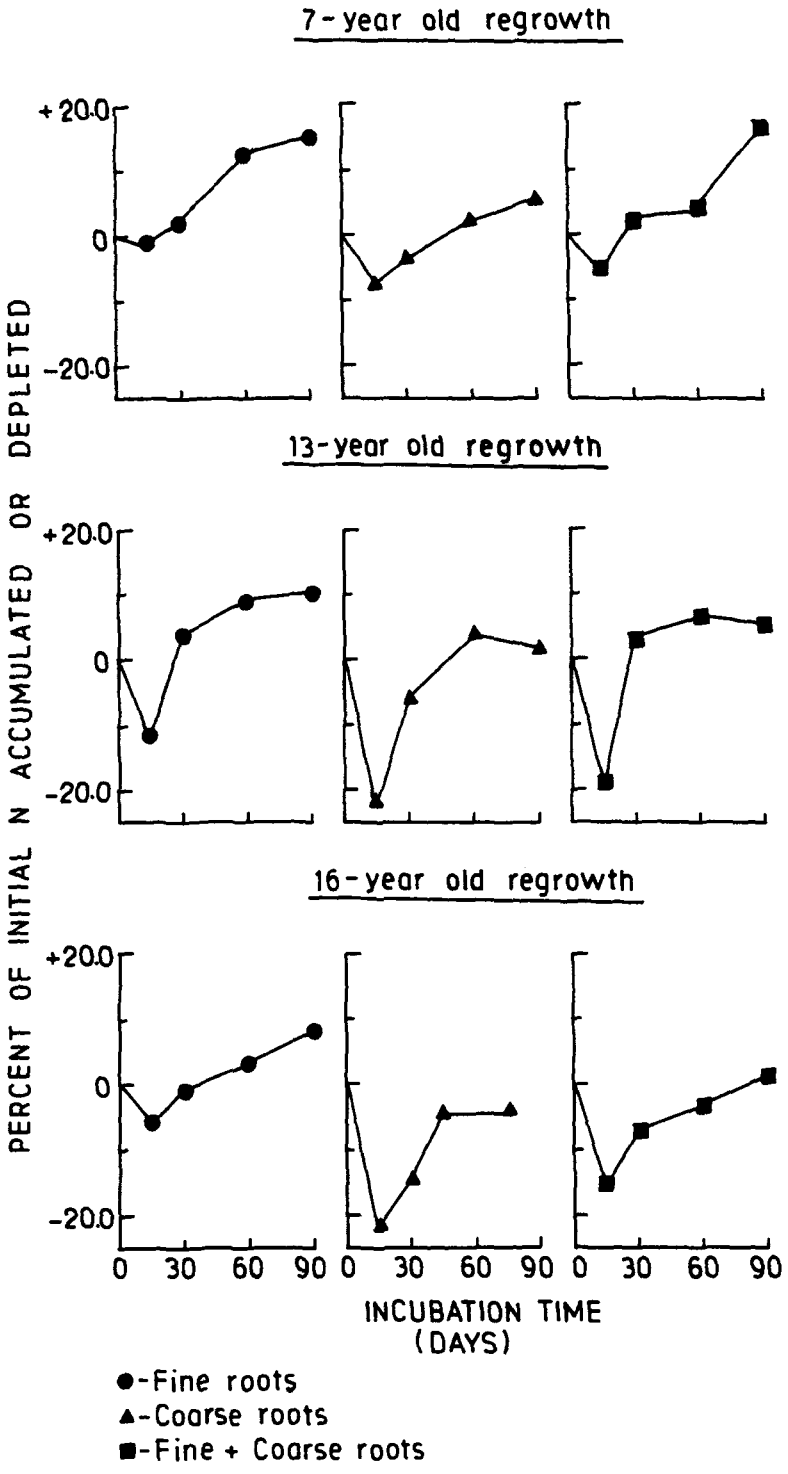

Figure 8.2: Net N accumulated or depleted in different treatments during 90 days of incubation.

Table 8.5. Correlation matrix for the relationships among initial chemical composition of roots (n=9) in the three forest regrowths.

Chemical composition	C	N	P	Lignin	C/N	Lignin/N
C	1.000	-0.747**	0.0027	0.871**	0.886***	0.734**
N		1.000	-0.331	-0.818**	-0.939***	-0.882***
P			1.000	-0.121	-0.562*	-0.281
Lignin				1.000	0.862**	0.632*
C/N					1.000	0.721**
Lignin/N						1.000

*, P<0.05; **, P<0.01; ***, P<0.005

RELATIONSHIP BETWEEN ROOT CHEMISTRY AND PERCENTAGE OF INITIAL-N ACCUMULATED OR DEPLETED DURING INCUBATION

The percent of N accumulated or depleted was positively correlated to initial N concentration during each sampling period when the data were pooled together irrespective of time (Tables 8.6, 8.7) then also the same relationship was revealed. P concentration also showed positive correlation with N accumulation/depletion, however, the relationship was not significant. Lignin showed a strong ($P<0.001$) negative correlation with the percent of initial N mineralized or immobilized; C concentration, C/N and lignin/N ratios also showed similar relationships (Tables 8.6, 8.7).

DISCUSSION

N and lignin concentrations in fine and coarse roots are well within the range (0.25-1.67% N; 20.8-59.1% lignin) reported for various forest ecosystems of the world (McClaugherty *et al.* 1984, Vogt *et al.* 1991). The higher N concentration in the fine roots than the coarse roots is in agreement with Vitousek *et al.* (1994).

The changes in soil pH during incubation may be attributed to the abundance or meagreness of the inorganic nutrients and the decomposition rate of the plant residue added (Vitousek *et al.* 1994). Ammonium is the dominant form of inorganic-N in the soils of all the three forest regrowths. Though the ammonification rates are higher than nitrification rates in all treatments, nitrate-N showed a steady increase with the incubation period. However, ammonium-N increased only up to 15 days of incubation, afterwhich, due to inconsistency in the data, no definite pattern of ammonification was descernible. The

Table 8.6. Correlation coefficients (r) from regression of chemical properties of the roots added to the soil with fraction of root nitrogen mineralized/immobilized after different periods of incubation (n=9).

Incubation time (days)	Chemical properties of roots					
	C (%)	N (%)	P (%)	Lignin (%)	C/N	Lignin/N
15	-0.61*	0.48	0.11	-0.83***	-0.59	-0.69
30	-0.56	0.84***	0.22	-0.70**	-0.81***	-0.82***
60	-0.54	0.88***	0.36	-0.59	-0.78**	-0.72**
90	-0.69*	0.84***	0.30	-0.89***	-0.84***	-0.89***

*, P<0.05; **, P<0.01; ***, P<0.005

Table 8.7. Relationship of initial root-N accumulated or depleted (%) with initial chemical composition of fine and coarse roots (n=36).

Variates	Regression equation	r	P
C (%)	Y=0.725-34.94X	-0.268	NS
N (%)	Y=42.23+39.80X	0.326	0.05
P (%)	Y=168.74+8.49X	0.116	NS
Lignin (%)	Y=21.54-0.84X	-0.840	0.001
C/N	Y=20.25-0.422X	-0.337	0.05
Lignin/N	Y=13.69-0.530X	-0.352	0.05

fluctuations in the ammonium concentration could be attributed to its immobilization by the roots. In this regard, Nadelhoffer *et al.* (1984) have reported that the forest tree roots readily take up the ammonium form of inorganic-N compared to nitrate. Hence, there is a high chance of nitrate leaching under *in situ* conditions. Net ammonification rates as observed under laboratory conditions in the present study ranged from 0.17-0.46 $\mu g\ g^{-1}\ day^{-1}$ and are much higher than those observed under *in situ* conditions in the same soils (0.14-0.21 $\mu g\ g^{-1}\ day^{-1}$, Chapter 6). The range for net nitrification rates in this study (0.12-0.25 $\mu g\ g^{-1}\ day^{-1}$) are, however, comparable to those obtained under the field conditions (0.11-0.15 $\mu g\ g^{-1}\ day^{-1}$). Fine root addition caused *ca.* 20-52% increase in net ammonification rate (Table 8.5), whereas addition of coarse roots increased the ammonification rate only in the soil of the 7-year old forest regrowth. In soils of the 13- and 16-year old regrowths, a net decrease was observed in the ammonification rates with respect to the control. This can be attributed to the differences in initial N concentration of fine and coarse roots. The higher N concentration in fine roots compared to the coarse roots might result in faster mineralization of the former during incubation. Higher percent increase in ammonification and nitrification rates in the soil of the youngest regrowth can also be attributed to the same reasons. The significant positive relationship (Table 8.7) observed between the initial N concentration and net ammonification, nitrification and mineralization rates further strengthens the above hypothesis.

Initial N concentration also showed positive correlation with the N accumulation/depletion regardless of the incubation periods, which is in agreement with Frankenberger & Abdelmagid (1985), Tian *et al.*

(1992) and Constantinides & Fownes (1994). Vitousek *et al.* (1994) reported that litter with relatively low P concentration had lower decomposition rates. In the present study, there was no significant relationship between percentage of initial root-N accumulated/depleted and initial P concentration. However, a strong negative correlation between initial root-N accumulated/depleted and lignin concentration was observed, which is in agreement with Melillo *et al.* (1982) and Melillo & Aber (1984). Lignin/N ratios (13.42-51.05, Table 8.2) are much higher than those reported (<10) by Palm & Sanchez (1991) and Oglesby & Fownes (1992). They found weak negative correlation between lignin/N ratio and mineralization which they attributed to the narrow range of lignin/N ratio. However, in the present study there was a strong negative correlation between N mineralization/immobilization with lignin/N ratio.

It has been established that materials with high C/N (>25) ratio are of low quality and generally immobilize N as it decomposes (Myers *et al.* 1994). On the contrary, material with low C/N (<25) ratio as found in case of legumes, is considered to be of high quality and it rapidly releases nutrients. The immobilization or accumulation of N in fine and coarse roots during initial 15 days of incubation may be attributable to the former reason. The greater release of N from the fine roots of the 7-year old regrowth as compared to the 13- and 16-year old regrowths could be associated to the higher N concentration and lower C/N ratio in roots of the youngest regrowth. The greater release of N from the fine roots than coarse roots during the course of incubation could also be attributed to the same reason.

In the 'fine root' plus 'coarse root' treatments (1:1), the greater mineralization or release of N from the soil of the 7-year old

regrowth than the 13- and 16-year old regrowths observed in this laboratory experiment conforms well with the results of a field study carried out by Arunachalam *et al.* (1996c) in the present study sites. It is quite evident from the foregoing discussion that fine and coarse roots contribute significantly to N mineralization in the forest soils and the process is regulated by the initial chemical composition of the roots as well as by the age of the forest regrowth.

CHAPTER 9

EFFECTS OF SOIL pH, TEMPERATURE AND MOISTURE ON N MINERALIZATION

INTRODUCTION

Soil pH is a crucial determinant of microbial growth and activity (Alexander 1977). Variation in soil pH could be attributed to cation exchange capacity and nutrient availability. Ammonium and nitrate ions are the most dynamic forms of nitrogen, which is a limiting nutrient in forest ecosystems. A few workers have attributed increasing soil organic matter and humus content to soil acidity (Nommik 1978, Shah *et al.* 1990). Bacterial and fungal growth is reported to be optimum in the pH range of 4.5-6.1. N mineralization, a microbe-mediated process, is therefore, expected to vary with difference in soil pH. However, there has been little effort to characterize the nitrogen mineralization pattern along pH gradient, especially in forest soils. Nevertheless, effects of liming, and addition of wood ash and gypsum have been studied with reference to soils under agricultural systems (Laudelout 1993, Huettle & Zoettle 1993, Zelles *et al.* 1987, Persson *et al.* 1989).

Temperature also affects microbial growth and activity, which are best at optimum temperatures beyond which there is progressive inactivation. Thus, it is expected that microbial activities in soil should respond to changes in temperature. Many studies have quantified

the influence of temperature on litter decomposition (Witkamp & Van der Drift 1961, Witkamp & Frank 1969) and N mineralization (Kladivko & Keeney 1987, Foster 1989). These studies have established that the decay or mineralization rates increase exponentially with soil temperature, over a range of 10-30°C. Scholes *et al.* (1994) reported that tropical soil temperatures also vary in the similar range, and pointed out that the relative impact of temperature on N mineralization is less understood.

The enhancement of CO_2 evolution and N mineralization following rewetting of dry soil has long been recognized as an important phenomenon in the process of C and N turnover (Birch 1960) and has been well established by laboratory studies (Vangestel *et al.* 1991, 1993, Clein & Schimel 1994). According to Birch (1960, 1964), enhanced production of mineral-N during rewetting of dry soil is a result of increased availability of organic substrates either due to chemical reactions or due to death of soil microbial cells during drying caused by water stress. Harris (1981) used the term 'downshock' and 'upshock' to describe the physiological water stress during drying and subsequent rewetting events, respectively. He also hypothesized that the thickness of the cell wall is the major determinant of microbial ability to withstand rapid change in water potential in soil.

The Shillong plateau is characterized by distinct seasonal cycle: dry winter and spring, and rainy season with heavy rainfall. Soil moisture level also varies widely ranging from 10% in dry periods to 60 % during wet period. In this context, the analysis of drying and rewetting on mineralization could be quite relevant.

In view of the above facts, a few small experiments were carried out to analyse the effects of soil pH, temperature and moisture content on N mineralization rates in the forest regrowths of three different ages, and the results are presented in this chapter.

METHODS

EFFECT OF SOIL pH

The mineral soil (0-10 cm depth) was collected randomly from 10 different points in each forest stand and brought to the laboratory for further processing. The soil from each stand was sieved through 2 mm mesh screen and immediately analysed for pH, moisture, ammonium and nitrate concentrations. 100 g of sieved soil was taken in polyethylene beakers and adjusted for different pH ranges by adding finely ground calcium carbonate or 3% sulphuric acid to raise or to lower the soil pH, respectively (Thomposon & Troeh 1975). The reagents were thoroughly mixed with the soils and left undisturbed for one month at 25±1°C to stabilize the soil pH. Then the soils having different pH were incubated at 25±1°C for another 30 days. Throughout the incubation period, moisture content was kept more or less constant by adding deionized water in calculated amounts (beaker+soil was weighed after every 3rd day and loss in weight from the initial weight was taken as loss of moisture content). After 1 month, soils were analysed for final pH, moisture content and nitrate and ammonium concentration. Three replicates were used for each treatment and the results are expressed on oven-dry weight basis.

Computation and statistics

Initial values of ammonium and nitrate-N were subtracted from the final values obtained after 60 days of incubation (first 30 days for pH stabilization and next 30 days of incubation) and divided by the number of days of incubation to get the net ammonification and nitrification rates, respectively. These two rates were summed to get net N mineralization rate. Linear regressions were worked out to find out the relationship between soil pH and ammonification, nitrification and net N mineralization.

EFFECT OF SOIL TEMPERATURE

Equal quantity (100 g) of sieved soil from each site (obtained in the manner described under soil pH effect study) was taken in each of the 63 polyethylene beakers (250 ml) and incubated for 30 days at different temperatures *viz.* 5, 10, 15, 20, 25, 30 and 40°C . Soils from each forest regrowth were analysed for initial moisture, ammonium and nitrate-N. For soils from each regrowth three replicate beakers were maintained for each temperature treatment. After 30 days of incubation, soils from the three beakers representing a particular temperature treatment were mixed, which was homogenized to form a composite sample and analysed for ammonium- and nitrate-N. The results are expressed on oven-dry weight basis.

Computation and statistics

The initial concentrations of ammonium and nitrate were deducted from the respective final concentrations and divided by the number of incubation days to get the ammonification and nitrification rates, respectively on per day basis. Q_{10} was calculated according to Scholes *et al.* (1994), by dividing the rate at a given temperature by the rate at temperature 10°C lower. Linear regressions were worked out following Zar (1974) to test the relationship between ammonification, nitrification and net N mineralization rates with temperature.

EFFECT OF SOIL MOISTURE CONTENT

Soil samples were collected randomly from ten points in the three study sites mentioned in Chapter 3. The soils from each stand were bulked to form a composite sample and brought to the laboratory for further processing and analysis. The bulked soil was sieved through 2mm mesh screen and sub-samples were immediately analysed for initial moisture content, ammonium and nitrate-N. Remaining sieved soil was

placed intact in sieves (20.5 cm diameter, 6.5 cm height) of 0.5 mm mesh size. The sieves were floored with blotting paper. Each sieve was placed in a tray (45 cm length, 30 cm breadth, 7.5 cm height) full of deionized water for saturating the soil. After soil became fully saturated with water, the sieves were removed from the tray, allowed for 24 h drainage and were kept undisturbed under laboratory conditions. For each forest soil, three replicated sieves were used. Soils were allowed to air-dry and were analysed periodically for soil moisture level, and ammonium and nitrate concentrations. When the soil moisture content decreased to *ca.*<10% on the 12th day, soils in each sieve were brought to the field capacity by adding deionized water. Soil samples were then left undisturbed for 24 h to drain-off excess water and then again the moisture, ammonium and nitrate-N were measured on the 13th day. The soil samples in the sieves were again left for air-drying at room temperature under laboratory conditions and the mineral-N and water content in the drying soils were measured periodically till 40th day when the soil moisture content declined to *ca.* 6, 7 and 12% in the 7-, 13- and 16-year old forest soils, respectively.

Computation and statistics

Ammonification, nitrification and net N mineralization rates were calculated by subtracting the final values of ammonium and nitrate concentrations from their initial values and dividing it by the number of days of incubation, to express the result on per day basis. Linear regressions were used to find out the relationship between soil moisture and N mineralization.

RESULTS

SOIL pH

Ammonium and nitrate concentration

Ammonium concentration declined with the increase in pH, while a reverse trend was observed in nitrate concentration regardless of the regrowth age (Table 9.1). However, the proportion of ammonium and nitrate-N varied due to pH along the age gradient of the forest regrowth. For instance, nitrate-N tended to be more than the ammonium level with the increase in soil pH and as it approaches towards neutral in the soils from the 7- and 13-year old regrowths, whereas the soils from the 16-year old regrowth showed a reverse trend. The ammonium-N concentration in the soils used for the study showed an increasing trend with decrease in soil pH. On the other hand, nitrate-N concentration tended to decrease with decreasing soil pH. In the control treatment (field soils where neither $CaCO_3$ nor dilute H_2SO_4 was added), the proportion of nitrate-N was *ca.* 30-40% of the total inorganic-N (ammonium+nitrate); proportion of nitrate-N progressively decreased with the regrowth age, being lowest in the 16-year old regrowth (Table 9.1).

Net N mineralization

Ammonification and nitrification rates increased with age of the regrowth. Further, the ammonification rate showed a declining trend with increase in soil pH irrespective of the regrowth age, whereas a reverse trend was observed in the case of nitrification (Table 9.2). Compared to the control, in the soil at more or less same pH there was 21% increase in the N mineralization rate in the case of 7-year old regrowth, 8% in 13- and 64% in the case of 16-year old regrowth.

Maximum N mineralization rate was obtained at pH 7.1 in the case of soil from the 7-year old regrowth, at 6.51 in the 13-year old

Table 9.1. Concentration ($\mu g\ g^{-1}$) of ammonium, nitrate and total inorganic-N (ammonium+nitrate) in soils as influenced by pH.

7-year old regrowth				13-year old regrowth				16-year old regrowth			
pH	Amm.	Nit.	Tot.	pH	Amm.	Nit.	Tot.	pH	Amm.	Nit.	Tot.
5.35*	13.99	9.35	23.34	5.25*	15.59	11.42	27.01	4.72*	24.42	9.85	34.27
4.70	16.09	7.65	23.74	4.10	25.47	8.93	34.40	3.92	59.39	4.77	64.16
4.98	15.37	9.57	24.94	4.59	17.75	10.71	28.46	4.35	60.01	6.46	66.47
5.30	13.56	12.74	26.30	5.00	16.92	11.65	28.57	5.00	55.24	9.78	65.02
5.92	12.42	12.92	25.34	6.08	14.69	16.54	31.23	5.57	45.77	17.62	63.39
6.56	11.09	14.59	25.68	6.51	13.98	16.90	30.88	5.98	44.99	17.39	62.38
7.10	10.88	16.01	26.89	7.01	12.41	16.91	29.32	6.53	43.04	19.17	62.21
7.30	11.60	12.84	24.44	7.36	11.87	18.61	30.48	6.98	41.07	25.43	66.50

Amm.-Ammonium; Nit.-Nitrate; Tot.-Ammonium+Nitrate

*pH of field soil in which neither $CaCO_3$ nor H_2SO_4 was added.

Table 9.2. Ammonification and nitrification rates ($\mu g\ g^{-1}\ day^{-1}$) of soils from the three forest regrowths with change in soil pH.

7-year old regrowth			13-year old regrowth			16-year old regrowth		
pH	Ammonification	Nitrification	pH	Ammonification	Nitrification	pH	Ammonification	Nitrification
5.35*	0.188	0.167	5.25	0.238	0.244	4.72	0.333	0.247
4.70	0.258	0.110	4.10	0.558	0.161	3.92	1.499	0.078
4.98	0.234	0.174	4.59	0.300	0.220	4.35	1.520	0.134
5.30	0.173	0.280	5.00	0.273	0.252	5.00	1.361	0.245
5.92	0.135	0.286	6.08	0.198	0.415	5.57	1.045	0.506
6.56	0.091	0.341	6.51	0.175	0.427	5.98	1.019	0.499
7.10	0.084	0.389	7.01	0.122	0.427	6.53	0.954	0.558
7.30	0.108	0.283	7.36	0.104	0.484	6.98	0.888	0.767

* pH of field soil in which neither $CaCO_3$ nor H_2SO_4 was added.

Table 9.3. Linear regression equations and correlation coefficients (r) depicting the relationship between soil pH and ammonification, nitrification and net N mineralization rates ($\mu g\ g^{-1}\ day^{-1}$) for the soils from the three forest regrowths.

Regrowth age	Regression equation	r	df.	P
Soil pH x Ammonification rate				
7-year old	Y=6.36-2.78X	-0.242	6	NS
13-year old	Y=6.19-1.68X	-0.233	6	NS
16-year old	Y=5.36-0.31X	-0.125	6	NS
Soil pH x Nitrification rate				
7-year old	Y=4.36+7.19X	0.954	6	0.001
13-year old	Y=3.80+7.36X	0.951	6	0.001
16-year old	Y=4.21+4.22X	0.962	6	0.001
Soil pH x net N mineralization rate				
7-year old	Y=4.45+4.19X	0.665	6	NS
13-year old	Y=4.48+2.63X	0.488	6	NS
16-year old	Y=4.38+1.09X	0.632	6	NS

NS-not significant

regrowth and at *ca.* 7.0 in the 16-year old regrowth (Figure 9.1) In soil from the 16-year old regrowth, nitrification rate was lower in the pH range of 3.9-5.0 whereas it increased at pH > *5 to* ca. 7. The increase in mineralization with the increase in was far greater in the 16-year old regrowth compared to the 13- and 7-year old regrowth, where the increase was minimal (Table 9.2).

Relationship of net N mineralization rate with soil pH

Though the ammonification rate declined with soil pH, there was no significant correlation. On the contrary, nitrification rate showed a strong positive correlation ($P<0.001$) with soil pH (Table 9.3). In all the three forest regrowths, the net N mineralization rate was positively correlated with soil pH, however, the relationship was not significant.

SOIL TEMPERATURE

Soil moisture content invariably declined with increase in temperature. However, ammonium and nitrate concentrations increased with the increase in soil temperature up to 30°C after which, it declined sharply in soils from all the three regrowths (Table 9.4). The variations in inorganic-N between various incubation temperatures (Figure 9.2) were significant ($P<0.05$). Ammonium-N concentration was always higher than nitrate-N in all the treatments. Generally, ammonification and nitrification rates were lower at 5 and 10°C; soils of the 16-year old regrowth showed only immobilization at these temperatures. At low temperatures ammonification rates were lower compared to the nitrification rates. At temperatures higher than 15°C, however, ammonification rate was greater than nitrification. Overall, in all the three forest soils studied ammonification, nitrification (Table 9.5) and net N mineralization rates (Figure 9.3) showed an increasing trend with incubation temperature up to 30°C above which

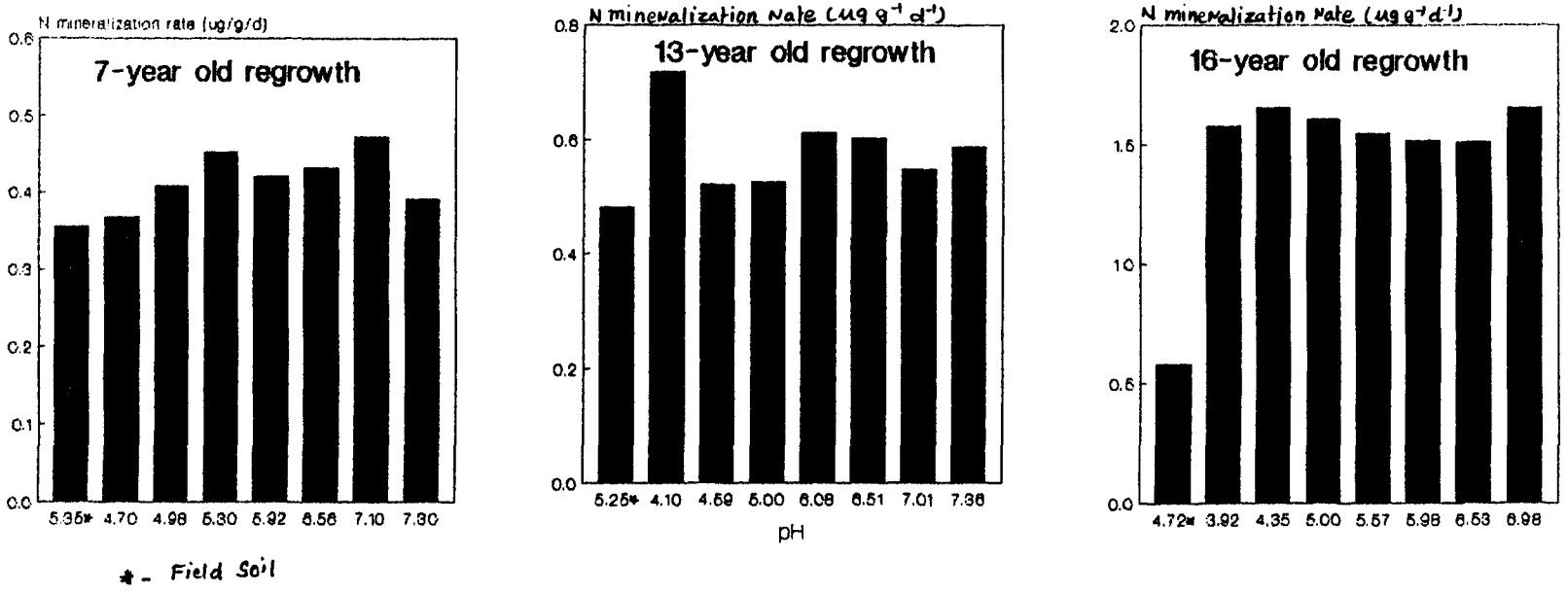

Figure 9.1: N mineralization rates (μg g^{-1} day^{-1}) at different pH levels in soils from the three forest regrowths.

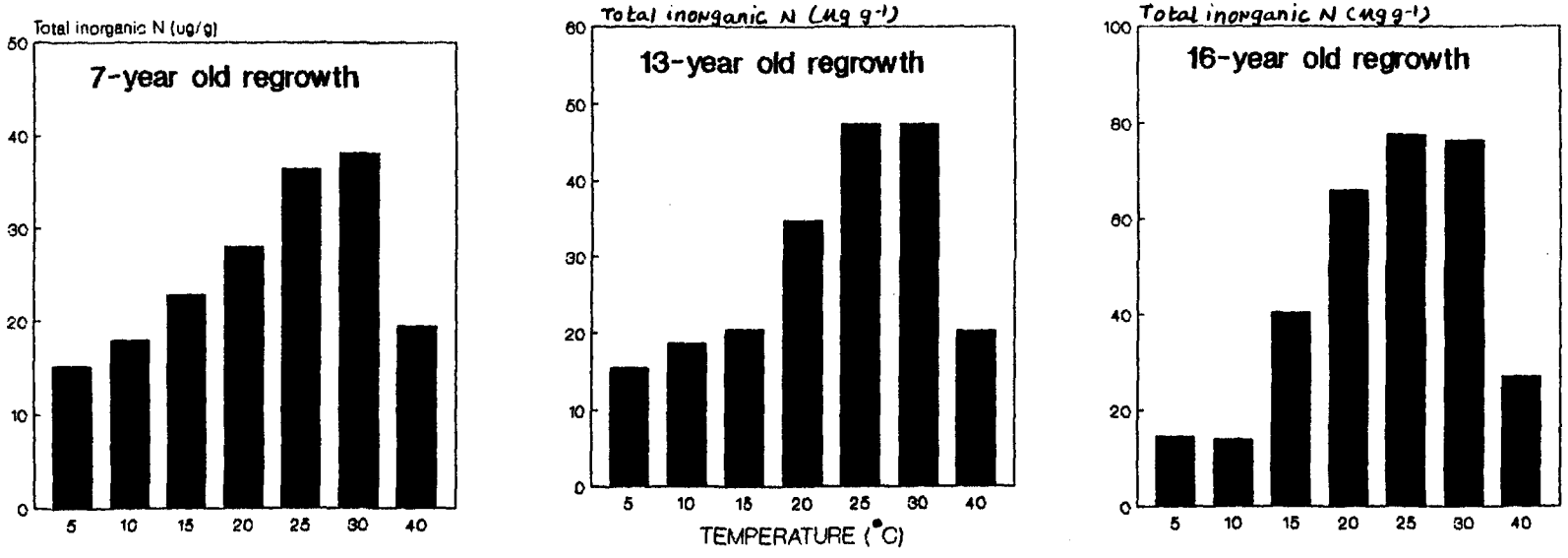

Figure 9.2: Concentration of total inorganic N (ammonium+nitrate) (μg g^{-1}) at different temperatures in soils from the three forest regrowths.

Table 9.4. Soil moisture content (%), ammonium, nitrate and total inorganic-N (ammonium+nitrate) concentrations ($\mu g\ g^{-1}$) in the soils incubated for 30 days at different temperatures.

Incubation temperature (°C)	7-year old regrowth			13-year old regrowth			16-year old regrowth		
	SMC	Ammonium	Nitrate	SMC	Ammonium	Nitrate	SMC	Ammonium	Nitrate
Initial	22.7	7.31	5.62	47.2	8.86	5.96	55.0	12.16	8.31
After 30 days									
5	40.0	8.30	6.93	36.2	8.95	6.59	53.8	7.55	7.16
10	25.4	9.79	8.12	34.8	9.63	8.98	51.0	7.28	6.82
15	27.1	12.63	10.23	26.6	11.68	8.85	43.5	24.32	16.14
20	24.1	15.63	12.42	24.4	20.22	14.59	37.8	44.55	21.71
25	22.1	20.04	16.47	24.4	26.75	20.78	32.2	49.68	28.05
30	22.0	22.21	16.03	22.0	26.18	21.22	28.1	48.32	28.16
40	13.1	11.32	8.11	16.1	10.23	10.04	20.2	18.67	8.32

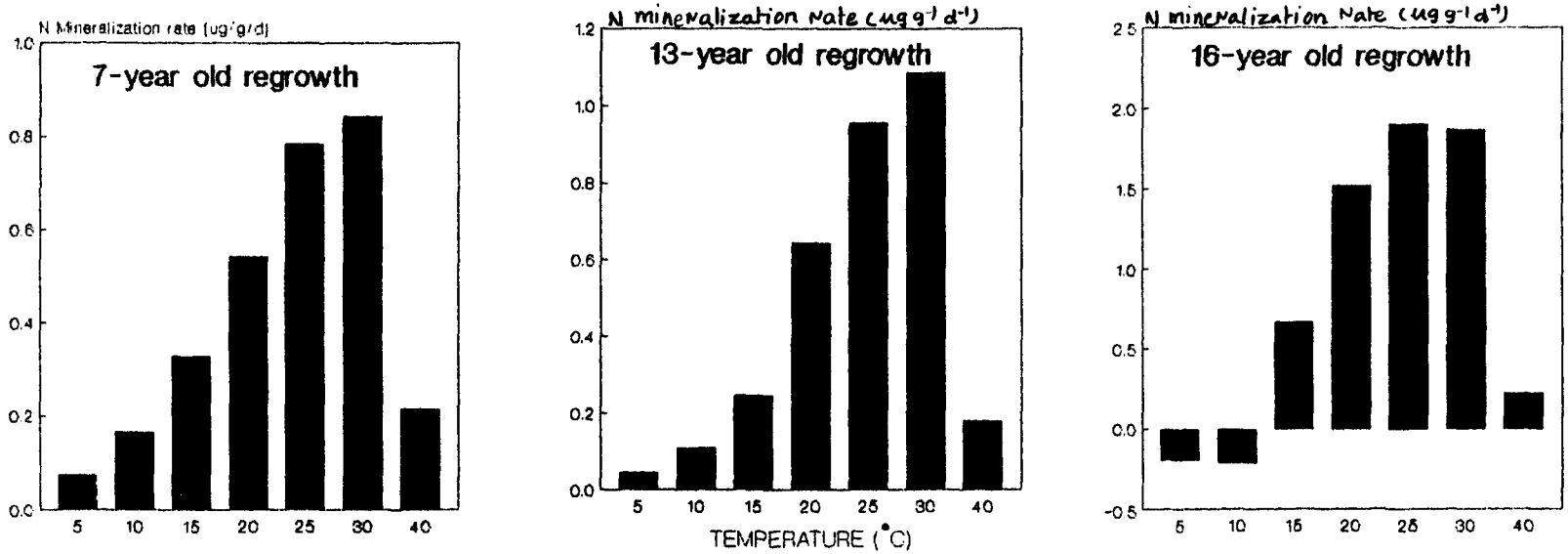

Figure 9.3: N mineralization rate ($\mu g\ g^{-1}\ day^{-1}$) at different temperatures in soils from three forest regrowths.

Table 9.5. Ammonification (1) and nitrification (2) rates (μg g^{-1} day^{-1}) in the soils of the three forest regrowths incubated at various temperatures.

Incubation temperature (°C)	Age of the forest regrowth					
	7-year old		13-year old		16-year old	
	1	2	1	2	1	2
5	0.033	0.043	0.003	0.021	-0.153	-0.038
10	0.082	0.083	0.025	0.100	-0.162	-0.049
15	0.177	0.153	0.093	0.096	0.405	0.261
20	0.277	0.266	0.378	0.286	1.079	0.446
25	0.424	0.361	0.596	0.493	1.250	0.658
30	0.496	0.347	0.577	0.508	1.205	0.661
40	0.133	0.083	0.045	0.136	0.217	0.003

i.e. at 40°C the rates declined sharply. The optimum temperature range for N mineralization was 25-30 °C.

Correlation coefficients (r) obtained by regressing the rates of ammonification, nitrification and net N mineralization with incubation temperatures are all significant (Table 9.7). The Q_{10} values ranged between -3.46 and 7.91; negative values were observed in the soils of the 16-year old stand. Generally, Q_{10} values between 30° and 20°C were close to 2.0 (Table 9.6).

SOIL MOISTURE CONTENT

Moisture content of soils from 7-, 13- and 16-year old regrowths declined to 7, 9 and 12% after 12 days of air-drying. However, when the soils were brought back to the field capacity by rewetting them on the 13th day and were subsequently allowed to air-dry, they took 28 days to reach the abovementioned moisture levels.

Mineral N concentration and net N mineralization

During the course of air-drying, both ammonium- and nitrate-N concentrations showed a steady increase (Table 9.8). Within 24 h of rewetting *i.e.* on the 14th day after incubation there was *ca.* 30-50% increase in the inorganic-N level over the values recorded on the 12th day. And this 'upshock' in mineral-N concentration constantly showed an increasing trend with subsequent air-drying. Ammonification rate tended to decline with the decrease in soil moisture during the course of air-drying, and it sharply increased after rewetting the soil, and again it started decreasing as the soil was allowed to air dry (Table 9.9). Nitrification (Table 9.9) and net N mineralization (Figure 9.4) rates also showed a similar trend.

Relationship between N mineralization and soil moisture

Regression analysis was carried out to find out the relationship between soil moisture and ammonification, nitrification and net N

Table 9.6. Temperature coefficients (Q_{10}) for the N mineralization rates under various temperature ranges.

Temperature range (°C)	7-year old stand	13-year old stand	16-year old stand
5-15	4.35	7.91	-3.46
10-20	3.03	5.28	-5.63
15-25	2.37	5.73	2.86
20-30	1.63	1.63	1.56
30-40	0.26	0.17	0.12

Table 9.7. Relationships between ammonification, nitrification and net N mineralization rates ($\mu g\ g^{-1}\ day^{-1}$) and incubation temperature (°C) in forest regrowths of three different ages.

Regrowth age	Regression equation	r	df	P
Temperature x Ammonification				
7-year old	Y=9.67+67.45X	0.943	6	0.005
13-year old	Y=13.56+39.81X	0.852	6	0.05
16-year old	Y=14.45+15.82X	0.855	6	0.05
Temperature x Nitrification				
7-year old	Y=8.84+79.39X	0.951	6	0.005
13-year old	Y=11.85+54.11X	0.884	6	0.01
16-year old	Y=13.45+34.63X	0.878	6	0.01
Temperature x net N mineralization				
7-year old	Y=9.322+37.07X	0.945	6	0.005
13-year old	Y=12.77+23.08X	0.869	6	0.05
16-year old	Y=14.32+11.61X	0.862	6	0.05

Table 9.8. Concentration of ammonium and nitrate-N ($\mu g\ g^{-1}$) at different moisture levels (%) in the soils of 7-, 13- and 16-year old forest regrowths.

Incubation time (day)	7-year old regrowth				13-year old regrowth				16-year old regrowth			
	SMC	Ammonium	Nitrate	Total	SMC	Ammonium	Nitrate	Total	SMC	Ammonium	Nitrate	Total
Initial	21	6.21	6.73	12.94	28	15.09	8.62	23.71	40	25.12	11.78	36.90
3	17	10.93	8.92	19.75	22	17.18	12.31	29.49	33	30.32	16.82	47.14
6	15	12.81	9.89	22.70	16	18.62	15.21	33.83	25	33.14	20.36	53.50
9	10	13.67	10.62	24.29	13	18.98	16.62	35.54	17	32.87	23.82	56.69
12	7	13.97	10.66	24.63	9	20.25	17.12	37.37	12	34.12	25.02	59.14
Soils were rewetted on the 13th day												
14	47	38.82	18.68	57.56	52	45.77	25.72	71.49	56	62.75	25.87	88.62
15	44	60.61	36.08	96.69	49	58.39	38.71	97.10	52	72.32	32.32	114.64
17	41	74.32	48.19	122.51	44	73.89	44.82	118.71	48	90.39	51.08	131.47
21	36	80.20	54.16	134.36	39	78.93	57.21	136.14	39	99.62	48.03	147.65
25	29	84.21	56.82	141.03	31	82.72	66.38	151.11	34	103.68	52.03	155.70
29	24	86.83	60.10	146.93	27	84.10	70.32	154.42	29	109.86	55.32	165.18
33	17	89.02	62.01	151.03	17	85.82	72.31	158.13	17	112.12	58.12	170.24
40	6	89.76	63.18	152.88	7	86.28	74.68	160.96	12	112.93	59.39	178.92

Total – Ammonium + Nitrate, SMC - Soil moisture content

Table 9.9. Ammonification and nitrification rates (μg g^{-1} day^{-1}) at different moisture level (%) in the soils of 7-, 13- and 16-year old regrowths.

Incubation time (day)	7-year old regrowth			13-year old regrowth			16-year old regrowth		
	SMC	Ammonification	Nitrification	SMC	Ammonification	Nitrification	SMC	Ammonification	Nitrification
3	17	1.54	0.73	22	0.69	1.23	33	1.73	1.68
6	15	0.66	0.32	16	0.48	0.96	25	0.94	1.18
9	10	0.28	0.24	13	0.12	0.47	17	-0.09	1.15
12	7	0.10	0.01	9	0.42	0.16	12	0.41	0.40
Soils were rewetted on the 13th day									
14	47	12.42	4.01	52	12.73	4.31	56	14.31	0.43
15	44	21.79	17.40	49	12.62	12.99	52	19.57	6.45
17	41	6.85	6.05	44	7.75	3.05	48	4.03	4.38
21	36	1.47	1.49	39	1.26	3.09	39	2.30	1.73
25	29	1.00	0.66	31	0.94	2.29	34	1.01	1.00
29	24	0.65	0.82	27	0.34	0.98	29	1.54	0.87
33	17	0.54	0.47	17	0.43	0.49	17	0.56	0.70
40	6	0.10	0.16	7	0.06	0.33	12	0.11	0.18

SMC-soil moisture content

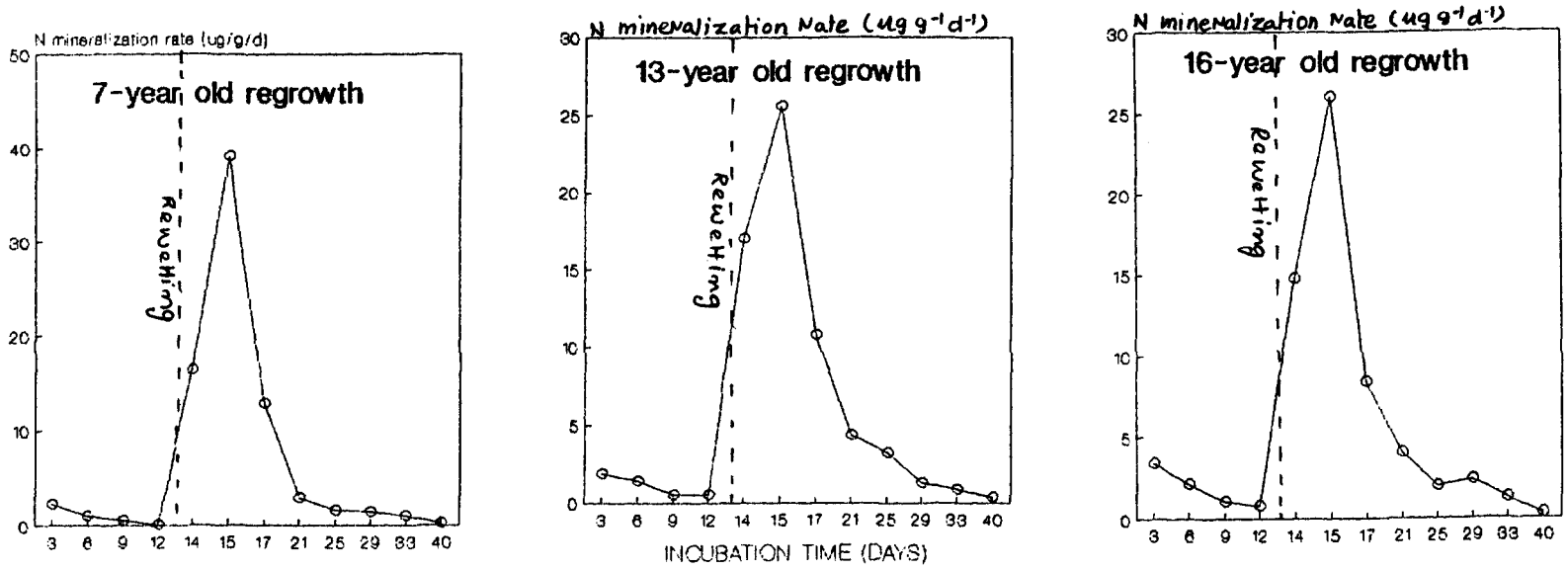

Figure 9.4: N mineralization rate (μg g^{-1} day^{-1}) from the dates of air drying/rewetting.

mineralization rates. Soil moisture showed a strong positive correlation with ammonification and nitrification rates (Table 9.10). Similar relationship was also found when the values of N mineralization was regressed against soil moisture, irrespective of forest regrowths (Figure 9.5).

DISCUSSION

SOIL pH

The declining trend in ammonium concentration with increasing soil pH could be due probably to its conversion into nitrate-N, as indicated by the increased nitrate concentration with increasing soil pH. This is further substantiated by the nitrification rates (Table 9.2). The rapid conversion of ammonium to nitrate-N could be due to the relative increase in nitrifier populations with the increase in pH towards neutral (Pennington & Ellis 1993). In this context, it may be mentioned that nitrifiers are active at a pH range of 4.0-8.5 (Baath *et al.* 1995). Ellis & Pennington (1989) reported active nitrification to occur in forest soil with a field pH of 4.3. In the 16-year old regrowth, where the field soil pH was 4.72, the nitrification rate was far greater as compared to the soils from the 7- and 13-year old regrowths where the pH of the field soil was 5.35 and 5.25, respectively. It is reported that nitrification rate is low in acidic soils (Haynes 1986, Chao *et al.* 1993). These workers have reported that low soil pH is one of the limiting factors for autotrophic nitrification. The low nitrification in the soil of oldest stand could thus be attributed to lower soil pH in this stand which might have favoured N immobilization and/or denitrification by indigenous microflora.

The net N mineralization in this study showed positive relationship with soil pH (Table 9.3). This is in contrarast with results of

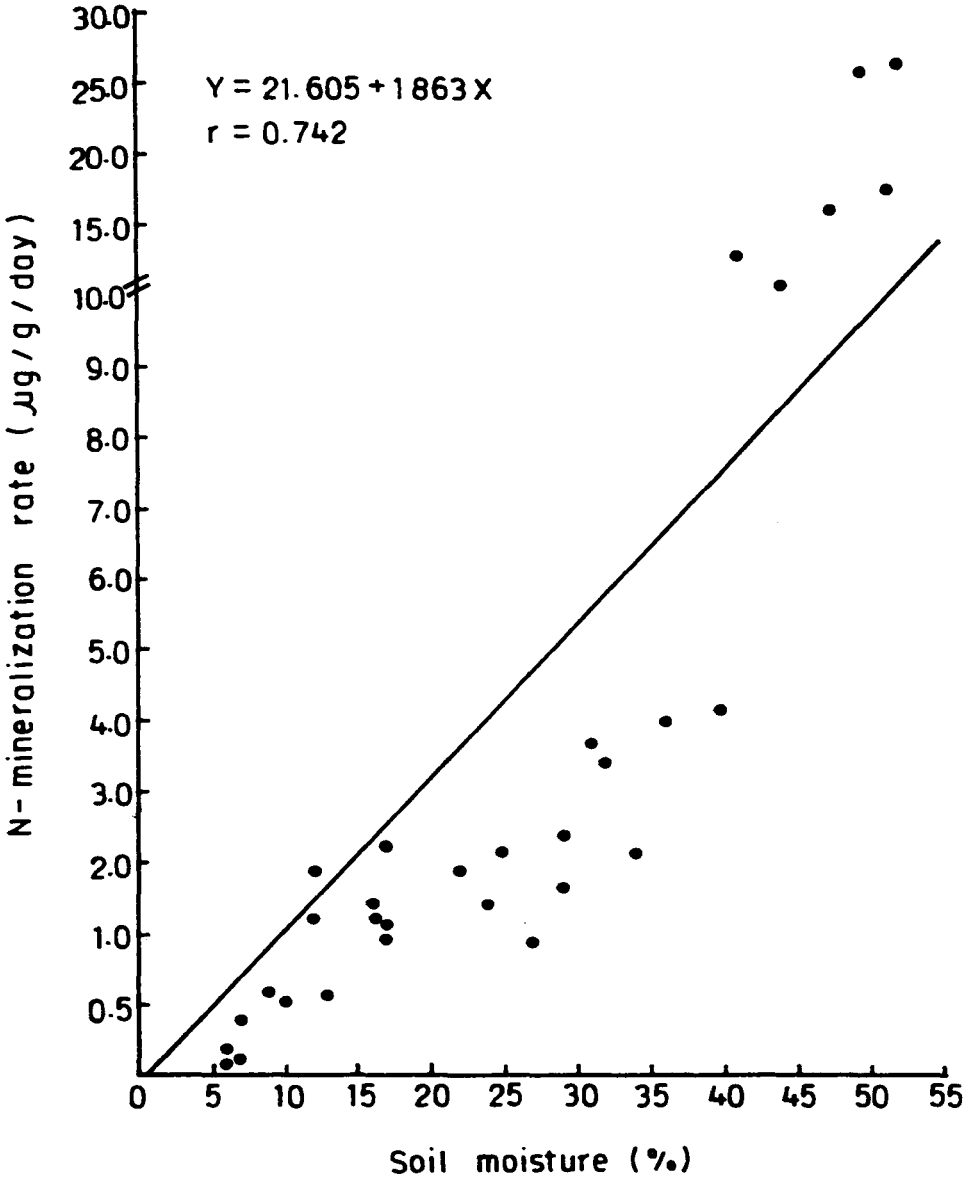

Figure 9.5: Regression graph showing relationship between N mineralization rate and soil moisture content.

Table 9.10. Linear regression equations and correlation coefficients (r) depicting the relationship of ammonification, nitrification and mineralization rates (μg g^{-1} day^{-1}) with soil moisture (%) in the 7-, 13- and 16-year old forest regrowths.

Forest stand age	Regression equation	r	df.	P
Soil moisture x Ammonification				
7-year old	Y=20.02+1.51X	0.638	10	0.05
13-year old	Y=21.95+2.06X	0.687	10	0.05
16-year old	Y=27.12+0.99X	0.442	10	NS
Soil moisture x Nitrification				
7-year old	Y=19.85+1.93X	0.659	10	0.05
13-year old	Y=20.33+3.14X	0.714	10	0.01
16-year old	Y=20.09+6.77X	0.838	10	0.001
Soil moisture x net N mineralization				
7-year old	Y=19.79+0.86X	0.658	10	0.01
13-year old	Y=20.40+1.58X	0.740	10	0.01
16-year old	Y=24.49+1.52X	0.710	10	0.05

NS-not significant

field study which showed an increase N mineralization rate in the 16-year old regrowth compared to the 7-year old regrowth despite the fact that was relatively lower in the former stand (see Chapter 6). This could be attributed to a number of factors operating under field situations such as uptake by plants, leaching, run-off, *etc.* Slower nitrification could, however, be a measure of N conservation, as nitrate is one of the most mobile ion in soil and is subjected to heavy leaching.

SOIL TEMPERATURE

Increase in ammonium and nitrate concentrations with the increasing temperature could be due to increase in ammonification and nitrification rates at the higher temperatures. Ammonium and nitrate concentrations were lower at the incubation temperature of 5-10°C where the ammonification and nitrification rates were very low. In the soils of the 16-year old regrowth only immobilization occurred. The fluctuation in N mineralization rates could also be explained on the basis of soil moisture content, pH, organic matter, *etc.*. Relationships among these variables have been already discussed in chapter 6.

The N mineralization rates increased with incubation temperature up to 30°C after which (*i.e.* at 40°C) it sharply declined. These results are in agreement with Scholes *et al.* 1994) who also reported increase in microbial activity with soil temperature over a range of 10-30°C. The sharp decrease in N mineralization rate at 40°C observed in all the three forest soils could be due to the denaturation of enzymes at that temperature. However, soils are seldom simulataneously hot and wet. In hot soils, therefore, the microorganisms are inactivated by lack of water rather than the high temperature.

Many workers use temperature coefficient (Q_{10}) to explain the

effect of temperature on microbial activity. Generally the Q_{10} values for biochemical reaction is in the order of 2 or 3. In the present study the values were mostly higher than 2 at low temperatures. However, at incubation temperature 20-30°C, Q_{10} values were close to 2. This indicates that the microbial activity in the soils tested are optimum at 20-30°C. Schleser (1982) found that there was a tendency for soil Q_{10} values to decrease with increasing temperature. The present finding relating to decline in Q_{10} values with the increase in incubation temperature is in agreement with Schleser's report.

SOIL MOISTURE CONTENT

In the present study, variation in soil moisture content affected the nutrient availability, especially of inorganic form of N *i.e.* ammonium and nitrate-N. Such variations, under field conditions could be attributed to leaching loss, microbial immobilization, plant uptake, *etc.* The present data, collected in laboratory study may not depict the mineral-N flux as could be obtained under *in situ* conditions. However, the results do reveal that the variation in the ammonium and nitrate concentrations with varying soil moisture could be attributed to drying and rewetting of soil which causes physical disruption of the soil structure and increases microbial mobility and diffusion of soluble organic compounds (Jenkinson & Powlson 1976, Keift *et al.* 1987).

Positive correlation has been obtained between soil moisture and N mineralization in dry tropical (Srivastava 1992b) and temperate (Orchard & Cook 1983) forest soils. The present laboratory experiment with subtropical forest soils also fully corroborates the findings under *in situ* conditions. Thus the data obtained in laboratory experiment could be considered as valid as that obtained under *in situ* studies, for predicting the influence of soil moisture on N mineralization. This is further confirmed by the 'upshock' effect

(sudden increase in N mineralization) of rewetting the dry soil. The flush in N mineralization after rewetting is comparable to the increased N mineralization after heavy rainfall in the field situations. This flush in mineral-N could be attributed to metabolism of organic substrates released by microbial death during drought and rewetting (Bottner 1985, Vangestel *et al.* 1993). Kieft *et al.* (1987) reported that the shock of the wetting event alone can kill from 17 to 58% of the microbial biomass, which may subsequently induce faster mineralization. This point is substantiated by substantial increase in N mineralization rate (*ca.* 57% in 7-, 47% in 13- and 33% in 16-year old regrowth, respectively) after rewetting the soils. Nitrification rate, however, did not show any marked increase, which could be due to creation of anaerobic sites due to poor aeration that is detrimental to nitrifier population (Chao *et al.* 1993).

CHAPTER 10

GENERAL DISCUSSION

Study of floristic richness and species diversity in the three forest regrowths showed that initial successional community that developed after partial tree cutting was characterized by predominance of grasses, weeds and *Pinus kesiya*. High light intensity and relatively low soil moisture did not allow the shade-tolerant species to develop in the early stages of secondary succession. However, with the development of canopy in the older regrowths, light intensity on the forest floor was reduced and there was a gradual increase in the soil moisture content due to the accumulation of litter on the forest floor, which might have favoured the growth and establishment of the shade loving species like mosses, ferns and *Schima khasiana etc.*. The density and basal area of the woody vegetation showed a remarkable increase with the age of the regrowth. The tree density increased from 180 trees ha^{-1} in the 7-year old regrowth to 480 and 1140 trees ha^{-1} in the 13- and 16-year old regrowths, respectively.

Populations of both bacteria and fungi also showed an increasing trend with the regrowth age. In total, 29 species of fungi were identified from soils of the three forest regrowths. Fungal species content increased from 17 in the 7-year old regrowth to 24 and 28 in the 13- and 16-year old regrowths, respectively. Greater species

diversity of soil fungi in the 16-year old regrowth is attributed to higher nutrient status and favourable microclimate for growth and establishment of fungi in this stand. Greater species richness and diversity of fungi in the soil of the 16-year old regrowth signals a relatively stable environment, while unstable environment in the 7- and 13-year old regrowths appears to have eliminated some of the species.

Although the three forest stands were located nearby and had similar toposequence, they differed markedly with respect to soil texture. The texture of the soil was sandy loam in the 7-year old stand, sandy clay loam in the 13-year old stand and clay loam in the 16-year old regrowth. A relatively dense vegetation cover in the 16-year old regrowth contributed to high organic matter thereby resulting in significantly higher percentage of clay particles in the 16-year old regrowth. A remarkable increase in soil organic matter (SOM), N and P in the soil from the 7-year old to 16-year old regrowth was related to dense growth of vegetation and to the increased input and decay rates of litter and fine roots (Arunachalam *et al.* 1996a).

Analysis of C, N and P immobilization in microbial biomass and mineralization in forest regrowths of three different ages clearly indicated the following trends along a recovery gradient following tree cutting. All the three microbial nutrients showed an increasing trend with stand age (Figure 10.1), which is accomplished by increased detrital accumulation, nutrient mineralization and higher clay content in the older regrowths. The increasing trend in microbial biomass with the ecosystem recovery is reflected in microbial population also. It could be thus said that microbial biomass and microbial population are a measure of ecosystem recovery. The spatial trends in microbial

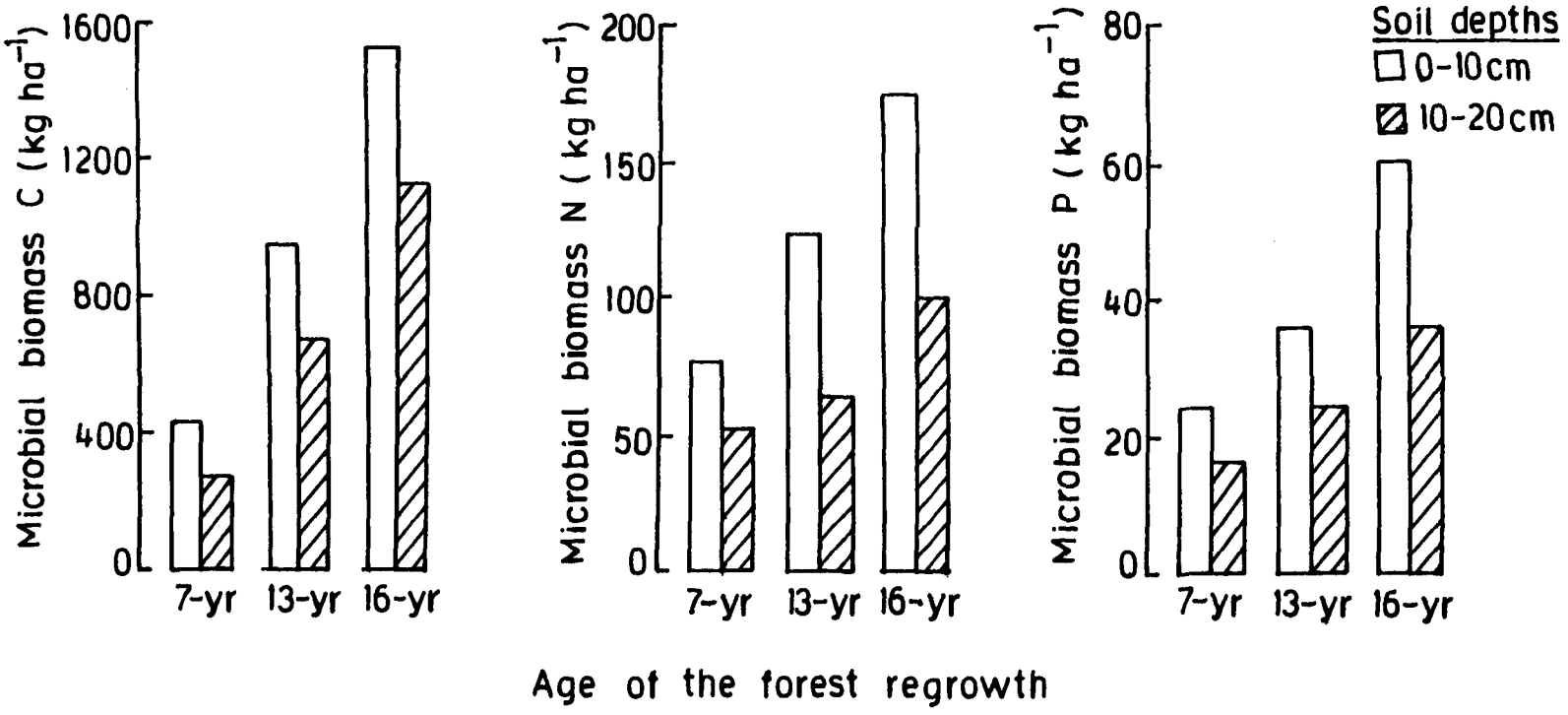

Figure 10.1: Microbial biomass C, N and P (Kg ha^{-1}) in two soil depths (□ 0-10 and ▨ 10-20 cm) in the three forest regrowths.

nutrients could also be well explained by the seasonal changes in microbial population and amounts of organic matter and nutrient stocks on the forest floor and in the soil.

Microbial biomass C and N in a mature forest (unpublished data) located 28 km away from the present study sites are 30 and 57% more than the microbial biomass C and N values, respectively in the 16-year old regrowth. Microbial biomass C increased at the rate of 9.4% yr^{-1} from 7- to 13-year old regrowth and at the rate of 13% yr^{-1} from 13- to 16-year old regrowth. The percent increase in microbial biomass N and P was 6.1 and 5.45% yr^{-1} from 7- to 13- year old regrowth and 10.5 and 12.2% from 13- to 16-year old regrowth, respectively. Microbial biomass C, N and P all showed similar seasonality with a peak during winter and trough during rainy season in all the three regrowths. Such a seasonal flux could be explained on the basis of seasonal changes in the rainfall and detrital decomposition. For example, peak in biomass during winter corresponds to the low decomposition rate and low rainfall. The microbial activity was also low and as a result, nutrient immobilization in the dormant microbial cells was promoted. On the other hand, low microbial biomass during rainy season coincided with rapid release of nutrients from decomposing detritus owing to high rainfall. The seasonal trends observed in the microbial biomass clearly indicates that microbial biomass conserves the nutrients when they are in excess and simultaneously, releases them when the requirements of plants are high.

N mineralization rate was 78% more in the mature undisturbed 'sacred grove' than the oldest regrowth. This can be attributed to the higher nutrient status and better nutrient availability in soil in the sacred grove (Das *et al.* unpublished). Such a comparative analysis

indicates that the system recovery is in progress even beyond 16-years of regrowth after tree cutting and it may take longer to reach a stable state.

The seasonal fluctuations in mineral nitrogen and mineralization rates were similar in all the regrowths. N mineralization rate peaked during rainy season when temperature and moisture conditions were favourable for growth and activity of microbes, whereas low mineralization rates were recorded during winter and spring. The level of inorganic-N (ammonium+nitrate) in soil showed opposite trend from that of mineralization rate. The low level of inorganic N recorded during rainy season in the present study could be ascribed to greater demands of these nutrients by the plants growing vigorously during rainy season. However, the losses due to leaching and denitrification cannot be underestimated as they are the important factors responsible for N loss during high rainfall period.

N mineralization rate obtained in the present study does not appear to have any effect on the secondary successional pattern, as there was a small difference in the rates between the three forest regrowths. The percentage increase in net N mineralization was low during 7 to 13 years of forest regrowth (1.5% yr^{-1}), which got doubled (*ca.* 3% yr^{-1}) during next three years of vegetation development. This indicates relatively faster rate of recovery in the later stages of secondary succession.

N mineralization rate was significantly influenced by soil moisture, temperature, pH and addition of organic material (leaf litter and fine & coarse roots) and the results have been discussed in detail in chapters 7-9. However, in the field situations, these parameters showed comparatively weaker correlation with N

mineralization, because under *in situ* conditions several factors interact simultaneously. Among the soil properties, moisture and temperature had greater influence on N mineralization as well as immobilization. The relative influence of leaf litter and fine & coarse roots on N mineralization was in the following order: fine roots > leaf litter > coarse roots. This differential influence is attributed to the initial chemical composition of the residues, particularly N and lignin.

Organic material with high initial N concentration and low lignin content (*e.g. S. khasiana*) mineralized at comparatively faster rate when compared to the resource with low initial N and high lignin content (*e.g. P. kesiya* & *R. arboreum*). Among the leaf litter *S. khasiana* which had highest N concentration and lowest lignin content started releasing N from the 30th day of incubation, whereas *R. arboreum* with low N and high lignin content did not release N during the entire 90 days of incubation. Similarly, among the roots, the addition of fine roots with comparatively high N and low lignin content than the coarse roots caused N mineralization at a faster rate. Among fine roots also, the roots from the 7-year old regrowth showed faster release of N than the older regrowths.

The ratio of immobilization:mineralization for the three nutrients (C, N and P) remained 1:1 without showing any temporal and spatial variations. Similar observation has been made by Rosswall (1976) while studying the influence of microorganisms on decomposition of plant residues and cycling of nutrients in global plant-soil system. The rates of microbial biomass C, N and P turnover (Table 10.1) showed no definite trend in relation to age of the forest regrowths. The variation between two annual cycles (1993-94 and 1994-

Table 1.0.1. Turnover rates (kg ha^{-1} yr^{-1}) of microbial biomass C, N and P in the three forest regrowths.

Age of forest regrowth	Soil depth (cm)	Microbial C			Microbial N			Microbial P		
		1993-94	1994-95	Mean	1993-94	1994-95	Mean	1993-94	1994-95	Mean
7-yr	0-10	0.62	0.56	0.59 (1.60)	0.53	0.49	0.51 (1.96)	0.63	0.76	0.69 (1.44)
	10-20	0.39	0.35	0.37 (2.70)	0.53	0.51	0.52 (1.92)	0.71	0.68	0.69 (1.44)
13-yr	0-10	0.38	0.29	0.40 (2.50)	0.54	0.51	0.53 (1.88)	0.69	0.66	0.63 (1.58)
	10-20	0.16	0.27	0.21 (4.76)	0.59	0.68	0.63 (1.58)	0.64	0.60	0.62 (1.61)
16-yr	0-10	0.52	0.54	0.53 (1.88)	0.64	0.52	0.58 (1.72)	0.47	0.68	0.57 (1.75)
	10-20	0.56	0.56	0.56 (1.78)	0.57	0.54	0.55 (1.81)	0.63	0.48	0.55 (1.81)

Values in parentheses are the turnover time (in years)

95) in microbial C, N and P turnover was within *ca.* 40%. The turnover time for microbial biomass C varied between 1.6-4.8 years, for microbial biomass N 1.6-2.0 and for microbial biomass P 1.4-1.8 years. The turnover rates per year (0.10-0.25) are higher than the value (0.09 yr^{-1}) reported by Rosswall (1976). However, the turnover of all the three nutrients (C, N and P) remained more or less the same in all the three regrowths. This indicates that the cycling of N and P and C is closely linked.

Generally, the turnover rates of microbial C and P were faster in the 16-year old regrowth. Similar reports were made in case of nutrient mineralization in fine roots (Arunachalam *et al.* 1996c) in the present study sites. The declining trend in P turnover during forest regrowth could, however, be a measure of nutrient conservation which will help promote P utilization and cycling within the system.

the contribution of microbial nutrients to total soil nutrient pool increased significantly with the age of the regrowth. The percent contribution of microbial biomass C to total soil organic-C ranged from 0.49-2.68, that of microbial biomass N to total Kjeldahl N 1.1-2.1% and percent contribution of microbial biomass P to total soil P was 2.8-7.9%. The higher contribution of microbial biomass P to total soil P in the present study has great significance because of low availability of P in the oxic soils of this region.

It may be concluded that immobilization of C, N and P in the microbial biomass increases with the increase in leaf litter and fine root input, and organic matter and nutrient build-up during forest regrowth following tree cutting. The immobilization:mineralization ratios of C, N and P remained constant (1:1) indicating that the microbial biomass turnover is complete irrespective of stand age. This signals the interlinkages of all these three nutrients in the regrowing forest stands.

SUMMARY

The moist deciduous, subtropical broadleaved forest located at higher altitude of Meghalaya have recently been subjected to different kinds of disturbance notably, cutting of trees for various purpose and shifting agriculture, giving rise to secondary successional plant communities.

Vegetation regrowth after disturbance depends mainly on the soil conditions, which deteriorate as a consequence of the disturbances. Recent studies have established that soil microbial biomass influences soil fertility level through such vital processes as immobilization and mineralization. So far, there is no holistic effort to quantify the microbial biomass and its role in soil organic matter (SOM) and nutrient circulation in humid tropical forests, particularly in India. Nevertheless, a few studies have been undertaken on these aspects in a dry tropical region of Indian subcontinent. The main objective of the present study was to understand the role of microbial biomass in SOM and N and P turnover in the forest regrowths developing after tree cutting. For this purpose, three forest stands 7-, 13- and 16-year old regrowths of a cut-over humid subtropical forest of Meghalaya were selected. The objecive was achieved by studying C, N and P immobilization in microbial biomass and their subsequent

mineralization. Besides, the changes in physico-chemical and biological properties of soil were also investigated. The effects of few critical variables such as soil pH, soil moisture and temperature, and quality of organic material were tested on soil N mineralization under laboratory conditions.

CHANGES IN SOIL PHYSICO-CHEMICAL PROPERTIES

Soil texture was sandy loam in the 7-year old regrowth, sandy clay loam in the 13-year old regrowth and clay loam in the 16-year old regrowth. CEC and WHC increased with the progression of vegetation recovery, however, soil temperature showed a reverse trend. Soil organic-C, N and available-P all increased significantly with the regrowth age, however, they all showed a reverse trend with the increase in soil depth.

MICROBIAL POPULATION DYNAMICS

Bacterial and fungal population along with species composition and diversity of soil microfungi were analysed at two soil depths in the three forest regrowths. Microbial population was maximum in the 16-year old regrowth and minimum in the 7-year old regrowth. However, in all the three forest regrowths, the population was significantly ($P<0.01$) lower in the subsurface soil layer than in the surface soil layer. Total number of fungal species increased from 17 in the 7-year old regrowth to 28 in the 16-year old regrowth and decreased with the increase in soil depth in all the three forest stands. *Trichoderma viride* and *Penicillium chrysogenum* were the only species present in all the three forest regrowths. In general, except a few, almost all the fungal species isolated were found in all the three forest

regrowths. Bacterial and fungal population showed significant ($P<0.05$) correlation with rainfall, however, the relationship with air temperature was not significant. Clay content, bulk density, WHC, SOC, TKN and available-P showed strong ($P<0.01$) positive correlation with the populations of bacteria and fungi.

MICROBIAL BIOMASS DYNAMICS

Seasonal dynamics of microbial biomass C, N and P studied in the 7-, 13- and 16-year old regrowths showed that the concentrations of all the three nutrients were maximum during winter and minimum during rainy season in both the soil depths. The surface soil layer had significantly higher concentrations than the subsurface layer. The mean annual concentrations of microbial biomass C, N and P increased with the age of the regrowth. The C:N and C:P ratios in microbial biomass also showed an increasing trend with the regrowth age. The percent contribution of microbial biomass C to soil organic-C (SOC), N to total Kjeldahl N (TKN) and P to total-P increased significantly from 7- to 16-year old regrowth. Microbial biomass C, N and P all showed positive correlation with the texture, organic-C, TKN and available-P content of the soil and negative correlation with the soil temperature and pH.

N AVAILABILITY AND MINERALIZATION

N availability and mineralization was studied monthly for a period of two years in the three forest regrowths. The mean concentrations of ammonium and nitrate-N was maximum in the 16-year old regrowth, followed by 13-year old regrowth, and the minimum values were obtained in the 7-year old regrowth. The total inorganic-N

(ammonium+nitrate) was maximum during winter and minimum during rainy season in the three forest regrowths, while a reverse seasonal trend was shown by ammonification, nitrification and N-mineralization rates. Net ammonification and mineralization rates were higher in the 16-year old regrowth than the 7- and 13-year old regrowths, however, nitrification rates recorded their highest values in the 7-year old regrowth followed by 13- and 16-year old regrowths, respectively. Concentration of inorganic-N and N-mineralization rate were significantly greater ($P<0.01$) in the top 10 cm soil layer than the subsurface layer (10-20 cm). Ammonium was the dominant form of inorganic N in all the three forest regrowths at all sampling dates. Ammonification and N mineralization rates showed positive correlation with the density and basal area of the woody vegetation, while nitrification rate showed a strong negative correlation. Further, N-mineralization was influenced by rainfall, soil moisture and temperature, and microbial population. The percent increase in net N-mineralization was higher (3% yr^{-1}) during 13-16 years of forest regrowth than during 7-13 years (1.5% yr^{-1}) of forest regrowth.

EFFECT OF SOIL MOISTURE, TEMPERATURE AND pH ON N MINERALIZATION

Rewetting of soils caused *ca.* 30-50% increase in inorganic-N level (ammonium+nitrate-N) over the values recorded after 12 days of air drying the soils. Net ammonification, nitrification and mineralization rates showed a declining trend with the decreasing soil moisture content during the course of air-drying, and a flush in N mineralization rate was observed after rewetting these soils.

Studies on the effect of temperature on N-mineralization showed that the concentrations of ammonium and nitrate in the soil increased

with the increasing soil temperature up to 30°C, but there was a sharp decrease in the concentration of ammonium and nitrate at 40°C in the soils of all the three forest regrowths. Rates of N mineralization also followed similar trend. The q_{10} values ranged between -3.46 and 7.91. The Q_{10} values were close to 2.0 at the incubation temperature of 20-30°C.

Effect of different pH levels on N availability and mineralization revealed that ammonium concentration declined with the increase in pH, while a reverse trend was observed in case of nitrate concentration in all the three forest soils. N mineralization rate showed a declining trend as the soil pH increased and approached neutral, and it showed an increasing trend with decreasing pH along the regrowth age. Though ammonification rate showed a negative correlation with soil pH, the relationship was not a significant. On the contrary, nitrification rate showed significant positive ($P<0.01$) correlation with soil pH.

RESOURCE QUALITY OF LEAF LITTER AND FINE & COARSE ROOTS

Pinus kesiya and *Quercus dealbata* were the common species present in all the three regrowths. *P. kesiya* generally had greater concentrations of carbon and lignin than the *Q. dealbata* however, N and P concentration showed a reverse trend. In the 16-year old regrowth *Schima khasiana* had the highest concentration of N and P followed by *Q. griffithii*, *Q dealbata* and *Rhododendron arboreum* in the decending order. The highest lignin content was recorded in case of *P. kesiya* leaf litter followed by *R. arboreum* and minimum in case of *Q. dealbata* and *S. khasiana* litter. Concentrations of C, P, and lignin, and C/N as well as lignin/N ratios in the fine and coarse

roots were significantly ($P<0.05$) higher in the 16-year old regrowth compared to the root material from the 7-year old regrowth. However, the concentration of N followed a reverse trend. Coarse roots generally had higher C and lignin content compared to the fine roots. On the contrary, Concentration of N and P and C/N and lignin/N ratios were greater in the fine roots than the coarse roots of all the three forest regrowths.

N MINERALIZATION AS INFLUENCED BY THE ADDITION OF DIFFERENT ORGANIC MATERIALS

Release of N was highest from the leaf litter of *S. khasiana*, which started releasing N from 30th day of incubation. *Q. griffithii* litter also showed net release of N after 60 days of incubation. however, *P. kesiya* litter exhibited net immobilization of N throughout the the 90 days of incubation in the soils from 13- and 16-year old regrowths. Leaf litter of *S. khasiana* showed maximum release of N followed by *Q. griffithii*, and the maximum immobilization was recorded in the leaf litter of *R. arboreum* followed by *P. kesiya*. Release of N from the roots followed different trend with incubation time. Coarse roots generally immobilized N from the soil under controlled laboratory conditions. In the soil+fine root treatment, there was a net release of N from the 30th day onwards. The release of N from the fine roots of younger stand was significantly ($P<0.05$) greater than the 13- and 16-year old forest regrowth. The percent of N accumulated or depleted from the leaf litter and roots was positively correlated to initial N concentration and negatively to lignin content of the resource. Concentration of C, C/N ratio and lignin/N ratio also showed negative correlation with the percent of initial N mineralized or

immobilized.

CONCLUSIONS

It is concluded that immobilization of C, N and P in the microbial biomass increases with the increase in litter and fine root input, and organic matter and nutrient build-up, during forest regrowth. Fungal species diversity increases with the progression of forest regrowth. N mineralization rate increased with the regrowth age. Soil moisture, pH and temperature, and root and leaf litter quality are major determinants of N mineralization. Microbial C and N turnover is more in the 16-year old regrowth, while P turnover in the 7-year old regrowth. Ths immobilization:mineralization ratio which remained constant (1:1) indicates that the microbial biomass turnover is complete in itself irrespective of the age of the forest regrowth. The results reveal that microbial biomass contributes significantly to SOM and nutrient build-up during the recovery of the cut-over subtropical humid forest ecosystem.

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