Thesis
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SOIL BIOLOGICAL STUDIES IN CONTRASTING TYPES OF VEGETATION IN CENTRAL AMAZONIAN RAIN FORESTS

A thesis presented for the degree of

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by

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I hereby declare that this thesis has been composed by myself and except where otherwise stated the work contained herein is my own.

Regina C. C. Luizão

"The complexity and beautiful order of the microbiological world is so wonderfully constructed that it appears to be part of a divinely ordained system." (Dr H.J. Shaughnessy).

"How many your works are, Jehovah ! All of them in wisdom you have made. The earth is full of your productions." (Psalms 104, 24).

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Abstract

Studies were carried out in a lowland evergreen rain forest (LERF), on an ultisol, in the 'Reserva da Campina', 45 km north of Manaus, and in two facies of the highly distinct formation called heath forest, on spodosols. The spodosols had a layer of mor humus of thickness varying from nil in some parts in the smaller facies of heath forest (SHF) to 35 cm in the taller facies (THF). The overall aim was to investigate the forest soil biota and its role in nutrient turnover by comparing the SHF, THF and LERF. Microbial biomass, soil respiration and nitrogen transformation rates were measured in the three forest types in both wet and dry seasons. Field and laboratory fertilization experiments were made to investigate potential limiting nutrients for microorganisms and plants. The role of fine roots in decomposition and litter animal colonization was assessed in litter bag studies.

SHF soils have a small microbial population with no net nitrification in any season. THF soils showed a variable microbial population adapted to high acidity, which immobilises nitrogen during the wet season, but which allows a net release during the dry season. LERF showed the most diverse population which causes mineralization and nitrification in both seasons. A bioassay with nutrient addition showed that the low pH, and nitrogen and sulphur supply were likely to be limiting nitrogen dynamics in all forest types, but especially in THF and LERF. The ingrowth bags showed that despite the lower values of fine root growth in the SHF (particularly when the white sand of the spodosol was used as the substrate), the roots showed

in all plots an increased production with added calcium as carbonate or sulphate. In the decomposition bioassay to evaluate the role of roots in the nutrient turnover it was shown that in all forest types there was no effect of roots on the mass loss of *Clitoria* leaves but there was a significant effect on concentrations of some nutrients. In general, roots contributed to the accumulation of aluminium and iron and to a faster release and uptake of calcium, magnesium and zinc. A survey of the mycorrhizal associations in all forest types showed that both VAM and ECM fungi with some unknown VAM fungal species are common. VAM and ECM adaptation to low pH and high phenolic compounds in the soils may be important in the maintenance of these ecosystems.

Chapter I. General Introduction

The Amazon basin lies between the Guiana Shield to the north and the Brazilian Shield to the south. The basin is a vast depression originating along a zone of weakness in the Precambrian Shield. The upper Cretaceous and the mid-Tertiary were periods of intense fluvial sedimentation. The majority of the neogenic sediments were derived from the Precambrian Brazilian and Guiana shields weathering mantles and originated from the chemical decomposition of crystalline rocks (Bigarella & Ferreira 1985). Amazonia is the region which encompasses the Amazon basin and is drained by the Amazon river and its tributaries. Estimates of the size of Amazonia range from 5,000,000 km^2 (Sioli 1984) to 6,000,000 km^2 (Pires 1973). Pires (1973) estimated that 3,700,000 km^2 of Amazonia lie in Brazil, of which 3,374,000 km^2 were covered by forest. Amazonia is neither uniform nor simple geologically, topographically, or climatologically (Bigarella & Ferreira 1985), and contains a wide range of vegetation types, both forest and non-forest on different substrata and occupying various geomorphological positions. Most of Amazonia that is not seasonally flooded, is covered by the tropical lowland evergreen rain forest formation (*sensu* Whitmore 1984) often referred to in Brazil as *Terra Firme* forest. In the whole of Brazilian Amazonia oxisols cover 39.1% of the area and ultisols 29.9 % (Richter & Babbar 1991) but in central Amazonia, oxisols cover 60 % of the area (Dias *et al.* 1980). A substantial proportion of Amazonia however is covered by a highly distinct forest formation known as *Bana* and *Caatinga* (in Venezuela) and *Campina* and *Campinarana* (in Brazil) which is the Heath forest of

Whitmore (1984). Heath forest also occurs in Borneo, where it is called kerangas and in small areas in Malaysia, and on coastal sands in Africa in Gabon, Cameroon, and Ivory Coast (Whitmore 1990).

Despite several individual attempts (Ducke & Black 1954; Lisboa 1975; Anderson 1978) to define the different physiognomy of heath forests in Amazonia, no definition is generally accepted. In this thesis I shall use the term 'heath forests' to refer to all forms of vegetation that develop in a climate suitable for mesophyllous lowland evergreen rain forest, but which often show distinctive features including microphylly and small vegetation stature (Whitmore 1989) and which occur on spodosols (Soil Survey Staff 1975) which are white sand soils mostly with a layer of mor humus.

Heath forests in Amazonia

The most extensive heath forests in the world are in the upper Rio Negro and Rio Orinoco in South America (Whitmore 1990). Heath forest spodosols are developed from siliceous sand, either coastal, alluvium or weathered sandstones. The podzolised quartz sands in the Rio Negro region in Venezuela are located between the flooded forests and lowland evergreen forests (Jordan 1985). Podsolization is the dominant soil forming process and a 'B' soil horizon enriched in organic matter is generally present. Because of the coarseness of the sand, heath forest soils are freely draining and have a low capacity to retain water (Bravard & Righi 1991). In Venezuelan heath forest an impermeable iron or humus pan has been described (Klinge & Medina 1979). Lateral drainage occurs quickly following storms, and within

a few days without rain, heath forest soils start to become very dry. Klinge & Medina (1979) observed in San Carlos de Rio Negro (Venezuela) that heath forest plants experience water stress in the dry season and suggested this as the cause for the change from tall *Caatinga* forest on soils with better water retention through the smaller stature low *Caatinga* to the shrubby *Bana* on soils with the worst water retention. Heath forest soils are very acidic, with a pH less than 4.0, and have a low buffering capacity owing to their low concentrations of iron and aluminium sesquioxides.

In Brazil the stunted facies of heath forest is called *Campina* and often lacks the mor humus layer; the taller facies is called *Campinarana*. According to Pires (1973) in the Brazilian Amazon area *Campina* covers 34,000 km^2 and *Campinarana* 30,000 km^2 (1.73% of the area). In this thesis the *Campina* is called stunted heath forest (which I shall normally abbreviate to SHF), the *Campinarana* is called tall heath forest (THF), and the surrounding *terra firme* forest is lowland evergreen rain forest (LERF).

The causes of heath forests

Low soil nutrients and periodic water shortages have been discussed as possible causes of the distinctive structure and physiognomy of the heath forests (Whitmore 1990). Vitousek & Sanford (1986) compared foliar and fine litterfall nutrients of various rain forests, and showed that nitrogen and phosphorus appear to cycle less in heath forest than in other lowland forests, and heath forest litterfall shows high C:N ratios which suggest that nitrogen is in short supply (Cuevas & Medina 1986, 1988) even though its total

amounts in the soil are not unusually low. In heath forest soils in San Carlos, Venezuela, and in Mulu, Sarawak, short periods of water shortage were observed although the soils were usually well supplied with water (Klinge & Medina 1979; Whitmore 1989). Phenolic compounds are abundant in heath forest leaves and litter, and these may be toxic or inhibit nutrient uptake when they leach into the soil (Rodrigues 1961; Proctor *et al.* 1983; Whitmore 1989). Also the very low soil pH has been suggested as a limiting factor in heath forest soils (Rodrigues 1961; Whitmore 1989).

Soil biota and nutrient cycling

Major soil processes are associated with production and decomposition, and these are, in turn, interlinked with the processes of immobilization and mineralization. These processes are biologically mediated and involve the microbial biomass which is only a small fraction (usually 2-4 %) of the total organic matter and which turns over rapidly (Jenkinson & Ladd 1981). There has been considerable interest in assessing the ecosystem role of the soil organisms (Swift *et al.* 1979). The soil microbial biomass forms the base of the detritus food-web and serves as a sink and source for most plant-available nutrients (Anderson & Domsch 1986; Jenkinson & Ladd 1981). Microbial biomass thus has the potential to influence plant growth and the amount of biomass is directly linked to the decay of plant litter (Anderson 1975; Beare *et al.* 1991). Microbial growth in soil is regulated by a multitude of biological, chemical and physical factors, which all interact in a complex fashion. The most important are nutrient availability, moisture, temperature

and grazing by soils animals. Soil invertebrates are very important in the generation and maintenance of the biological, chemical and physical character of the soil (Coleman 1985). In heath forests, trees are usually thought to be ectotrophically mycorrhizal and the mycorrhizal fungi inhibit litter-decomposing microorganisms, leading to litter, and later raw humus accumulation on the forest floor (Singer 1984; Gadgil & Gadgil 1975). This humus layer usually supports an abundance of fungal hyphae and small invertebrates (Collins 1984). Such invertebrates, most of them microbe-feeding, function as regulators of microbial processes through comminution, translocation, defecation, and inoculation of microbial propagules (Visser 1985). Without the combined role of the microflora and the microfauna, together with the collembola, mites and other associated mesofauna, the soil would become a repository of dead plant remains with no recycling of nutrients (Wild 1988).

Heath forests are easily and irreversibly degraded by human disturbance. Once heath forest is felled the soil degenerates very quickly. The surface humus layer is either eroded, burned, or oxidized (Whitmore 1990). The small amount of clay in the soil washes down the profile to leave almost pure silica sand, which has virtually no exchange capacity. The soil is unbuffered and can become increasingly acid. Without vegetation cover or surface humus the white sand can get very hot in the sun. This rapid and easy soil degradation is one of the reasons why agriculture on old heath forest sites is impossible (Whitmore 1990).

However, even degraded heath forests may have biological activity in vegetated patches which often include areas covered by lichens (Lisboa 1975). Several studies have been made on the soil and vegetation of Amazonian heath forest: in Guiana (Richards 1958), Surinam (Heyligers 1963), Venezuela (Klinge & Medina 1979) and, in Brazil (Rodrigues 1961; Anderson 1981), but few have examined aspects of the soil organisms and their role in soil processes (Singer & Araujo 1978; Cuevas & Medina 1986). Among these studies, those in Venezuela were made on heath forests which are located on imperfectly drained soils, subject to periodic waterlogging. No studies on soil biology and on microbially-mediated soil processes have been made on the better-drained heath forests of Central Amazonia even though they would help in the understanding of these ecosystems and provide tools for their wiser management and protection.

Aims of this study

This study investigates the heath forest soil biota and their role in nutrient turnover by characterizing and comparing some biological aspects of the SHF, THF, and LERF at the Reserva da Campina, 45 km north of Manaus. Chapter II concentrates on the characterization of the study sites, including climate, soils and vegetation. Chapters III and IV characterize microbial populations and relate them to soil processes, especially nitrogen transformations. Chapter V examines the influence of fine roots on litter decomposition and litter animal colonization, while Chapter VI investigates which nutrients possibly limit fine root growth. Chapter VII is mainly

descriptive and includes information on mycorrhizal fungi. A general discussion of the results is given in Chapter VIII, followed by concluding remarks.

Chapter II. Study Site Area

This study was carried out in the 'Reserva Biológica da Campina' (Figs 2.1 and 2.2) a station which belongs to Instituto Nacional de Pesquisas da Amazônia (INPA). The 'Reserva' is about 900 ha from which 150 ha are of heath forests and the rest is lowland evergreen rain forest. The station is located 45 km north of Manaus at about 2° 36' S and 60° 01' W (IPEAAOc 1971). The topography is essentially flat with a mean altitude of 44 m above sea level.

CLIMATE

Most of Amazonia has a humid, warm climate, but it is not a homogeneous climatic region because of an important diversity in temperature, and seasonality and amount of rainfall (Bigarella & Ferreira 1985). The Manaus area has a climate which corresponds to the 'Am' type of the Köppen classification defined as follows: 'A' means a tropical rainy climate, where mean monthly temperatures are never below 18 °C; 'm' means that there is a relatively long dry season, but the total annual rainfall is enough to prevent visible effects on the vegetation (IPEAAOc 1971).

Rainfall

Annual rainfall varies within the Amazon basin from 1,500 mm to over 3,500 mm. In the Manaus area, it is about 2,100 mm with a rainy season from December to May, and a drier period from June to November. The mean

rainfall from 1911 - 1980 was 551 mm in the drier season (June to November), and 1,554 mm in the rainy season (December to May) (Ribeiro & Adis 1984). The rainiest months tend to be March and April, with about 300 mm each, while the drier months July, August, and September, normally receive less than 100 mm each (IPEAAOc 1971).

In a *Terra Firme* watershed forest catchment located 9.5 km NW of the study sites, rainfall interception by the vegetation was 28.6%, and 45.5% was transpired by the plants. The evapotranspiration was 74.1%, with a daily rate of 4.1 mm, and 25.9% was drained through the forest streams (Leopoldo *et al.* 1982). Corresponding data are not available for heath forests but it may be presumed that heath forest has more evaporation from the soil.

Fig. 2.2 shows the monthly and total rainfall in the Reserva da Campina during the period of the study (from January 1992 - September 1993), taken from the Meterological Station of CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira) located 3 km north of the Reserva.

Temperature and air humidity

In Central Amazonia the mean annual temperature is 26.7 °C; the mean values of the maximum and minimum temperatures are 31.2 °C and 23.7 °C (IPEAAOc 1971). Table 2.1 shows the mean monthly temperature and ranges of mean minima and mean maxima and the air humidity (%) in the period of the study. The absolute minimum temperature during the study was 18.0 °C and the maximum 34.8 °C. Data were provided by CEPLAC.

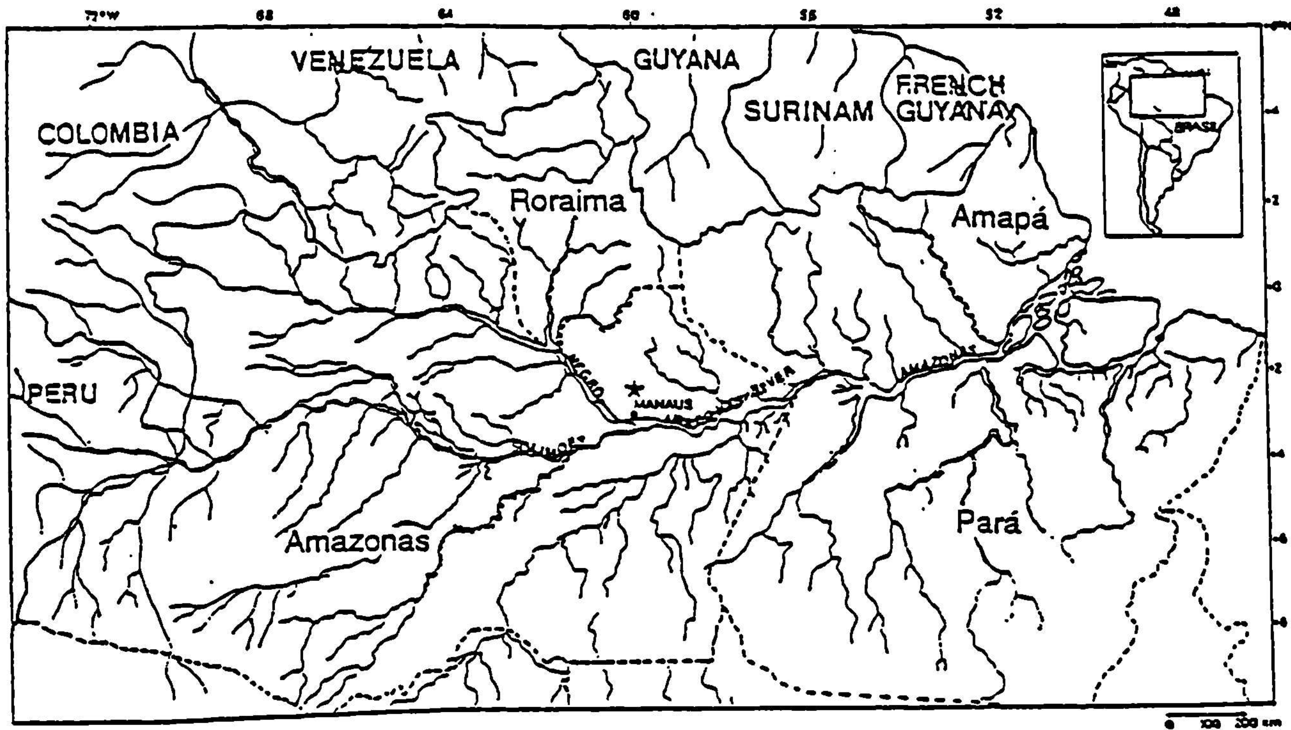
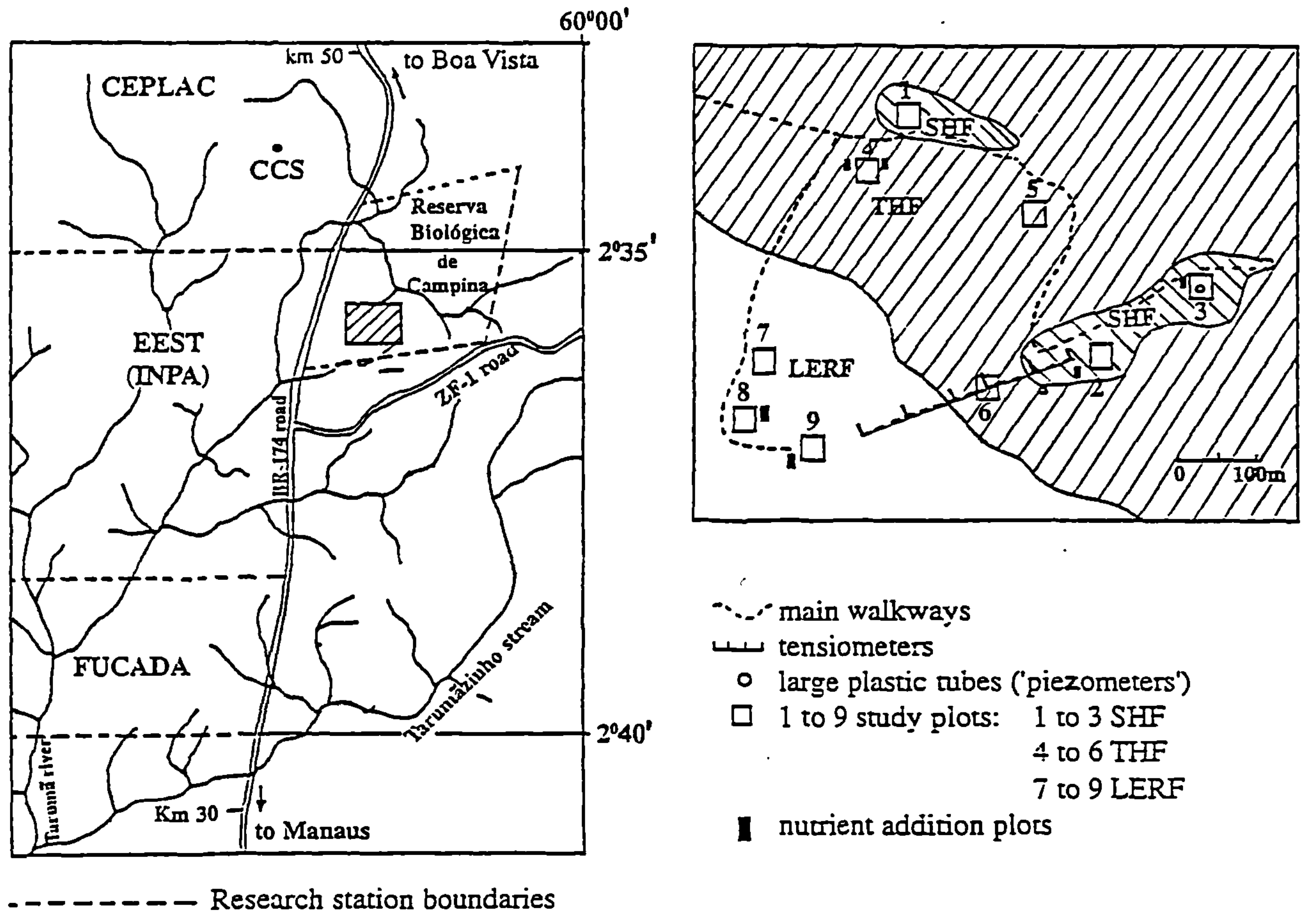


Fig. 2.1: Map of the Amazon Basin showing the location (*) of Reserva da Campina (above) and the main entrance of the Reserva da Campina (below)



CEPLAC - Cocoa Cropping Development Agency (Ministry of Agriculture)

EEST - Tropical Forestry Experimental Station of INPA (National Institute for Amazonian Research - Ministry of Science and Technology)

FUCADA - Model Farm for the Agricultural District (Ministry of Regional Development)

CCS - CEPLAC's climatological station

Fig 2.2. Details of the location of the Reserva Biológica da Campina and of the study plots within it.

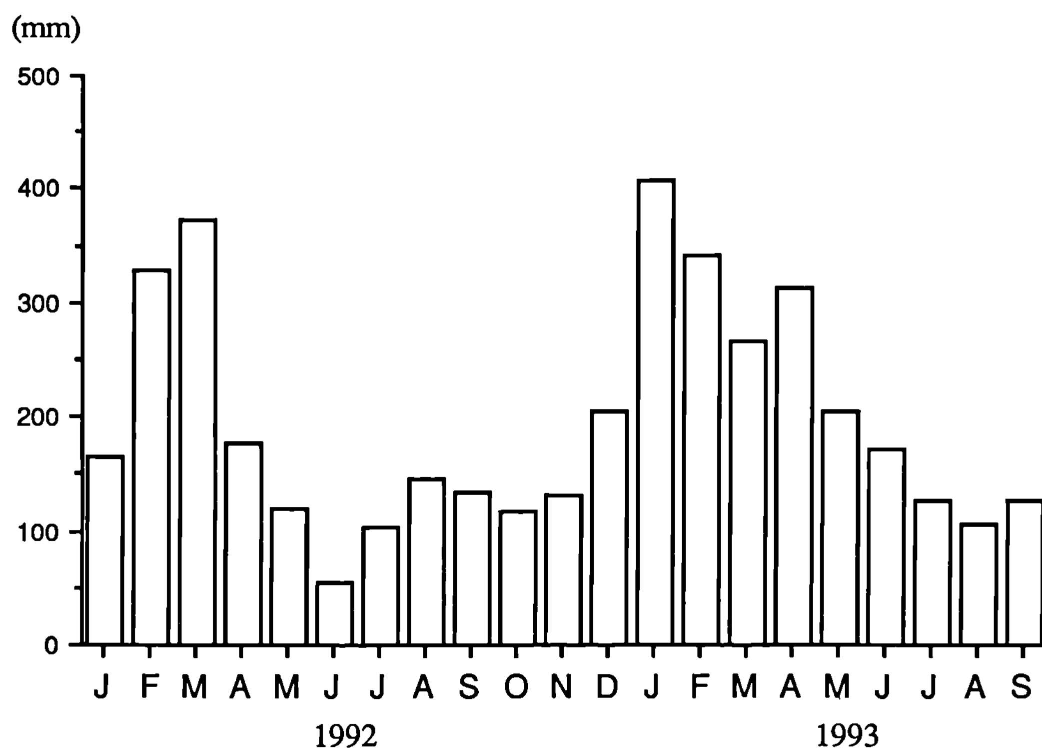


Fig. 2.3: Monthly rainfall (mm) in the Reserva da Campina during the period of the study. Data provided by CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira).

Table 2.1: Mean monthly temperatures (°C) (with the ranges of the mean minima and mean maxima, in parenthesis) and relative humidity of the air (% RH) near the Reserva da Campina in the period of the study. Data provided by CEPLAC.

Years	Months	Temperatures (°C)	% RH
1992	January	26.6 (22.2 - 31.6)	86
	February	25.8 (23.0 - 31.3)	87
	March	25.5 (23.1 - 30.3)	89
	April	25.8 (23.6 - 30.8)	87
	May	26.4 (23.2 - 31.6)	86
	June	25.8 (21.9 - 32.4)	84
	July	25.0 (21.9 - 32.4)	85
	August	24.6 (21.1 - 30.8)	87
	September	26.0 (22.1 - 33.0)	83
	October	26.1 (22.3 - 32.8)	84
	November	26.0 (22.7 - 32.4)	86
	December	25.3 (22.8 - 30.1)	90
1993	January	24.9 (22.4 - 30.1)	90
	February	24.9 (22.4 - 30.6)	89
	March	24.8 (22.5 - 30.0)	89
	April	25.2 (21.8 - 30.5)	90
	May	25.8 (22.1 - 31.6)	88
	June	25.5 (22.1 - 31.3)	88
	July	25.1 (21.4 - 31.6)	86
	August	24.9 (21.4 - 31.7)	87
	September	25.8 (21.4 - 32.3)	86

VEGETATION

At 'Reserva da Campina' the SHF is developed on the most extreme soil and has a peculiar flora with a distinctly open and stunted physiognomy. It has a xeromorphic aspect, thick leaves, thick bark, and an abundance of lichens and mosses on the branches and on the soil surface (Pires & Prance 1985). The SHF (Fig. 2.4) has open areas with 'islands' with communities of plant species such as the 'casca-doce' *Pradosia schomburgkiana* (Sapotaceae), *Protium heptaphyllum* (Burseraceae) and *Hirtella racemosa* (Chrysobalanaceae), all with small (< 10 cm) diameter stems and low stature (Anderson *et al.* 1975). The high light penetration is, according to Takeuchi (1960), the cause of the large numbers of epiphytes in the SHF. The gnarled, crooked trees are loaded with epiphytes of the Araceae, Bromeliaceae, Gesneriaceae, Orchidaceae and pteridophytes (Prance 1989). The open areas of sand are covered by the blue-green alga *Stigonema tomentosum* and lichens (mainly *Cladonia* spp.) which, perhaps decrease the soil temperature, and favour seed establishment of woody plants (Prance 1989). The SHF also shows some shaded dense areas dominated by the 'macucu' *Aldina heterophylla* (Caesalpiniaceae) which grows up to 10 m high (Anderson *et al.* 1975).

Fig. 2.4: Views of the SHF (above) showing the bare exposed sand among the shrubby vegetation, and the THF (below) showing many small-diameter trees.

THF is a closed forest on spodosol similar and adjacent to the SHF but in the THF the sandy area is fully colonized by trees and shrubs (Fig. 2.4). In the plots, *Pradosia schomburgkiana* is generally found as isolated individuals with a higher stature (ca. 12 m) and which may have thick layers of humus below them. Anderson *et al.* (1975) found 45 species of woody plants in the THF associated with 34 species of bryophytes and some lichens (Lisboa 1975). Janzen (1974) reported that heath forests are unproductive ecosystems which he believed was the cause of their small numbers of animals. Additionally, he suggested that plant species in SHF and THF are characterized by high concentrations of toxic substances, *e.g.* polyphenols, which inhibit herbivores. Lisboa (1975) mentioned a special allelopathic chemical produced by *Pradosia schomburgkiana* which inhibits other seedlings.

The floristic composition of Amazonian heath forests is variable from site to site (Braga 1979). Anderson (1981) estimated that 54.5% of the vascular flora of the heath forests in Central Amazonia is endemic to spod^P16L16osols and usually shows a moderate degree of sclerophylly (Rodrigues 1961). A substantial proportion of the species also occurs in lowland evergreen forest on oxisols (23.6%) and in *igapó* (black-water inundated forest) (20%); only a few species (2.6%) occur also in *várzea*, (a white-water nutrient-rich inundated forest). Table 2.2 shows a list of plant species found in both SHF and THF from Anderson (1978) and Braga (1979). Table 2.3 shows some of the plant species which are common to all three forests, SHF, THF and LERF (Anderson 1978).

Table 2.2: Some species of plants found in both SHF and THF in Reserva da Campina, INPA. (Braga 1979). * Also recorded by Anderson (1978).

Plant species	Family
* *Aldina heterophylla* Spr. ex Benth.	Leguminosae
* *Annona nitida* Mart.	Annonaceae
Borreria capitata (R. et P.) Dc.	Rubiaceae
Clusia aff. *columnaris* Engl.	Clusiaceae
Clusia grandiflora Splitg.	Clusiaceae
Conomorpha cf. *grandiflora* Mez.	Myrsinaceae
* *Doliocarpus spraguei* Cheesm.	Dilleniaceae
* *Erythroxylum campinense* Amaral Jr.	Erythroxylaceae
* *Eugenia patrisii* Vahl.	Myrtaceae
* *Pradosia schomburgkiana* (Mart ex Miq) Ducke	Sapotaceae
* *Henriettea maroniensis* Sagot	Melastomataceae
Heteropterys aff. *acutifolia* Adr. Juss.	Malpighiaceae
* *Hirtella racemosa* Lam. var. *racemosa*	Chrysobalanaceae
* *Humiria balsamifera* St Hil	Humiriaceae
* *Macrolobium arenarium* Ducke	Leguminosae
* *Mandevilla ulei* K. Schum	Apocynaceae
* *Manikara amazonica* (Hub.) Standl.	Sapotaceae
Matayba opaca Radlk.	Sapindaceae
* *Miconia lepidota* Dc.	Melastomataceae
Mouriri nervosa Plig.	Melastomataceae
* *Ormosia costulata* (Miq.) Kleinh.	Leguminosae
* *Ouratea spruceana* Engl.	Ochnaceae
* *Pagamea duckei* Standl.	Rubiaceae
* *Palicourea nitidella* (M. Arg.) Standl.	Rubiaceae
Parkia auriculata Spr. ex Benth	Leguminosae
* *Protium heptaphyllum* (Aubl.) March	Burseraceae
* *Qualea retusa* Spr. ex Warm.	Vochysiaceae
Sandemania hoehnei (Cogn.) Wurdack	Melastomataceae
* *Swartzia dolichopoda* Cowan	Leguminosae
Talisia cerasina (Bth) Radlk	Sapindaceae
* *Vernonia grisea* Baker	Compositae

Table 2.3: List of the plant species from SHF and THF on spodosols which are also found in LERF on ultisols in Reserva da Campina (Anderson 1978)

Plant species	**Family**
Tetrameranthus duckei	Annonaceae
Protium heptaphyllum	Burseraceae
Couepia racemosa	Chrysobalanaceae
Pera schomburgkiana	Euphorbiaceae
Humiria balsamifera	Humiriaceae
Aldina heterophylla	Leguminosae
Miconia lepidota	Melastomataceae
Palicourea nitidela	Rubiaceae
Talisia cerasina	Sapindaceae
Manikara amazonica	Sapotaceae
Simaba cuspidata	Simaroubaceae

PHYSICAL FEATURES

Geology

The geology of the area of the study sites is soft Tertiary sediments which belong to the Alter-do-Chão Formation, part of the Barreiras Group (Putzer 1984). The Barreiras Group was deposited as a result of successive erosive phases of extensive areas of the Palaeozoic basin and the Precambrian shields at the end of the Tertiary. In the Barreiras Group the rocks are sand-clay sediments of quartzitic and kaolinitic nature, with either coarse or fine texture and their sediments consist of arenites intercalated with argilites. During the Quaternary the sediments gave rise to the ground water spodosols and regosols (Dias *et al.* 1980). Their main constituents are resistant minerals such as kaolinite, quartz, and oxides of iron and aluminium (Chauvel 1982).

Soils

The interfluve spodosol areas, where the heath forests occur, are very common and extensive in the upper Rio Negro area of Brazil, the adjoining parts of Venezuela and Colombia, and the middle reaches of the Rio Branco drainage system (Sombroek 1984). Because of the coarseness of the sand, heath forest soils are freely draining and have a low water retention. They are nutrient-poor soils which are low in sexquioxides, poorly buffered, and highly acidic (Klinge 1967). In some heath forest soils, the seasonality is

amplified either by the flooding and associated anoxia of the soil, or drought caused by the excessive porosity of the sand (Pires & Prance 1985).

'Mor' acid humus often develops on the sandy soils resulting in podsolisation of the profile. The mor is poorly mixed with the mineral matter, has a slow decomposition, and supplies nutrients at a low rate. The humus develops in layers which are increasingly decomposed with depth. The spodosols in the THF and LERF have a well developed root mat but the THF shows a deeper humic layer. Findings of ceramic relicts in SHF (Prance & Schubart 1978) lends support to the idea that human influence destroyed the surface mor humus and left a pure white-sand with patches of low trees and shrubs (Lisboa 1975; Braga & Braga 1975) and associated algae and lichens. THF has a typical mor-like soil profile with a surface mor humus.

PLOT SELECTION

Three 50 m x 50 m replicate plots in each forest type were chosen in a stratified random way in the Reserva da Campina. Plots 1, 2 and 3 are in the SHF; plots 4, 5 and 6 are in the THF, and plots 7, 8, and 9 in the LERF (Fig. 2.2). The plots were marked out and subdivided into four quadrants of 25 m x 25 m. All nine plots were made permanent.

SOIL ANALYSES

In each plot, samples for soil analyses were taken from the upper layers of the soil which included all the organic layers (litter, root mat and humus) and the 10 cm of the mineral soil below the organic layers. The biological

measurements described in Chapter III and IV were made on these samples and analyses from them are given in Tables 2.4 - 2.6.

Additionally, Luizão (1995) provided soil chemical analyses from profiles on one pit dug outside but near each plot. These data are shown in Tables 2.7 - 2.9. The soils were all very acid: pH_{H2O} ranged from 3.4 (SHF, 10-20 cm depth) to 4.2 (LERF, 10-20 cm); pH_{KCl} from 2.4 (THF, 0-10 cm) to 3.4 (LERF, 10-20 cm). (Table 2.4). The LERF pH's were always a little higher than those in the SHF and THF. The organic layers are poorly represented in the SHF. In the THF and LERF, however, both the humus and the root mat form distinct and usually thicker layers (Table 2.5). The C:N ratios are high (25-82) in the litter and root mat and much less (13-20) in the other layers where the ratios are favourable for decomposition with net mineralization (Swift *et al.* 1979) (Table 2.6). Results from the pits (Tables 2.7-2.9) showed that the mineral soils of all study plots are low in clay, total nitrogen, total phosphorus, exchangeable potassium, sodium and magnesium, and have a low CEC (Table 2.10). Exchangeable aluminium is much higher in the LERF than in both the THF and SHF. However the upper layers (organic) of both the THF and LERF are higher in humus and show relatively high concentrations of nitrogen, phosphorus and exchangeable cations, except calcium, when compared with other acidic tropical soils (Table 2.10).

Table 2.4: pH_{H2O} and pH_{KCl} at two soil depths from SHF, THF and LERF. Values are means with ranges (n=5)

Depths (cm)	**SHF**		**THF**		**LERF**	
	pH_{KCl}	**pH_{H2O}**	**pH_{KCl}**	**pH_{H2O}**	**pH_{KCl}**	**pH_{H2O}**
0-10[1]	2.6 ± (2.4-2.8)	3.7 ± (3.4-3.9)	2.4 ± (2.2-2.6)	3.5 ± (3.2-3.7)	2.8 ± (2.6-3.0)	3.9 ± (3.7-4.1)
10-20[2]	3.2 ± (3.0-3.3)	3.4± (3.2-3.5)	2.8 ± (2.7-3.0)	3.9 ± (3.8-4.0)	3.4 ± (3.3-3.5)	4.2 ± (4.0-4.3)

[1] The 0-10 cm is an average depth which comprises all the organic material above the mineral soil- after sieving which removes the root mat and larger litter.
[2] The 10-20 cm comprises only mineral soil.

Table 2.5: The mean thickness (cm) of the litter layer, root mat, humus layer (mor in THF, moder in LERF), and the surface or near-surface layer of humus mixed with mineral matter. There were five subsamples in each replicate plot of SHF, THF and LERF.

Layer	SHF	THF	LERF
litter	1.7 (0 - 2.5)	3.0 (2.0 - 3.5)	2.4 (1.5 - 3.0)
root mat	0.5 (0 - 2.5)	6.0 (4.0 - 8.0)	3.9 (2.5 - 6.5)
humus	0.8 (0 - 1.5)	5.4 (2.0 - 12.0)	1.4 (0.0 - 3.0)
humus with mineral matter	1.5 (1 - 2.5)	2.8 (1.5 - 10.0)	1.2 (0.5 - 2.0)

Table 2.6: Percentage of organic carbon (C) and total nitrogen (N) and C:N ratio in the upper layers of the soils. There were two subsamples for each replicate plot of SHF, THF, and LERF.

	SHF			THF			LERF		
Layer	C	N	C:N	C	N	C:N	C	N	C:N
litter	34.6 ± 3.41	0.42 ± 0.11	82	38.3 ± 2.31	0.65 ± 0.14	59	31.9 ± 2.96	1.27 ± 0.41	25
root mat	23.8 ± 11.6	0.61 ± 0.23	39	37.7 ± 2.46	0.64 ± 0.05	60	27.7 ± 2.72	0.70 ± 0.11	40
humus	29.5 ± 2.60	2.34 ± 0.97	13	20.7 ± 14.7	1.15 ± 0.71	18	14.5 ± 3.87	1.09 ± 0.43	16
humus with mineral matter	5.40 ± 0.96	0.27 ± 0.05	20	5.32 ± 1.68	0.32 ± 0.08	17	7.67 ± 7.96	0.38 ± 0.32	20
mineral soil	0.66 ± 0.19	0.05 ± 0.02	13	1.04 ± 0.42	0.05 ± 0.02	20	1.30 ± 0.42	0.07 ± 0.02	19

Table 2.7: Soil chemical properties and particle size composition at a range of depths in pits outside of the plots in the SHF. Values are means for 3 pits, unlesss indicated otherwise.

	Depths of samples in the pits (cm)								
	0-3[1]	0-10[2]	3-10[1]	10-20	20-30	30-40	40-50	50-70	80-100
organic C	10.5	0.20	0.40	0.28	0.15	0.10	0.09	0.08	0.07
(%)	5.5-16		0.2-0.6	0.01-0.75	0.0-0.26	0.0-0.22	0.0-0.19	0.0-0.19	0.0-0.16
pH_{H2O}	3.7	4.7	4.3	4.5	4.9	4.9	5.0	5.0	5.4
	3.7-3.7		4.3-4.3	4.0-5.0	4.6-5.1	4.6-5.2	4.7-5.4	4.7-5.5	4.8-6.2
pH_{KCl}	2.6	3.5	3.2	3.4	3.6	3.8	4.1	4.4	4.9
	2.5-2.7		3.0-3.3	3.1-3.7	3.5-3.8	3.7-4.0	3.9-4.3	4.1-4.6	4.7-5.2
N total	4.0	0.40	0.20	0.37	0.30	0.23	0.17	0.17	0.1
(mg g^{-1})	2.1-6.0		0.2-0.2	0.2-.06	0.2-0.4	0.2-0.3	0.0-0.3	0.0-0.3	0.0-0.2
P total	200	39	44	29	23	23	22	24	22
(μg g^{-1})	200-200		43-44	24-34	21-25	19-29	20-25	16-33	16-27
Exchangeable cations (m-equiv kg^{-1})									
K^+	2.2	0.0	0.30	0.03	0.0	0.0	0.0	0.06	0.01
	1.3-3.1		0.2-0.4	0.0-0.1				0.0-0.18	0.0-0.02
Na^+	0.45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.2-0.7								
Ca^{2+}	0.90	0.5	1.5	0.41	0.82	0.19	0.37	0.75	1.79
	0.7-1.1		0.3-2.7	0.0-0.9	0.0-1.6	0.1-0.4	0.0-0.7	0.3-1.1	1.1-2.4
Mg^{2+}	3.8	0.06	0.37	0.08	0.10	0.05	0.06	0.18	0.20
	2.3-5.3		0.1-0.6	0.0-0.2	0.0-0.2	0.02-0.06	0.0-0.1	0.02-0.4	0.06-0.3
H^+	19.7	0.6	3.4	2.9	1.5	1.2	1.0	1.0	1.4
	14-25		3.4-3.4	1.4-5.1	0.9-2.1	0.8-1.6	0.9-1.1	0.4-1.6	1.1-1.5
Al^{3+}	0.0	0.0	1.0	0.0	0.2	0.0	0.0	0.0	0.0
			0.0-2.0		0.0-0.5				
CEC	27.1	1.16	6.57	3.40	2.78	1.44	1.47	2.01	3.4
	19-35		4.2-9.0	1.8-6.2	2.1-3.9	1.3-1.7	1.1-2.0	1.5-2.6	2.8-4.1
Base saturation	27.2	48.3	29.1	15.0	28.7	17.3	25.5	51.5	58.0
(%)	26-28		18.5-40	4.5-23	0-46	7-32	0-44	38-74	42-66
Total acidity	19.7	0.6	4.4	2.9	1.8	1.2	1.03	1.03	1.4
($H^+ + Al^{3+}$)	14-25		3.4-5.4	1.4-5.1	1.4-2.1	0.9-1.6	0.9-1.1	0.4-1.6	1.1-1.6
Particle fraction (%)									
clay (< 2 μm)	1.8	1.8	1.8	1.2	0.85	0.80	0.85	1.2	1.0
silt (2-62 μm)	4.1	4.1	4.1	0.46	0.25	0.0	0.85	0.0	0.01
sand (> 62 μm)	94.1	94.1	94.1	98.3	98.7	99.2	98.7	99.0	99.0

[1] values from two pits
[2] values from a single pit

Table 2.8: Soil chemical properties and particle size composition at a range of depths in the pits outside of the plots in the THF. Values are means and ranges for 3 pits, unless indicated otherwise.

	Depth of samples in the pits (cm)												
	0-3[2]	0-7[2]	0-12[2]	3-10[2]	10-20[1]	15-20[2]	20-30	30-40	40-50	50-60	60-70	70-80	80-100
organic C (%)	32.0	31.3	27.3	1.0	0.94	2.18	1.22	0.91	0.75	0.38	0.36	0.25	0.26
					0.4-1.4		0.2-1.8	0.2-1.4	0.0-1.4	0.1-0.6	0.1-0.6	0.1-0.4	0.2-0.4
pH $_{H2O}$	3.5	3.5	3.4	3.9	4.2	3.9	4.2	4.2	4.3	4.7	4.8	4.8	5.0
					3.9-4.4		3.5-4.7	4.1-4.3	4.1-4.5	4.6-4.8	4.3-5.2	4.5-5.0	4.6-5.3
pH $_{KCl}$	2.2	2.3	2.1	2.8	2.8	2.6	2.9	3.1	3.3	3.6	3.7	4.0	4.1
					2.7-3.0		2.7-3.3	2.9-3.3	3.1-3.6	3.5-3.7	3.5-3.9	3.8-4.2	4.0-4.1
N total (mg g^{-1})	12.2	15.2	9.9	0.6	0.45	0.50	0.20	0.03	0.03	0.09	0.03	0.05	0.03
					0.2-0.7		0.1-0.3	0.0-0.1	0.0-0.1	0.0-0.2	0.0-0.1	0.0-0.1	0.0-0.1
P total (µg g^{-1})	200	200	100	39	29	57	19	12	12	13	11	8.3	9.0
					12-46		0.0-34	0.0-21	1.0-18	0.0-20	1.0-16	0.0-17	0.0-15
Exchangeable cations (m-equiv kg^{-1})													
K^+	4.8	2.20	3.9	0.12	0.60	0.14	0.27	0.07	0.13	0.03	0.0	0.0	0.0
					0-1.2		0-0.8	0-0.2	0-0.4	0-0.05			
Na^+	5.0	3.20	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ca^{2+}	0.30	0.20	0.10	3.75	1.41	1.50	1.83	2.79	1.71	2.12	2.04	2.00	2.37
					0.2-2.6		0.0-3.7	1.5-5.1	0.7-3.2	1.1-3.5	1.6-2.7	0.7-2.6	0.7-4.0
Mg^{2+}	4.8	3.20	3.5	0.56	0.38	0.48	0.28	0.43	0.21	0.30	0.25	0.23	0.30
					0.3-0.4		0.0-0.5	0.3-0.7	0.1-0.4	0.2-0.4	0.2-0.4	0.1-0.3	0.1-0.5
H^+	26.4	62.9	61.1	5.6	7.6	11.9	7.3	4.9	3.2	3.7	1.0	1.0	1.4
					5.4-9.9		2.4-10	3.6-26	2.4-4.4	1.1-8.4	0.1-1.4	0.4-1.6	0.8-1.2
Al^{3+}	0.40	16.5	8.0	0.0	0.8	1.5	0.8	0.3	0.0	0.7	0.0	0.0	0.0
					0.5-1.0		0.5-1.5	0.0-1.0		0.5-1.0			
CEC	41.7	88.2	82	10.0	10.8	15.5	10.5	8.5	5.3	6.80	3.26	3.23	4.07
					9.4-12		7.2-13.5	6.7-9.6	3.7-6.4	4.1-10	2-4.5	1.8-4.5	2.7-6.0
Base saturation (%)	35.7	9.98	15.7	44.2	23.2	13.7	26.9	37.7	35.8	42.6	73.5	65.9	60.9
					10-32		0-59	19-62	22-62.6	22-63	56-95	45-88	31.77
Total acidity ($H^+ + Al^{3+}$)	26.8	79.4	69.1	5.6	8.4	13.4	8.1	5.2	3.2	4.4	1.0	1.0	1.4
					6.4-10		2.9-11	3.6-7.4	2.4-4.4	2.1-8.9	0.1-1.4	0.4-1.6	0.8-1.9
Particle fraction (%)													
clay (< 2 µm)	0.0	0.0	0.0	na	3.6	3.6	2.2	0.40	2.0	1.20	1.20	1.60	1.40
silt (2-62 µm)	0.0	0.0	0.0	na	0.04	0.04	1.38	1.29	0.0	9.07	0.0	0.81	0.30
sand (> 62 µm)	0.0	0.0	0.0	na	96.3	96.3	96.4	98.3	98.6	98.7	99.0	97.6	98.3

[1] Values from two pits
[2] values from a single pit

Table 2.9: Soil chemical properties and particle size composition at a range of depths in the pits outside of the plots in the LERF. Values are means and ranges of 3 pits, unless indicated otherwise.

	Depth of samples in the pits (cm)										
	0-3[2]	0-6[1]	3-10[2]	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-100
organic C (%)	18.4	24.6 15-35	0.77	1.30 0.3-2.1	1.10 0.8-1.3	1.06 0.7-1.4	1.08 0.8-1.4	0.94 0.6-1.2	0.85 0.5-1.1	0.60 0.4-0.9	0.64 0.4-1.1
pH $_{H2O}$	3.9	3.8 3.8-3.8	4.2	4.1 4.0-4.2	4.3 3.9-4.5	4.6	4.7 4.6-4.8	4.6 4.6-4.7	4.5 4.5-4.6	4.5 4.2-4.7	4.7 4.6-4.7
pH $_{KCl}$	2.7	2.8 2.7-3.0	3.4	3.5 3.4-3.6	3.9 3.9-4.0	4.1 4.0-4.1	4.2 4.1-4.2	4.2 4.2-4.3	4.2 4.2-4.3	4.2 4.2-4.3	4.2 4.2-4.3
N total (mg g^{-1})	9.0	11.7 7.5-16	0.8	0.6 0.1-1.0	0.3 0.1-0.7	0.57 0.2-0.8	0.51 0.02-0.8	0.24 0.01-0.5	0.35 0.2-0.5	0.17 0.01-0.4	0.23 0.1-0.4
P total (µg g^{-1})	200	250 200-300	18	28 23-32	18 15-20	12.3 9.0-15	7 5.0-8.0	5 1.0-7.0	9 1.0-17.0	5 2.0-7.0	3 3.0-4.0
Exchangeable cations (m-equiv kg^{-1})											
K^+	2.7	5.9 5.9-6.0	0.06	0.15 0.04-0.2	0.19 0.06-0.2	0.01 0.01-0.02	0.11 0.0-0.3	0.01 0.0-0.04	0.0	0.04 0.0-0.13	0.0
Na^+	5.4	4.8 4.1-5.6	0.0	0.08 0.0-0.2	0.0	0.0	0.27 0.1-0.7	1.07 0.0-2.4	0.75 0.0-1.5	0.09 0.0-0.3	0.0
Ca^{2+}	0.2	0.23 0.2-0.25	2.62	3.41 2.6-4.1	3.54 3.1-3.9	2.46 0.0-4.0	2.54 0.0-3.9	3.04 0.5-37	2.19 0.4-4.0	2.75 0.0-4.9	1.96 0.0-3.5
Mg^{2+}	2.8	2.5 2.2-2.8	0.71	0.61 0.5-0.6	0.47 0.4-0.5	0.55 0.5-0.6	0.43 0.3-0.6	0.56 0.4-0.7	0.47 0.4-0.5	0.68 0.4-1.1	0.38 0.2-0.5
H^+	26.6	3.0 0.0-0.6	3.9	4.5 3.9-6.1	1.6 0.9-2.6	1.5 0.4-2.6	0.7 0.0-1.9	1.0 0.0-2.1	0.8 0.9-1.6	1.6 1.1-2.1	0.9
Al^{3+}	39.0	62.0 35-89	14.0	10.6 7.5-14.8	13.5 12-14	11.7 10-12	12.3 10-14	11.3 8.5-13	10.0 7.5-13	9.0 7.5-10	9.1 8.7-11
CEC	76.7	78.6 56-102	21.3	19.4 16-25	19.3 19-20	16.2 16-17	16.3 15-18	17.0 14-22	14.2 13.5-15	14.2 12-16	12.3 11-15
Base saturation (%)	14.5	19.2 12-26	15.9	22.8 17-30	21.2 20-22	18.5 3.5-28	19.6 3.5-30	25.3 9.7-38	24.3 16-33	23.7 3.6-38	17.9 4.2-27
Total acidity ($H^+ + Al^{3+}$)	65.6	47.3 41-54	17.9	15.1 11-21	15.1 15-16	13.1 12-15	13.0 12-14	12.3 11-14	10.9 9.0-13	10.6 9.0-12	10.0 9.0-11
Particle fraction (%)											
clay (< 2 µm)	9.6	9.6	9.6	11.6	10.6	10.4	11.8	12.2	15.8	14.0	19.8
silt (2-62 µm)	19.8	19.8	19.8	7.51	6.01	10.4	13.3	15.3	11.5	9.87	12.5
sand (> 62 µm)	70.6	70.6	70.6	80.9	83.4	79.5	74.9	72.2	72.7	76.4	67.6

[1] values from two pits
[2] values from a single pit

Table 2.10 : Surface soil samples analyses from a range of tropical rain forest on acidic soils ($pH_{H2O} < 5$).

	Africa			Malaysia			Costa Rica	South America						
	Cameroon		Ghana	Pasoh	Danum	Mulu		Maracá	Surinam	Manaus		SHF	THF	LERF
	1	2	3	4	5	6	7	8	9	10	11	12	12	12
depth (cm)	0-8	0-10	0-15	2-20	1-25	0-10	0-15	0-10	0-7	0-8	0-20	0-5	0-9	0-6
pH_{H2O}	4.2	4.2	4.2	4.3	4.4	4.1	4.2	4.9	4.0	3.8	4.2	3.7	3.5	3.9
C %	3.6	2.7	2.2	0.5	0.3	11	8.9	0.5	2.4	2.0	-	7.1	30.2	22.6
N %	0.31	-	0.21	0.17	0.04	0.51	0.4	0.5	0.13	0.18	-	0.28	1.24	1.08
							$\mu g\ g^{-1}$							
P_{total}	-	234	-	200	100	120	940	61	86	-	-	146	167	233
$P_{extr.}$	-	10	6	0	-	-	2.2	5.1	6.1	0.27	-	-	-	-
							Exchangeable cations (m-equiv 100 g^{-1})							
K^+	0.17	0.07	-	0.58	0.10	0.25	0.17	0.07	0.07	0.05	-	0.15	0.36	0.49
Na^+	0.13	-	-	0.08	0.09	0.06	0.11	0.005	0.04	0.04	0.01	0.03	0.45	0.50
Ca^{2+}	0.87	0.10	-	0.28	0.25	0.04	1.10	0.23	0.38	0.21	0.04	0.08	0.02	0.02
Mg^{2+}	0.30	-	-	0.10	0.07	0.18	0.29	0.18	0.16	0.14	0.01	0.26	0.38	0.06
CEC	18	-	8	5.6	2.5	37	-	0.85	1.4	10.7	2.0	18.4	70.6	78.2
BS %	8	-	10	18.6	20.4	1.6	-	55.5	46.4	4	-	34.2	20.5	17.5
							Particle size of the mineral matter (%)							
sand	20	88	54	30	-	-	-	76	96	9	91	94	96	71
clay	64	8	27	49	-	-	80	12	4	81	7.7	1.8	2.4	9.6

[1] Hawkins & Brunt (1965) in Sambalang, soil type not determined
[2] Newbery *et al.* (1988) in Korup, soil type not determined
[3] Hall & Swaine (1976), soil type not determined
[4] Allbrook (1973), 'Durian series' soil
[5] Green (1992), Orthic Acrisol
[6] Proctor *et al.* (1983), Ultisol
[7] Grieve *et al.* (1990), Dystropept
[8] Thompson *et al.* (1992), Grossarenic plinthic Paleudult
[9] Poels (1987), Quartzipsammentic Ultic Haplorthox
[10] IPEAAOc (1972), Oxisol
[11] Bravard & Righi (1988), Ultisol
[12] Luizão (1995), soil analyses from the pits

Chapter III. Soil Microbial Biomass and Soil Respiration in SHF, THF, and LERF.

INTRODUCTION

Microbial biomass has been defined as the living component of the soil organic matter, excluding plant roots and soil animals larger than 5×10^3 μm^3 (Jenkinson & Ladd 1981). Soil microbes account for 1 - 3% of the total organic carbon in the soil (Diaz-Raviña *et al.* 1988; Jenkinson & Ladd 1981; Srivastava & Singh 1988) and are responsible for many of the decomposition and nutrient turnover processes. Microbial biomass has a turnover many times faster than the bulk of the organic matter and it can have an important influence on the availability of plant nutrients. Changes in microbial biomass occur more rapidly than in the bulk of the soil organic matter, and can be useful to predict longer-term trends in organic matter concentrations (Dalal & Meyer 1987; Powlson & Jenkinson 1981; Powlson *et al.* 1987; Voroney *et al.* 1981). A knowledge of the behaviour of the soil microbial fraction can assist in selecting the most appropriate land management practices (Paul & Voroney 1984; Nannipieri 1984).

Owing to their diversity, soil microorganisms are able to make numerous transformations under widely varying environmental conditions (Alexander 1961). Fertilizer applications to the soil affect the growth and activities of diverse groups of soil microorganisms. They can directly stimulate the growth of the general microbial population by supplying nutrients or indirectly

through root exudates from increased plant growth (Goyal *et al.* 1992). On the other hand, fertilizers may affect the composition of individual microbial communities in the soil (Khonje *et al.* 1989). During a study of the microfungi in a nitrogen-fertilized forest soil (Arnebrant *et al.* 1990) changes in species composition in plots treated with ammonium nitrate were similar to those previously reported in artificially acidified areas (Bååth *et al.* 1984). The use of urea, however, affected the microfungal community differently from ammonium nitrate. Since urea and ammonium nitrate have different effects on soil pH (Nômik & Moller 1981), it was suggested that pH was the main reason for the observed changes in species composition. When urea is hydrolysed, the resulting ammonia increases the pH so that the formation of nitrate in acid soils is greater from urea than from ammonium nitrate. An exact limiting pH cannot be ascertained since a variety of physico-chemical factors in soil alters plant response to acidity. Generally, calcium addition (lime) has been used in acid soils to reduce acidity. There is no information available on the size of microbial biomass and activity in acid heath forest soils and a nutrient addition experiment offers an opportunity to gain an insight into it.

The number of microorganisms in a soil cannot be regarded as an index of their activity since many organisms may be dormant at the time of sampling. The activity of the soil population is not a concept that can easily be given a quantitative definition (Wild 1988) but for many purposes it can be measured by the amounts of either carbon dioxide or heat evolved by the population. With the advent of the fumigation-incubation technique in which

carbon dioxide production is used to estimate microbial biomass (Jenkinson & Powlson 1976) this technique became widely employed as a measure of soil respiration (Brookes *et al.* 1985; MacFadyen 1970), with the assumption being that the carbon dioxide derives from the organic matter mineralized by microorganisms, and not from roots or soil invertebrates.

The objectives of this study were to investigate the size of the soil microbial biomass and its activity among the SHF, THF, and LERF to determine the relationships between it and the physical and chemical properties of the soil. Additionally, the relative effect on microbial activity of various nutrient additions was compared from the measurements of soil respiration.

MATERIALS AND METHODS

Microbial carbon estimated by fumigation-incubation

The fumigation-incubation (FI) method for measuring the carbon content of soil microbial biomass relies on the lysis of the microbes by a fumigant, usually chloroform, followed by a measurement of soil respiration (Jenkinson & Powlson 1976). A pulse of respiration is attributable to the mineralization of lysed microbes in which the magnitude of this pulse is directly related to the amount of microbial biomass in the soil sample. During the incubation, about 38 - 52% of the killed microorganisms are mineralized to CO_2 with differences between organisms and soils (Anderson & Domsch 1978; Nicolardot *et al.* 1984; Jenkinson 1988). A division by a correction factor (K_C) of 0.45 representing the proportion of the microbial biomass assumed to be mineralized under the conditions of the experiment has been recommended

by Jenkinson & Ladd (1981) and has been used generally (*e.g.* Diaz-Raviña *et al.* 1993; Vance *et al.* 1987)

In neutral and alkaline soils the carbon dioxide respired from a non-fumigated soil is subtracted from the amount of carbon dioxide respired by the fumigated soil (Jenkinson & Powlson 1976). However, in acid soils of pH < 5.5, such as those of the sites under study, basal respiration is slow to re-establish after fumigation, and the total amount of carbon dioxide respired from fumigated soils may be less than that respired from non-fumigated soil (Jenkinson *et al.* 1979; Williams & Sparling 1984). In this case, the subtraction of the carbon dioxide produced in the non-fumigated soil is inappropriate (Vance *et al.* 1987) since virtually all of the carbon dioxide respired in the fumigated samples over the 10 d following fumigation is derived from killed microbial biomass. For this reason microbial biomass carbon in acid soils is calculated as:

$$Bio\text{-}C = \frac{(CO_2\text{-}C)_{fum}}{K_C}$$

where Bio-C is the microbial biomass carbon; $(CO_2\text{-}C)_{fum}$ is the carbon from carbon dioxide produced in the fumigated samples during the incubation, and K_C is the correction factor of 0.45.

Soil sampling

In each of the three plots in SHF, THF, and LERF, five random locations were selected for the soil sampling. Using a 5-cm diameter soil corer, samples

were taken from the upper 20 cm of the soil and included the organic layers and the mineral soil below (Table 2.5). All samples, separated as the litter, root mat, humus and mineral layers were placed in plastic bags and brought to the laboratory to be immediately processed. However as an assumption of the FI method is that both litter and plant roots have to be excluded (Jenkinson & Ladd 1981), soil microbial biomass was initially measured only in the mineral samples.

One sampling time was chosen in each season: 5 April 1992 (mid-wet season) and 11 September 1992 (mid-dry season). Roots and litter were removed by hand and the samples were given a conditioning incubation of 5-7 d at 24 °C to allow metabolism to settle down before adjustment of the soil moisture to 55 % of its water holding capacity (Powlson *et al.* 1987).

Because of the very low values for microbial biomass and respiration measured in the mineral layer, five further random samples, each a composite including all the organic layers (Table 2.5) were taken from each plot at the beginning of the wet season (2 November 1993). The samples were sieved (2 mm) after collection and the larger fragments of litter and the surface roots were discarded.

The moisture content, pH and loss-on-ignition were measured for each soil sample. Soil moisture and loss-on-ignition were determined on 2-mm sieved sub-samples after oven drying at 105 °C followed by 3 h at 550 °C in a muffle furnace. Soil pH was determined in both a 1:2 (volume) paste of soil: distilled water and in 1:2 (volume) suspension of soil: 2 M potassium chloride.

Methodology for microbial biomass

Duplicate portions of moist soil (each containing the equivalent of about 25-g oven-dry soil), were either fumigated or stored unfumigated. The fumigations were done in large desiccators lined with moist filter paper and containing a beaker with 50 ml of ethanol-free chloroform and a few anti-bumping granules. Ethanol-free chloroform was prepared immediately before fumigation by passing 100-ml chloroform through a glass column containing 75-g aluminium oxide (Al_2O_3) (Vogel 1978). Using a vacuum pump, the desiccators were evacuated until the chloroform boiled vigorously. The desiccators, with their atmosphere of saturated chloroform vapour were then left in the dark at 25 °C for 20 h. The beaker of chloroform and the paper were then removed and the chloroform vapour removed by repeated evacuation of the desiccators. Fumigated samples were not re-inoculated, since an adequate microbial population normally survives fumigation to respire the organic carbon released, and unfumigated controls were prepared for incubation. The samples were placed in glass bottles together with beakers containing 10-ml 1 M sodium hydroxide to absorb carbon dioxide and also 10 ml of distilled water to offset the drying effect of the sodium hydroxide. All the bottles were stored in the dark in an incubator at 24 °C for 10 d. A set of blank incubations, in which the jar contained water and sodium hydroxide but no soil, were included in each experiment. At the end of the incubation the sodium hydroxide solution was titrated with 0.05 M hydrochloric acid. The amount of carbon dioxide evolved during incubation was calculated from the volume of acid required by titration to reduce the pH

from 8.30 to 3.70. The concentration of carbon as carbon dioxide in the sample was given by considering that 1 ml of 0.05 M hydrochloric acid is equivalent to 0.6-mg carbon dioxide in the sodium hydroxide solution (Jenkinson & Powlson 1976). Biomass-C was calculated using only the carbon dioxide produced in the fumigated samples. The carbon dioxide evolved in the non-fumigated samples is referred to as the soil respiration.

Assessment of the effects of nutrients added in the laboratory on the soil respiration

The possible effects of soil nutrient resource limitation on soil respiration in the SHF, THF and LERF were assessed by experimental nutrient additions under laboratory incubation conditions. The samples (2 November) which had been used for the measurements of microbial biomass were bulked and carefully mixed for each plot. A 50-g sub-sample of each bulk sample was then weighed into a 250-ml screw-cap flask and randomly allocated to one of twelve treatments as described in Table 3.1. All nutrients were added as a 6-ml solution to the 50-g samples. In the control only 6 ml of distilled water were added. The flasks were arranged in a randomized block design and then incubated at 24 °C for 10 d. Evolved carbon dioxide was measured by titration as described previously.

Table 3.1: Details of the chemicals added to the soils for the laboratory nutrient-addition experiment.

Treatments					
1 P	-	5 mg NaH_2PO_4	-	-	-
2 K	-	-	50 mg KCl	-	-
3 Ca_1	-	-	-	200 mg $CaCO_3$	-
4 Ca_1	-	-	-	200 mg $CaSO_4$	-
5 S	-	-	-	-	176 mg Na_2SO_4
6 N	100 mg $CO(NH_2)_2$	-	-	-	-
7 N+P	100 mg $CO(NH_2)_2$	5 mg NaH_2PO_4	-	-	-
8 N+K	100 mg $CO(NH_2)_2$	-	50 mg KCl	-	-
9 N+Ca_1	100 mg $CO(NH_2)_2$	-	-	200 mg $CaCO_3$	-
10 N+Ca_2	100 mg $CO(NH_2)_2$	-	-	200 mg $CaSO_4$	-
11 N+S	100 mg $CO(NH_2)_2$	-	-	-	176 mg Na_2SO_4
12 control	-	-	-	-	-

Assessment of the effects of nutrients added in the field on the soil respiration

On 1 March 1993, a long-term fertilizer addition experiment was established by Luizão (1995) in areas adjacent to the plots (Fig 2.2). This gave the opportunity to evaluate the effect of *in situ* nutrient additions on soil respiration.

The experimental design consisted of two ca. 40 m^2 plots close to the permanent plots in SHF, THF and LERF. At the THF and LERF sites, small natural gaps were used, after clearing fallen branches and trunks. At the SHF sites, open spaces between the 'islands' of vegetation were used. The selected plots were all flat, and it could be assumed that there was no lateral movement of the applied treatments. Twenty-eight 1 m x 1 m sub-plots were marked in each of the plots for nutrient addition and control treatments. Two replicates of each of seven treatments (including a control) were used in each of the plots. The seven treatments were randomly located inside each plot. The rates of chemical addition used were the ones generally recommended for growing seedlings in forestry nurseries and were in the lower part of the range used elsewhere for mature trees in forestry (Tanner *et al.* 1990). There were seven treatments: nitrogen, as 150 kg ha^{-1} of urea (NH_2CONH_2); phosphorus, as 50 kg ha^{-1} of sodium phosphate (Na_3PO_4); potassium, as 60 kg ha^{-1} of potassium chloride (KCl); calcium, as 2,000 kg ha^{-1} of calcium chloride ($CaCl_2$); calcium, as 2,000 kg ha^{-1} of calcium carbonate ($CaCO_3$); a mixture of the nitrogen, phosphorus, potassium, and calcium carbonate treatments; and a control with no added chemicals.

The urea, sodium phosphate, potassium chloride and calcium chloride were added in solution in 1.5 litres of distilled water, while calcium carbonate powder was first spread on the soil surface and then watered in with 1.5 litres of distilled water. Controls received 1.5 litres of distilled water. The nitrogen was added three times (0, 50 and 90 d), at which one third of the total addition was applied each time, while the other chemicals had their addition split, half at 0 and 50 d. The chemicals were added to the soil during the rainy season in 1 March (0 d), 19 April (50 d) and 31 May 1993 (91 d)

Only two replicate sub-plots for each nutrient within each plot were used because of the limited availability of suitable natural gaps. Since the microbial population responds rapidly to changes in soil (Powlson *et al.* 1987), two sampling times were chosen to reflect short-term (60 d) and medium-term (180 d) effects of nutrient additions on soil respiration. For the short-term measurements, soil samples from all treatments were taken. For the medium-term measurements, however, owing to the lack of enough respiration flasks, the treatments with phosphorus, potassium, and calcium chloride alone were not sampled. Soil samples were taken only from the mineral soil layer from each quadrat and immediately brought to the laboratory for carbon dioxide measurements following the procedure described earlier.

Statistical analysis

The data were subjected to analyses of variance, with all data transformed to $\log_{10n}$ (x + 1) to homogenize the variance inherent in measuring chemical variables (Zar 1974). Also, regression analyses of the soil respiration and microbial biomass with soil pH, soil moisture and LOI (loss-on-ignition), were carried out to explain differences in the measurements. Means of different treatments were compared by a one-way analysis of variance followed by the Dunnet's test. All analyses were made using the statistical software MINITAB.

RESULTS

Microbial biomass and soil respiration between types of soil

Soil respiration in organic samples was only measured in the wet season sampling. Soil respiration in both mineral and organic samples is shown in Fig. 3.1. Mineral-soil samples from the SHF plots had twice the respiration rate ($p < 0.05$) of the organic samples. In the THF plots though the soil respiration in the mineral samples was about half that of the organic samples, the high variability in the organic samples did not allow for significant differences. There were no significant soil respiration differences between the organic and mineral samples in the LERF plots. In the mineral layer, soil respiration rates in SHF (59 µg C g^{-1}) and LERF (56 µg C g^{-1}) were not significantly different, whereas the THF soils had a significantly ($p < 0.01$) lower value of 36 µg C g^{-1}. In the organic layer, the mean values of soil

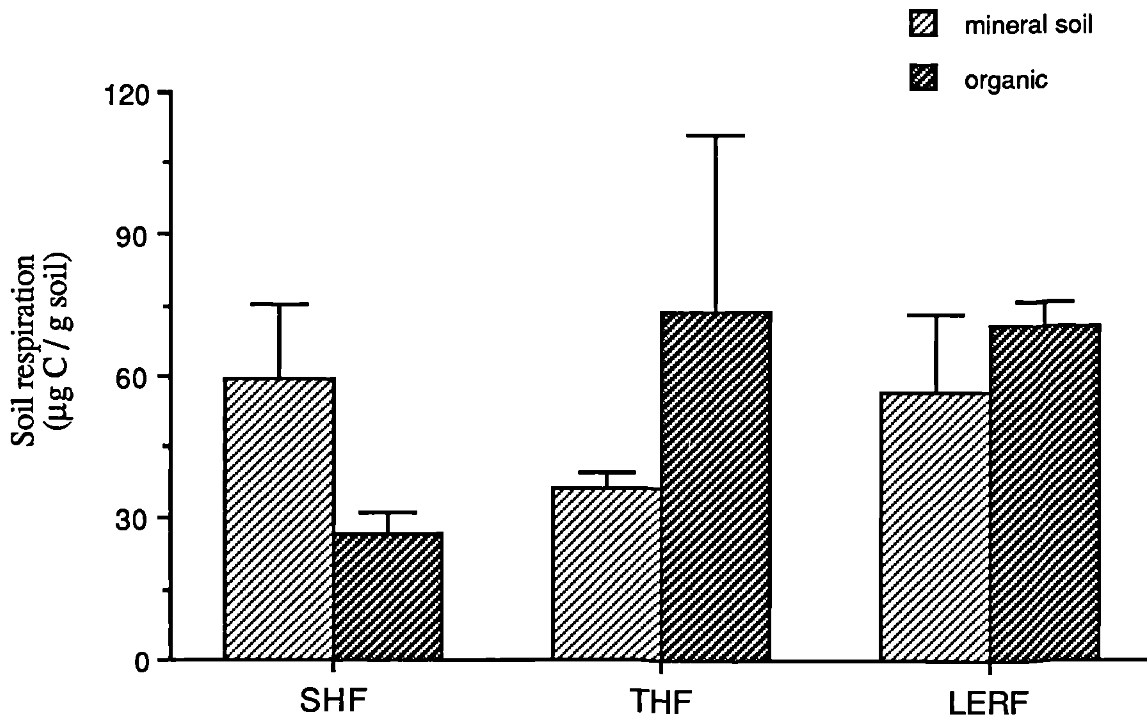


Fig. 3.1: Soil respiration (µg C g^{-1} oven-dry soil 10 d^{-1}) in the mineral soil and organic samples during the wet season in SHF, THF and LERF. Values are means ± SD of the three replicate plots in each forest type.

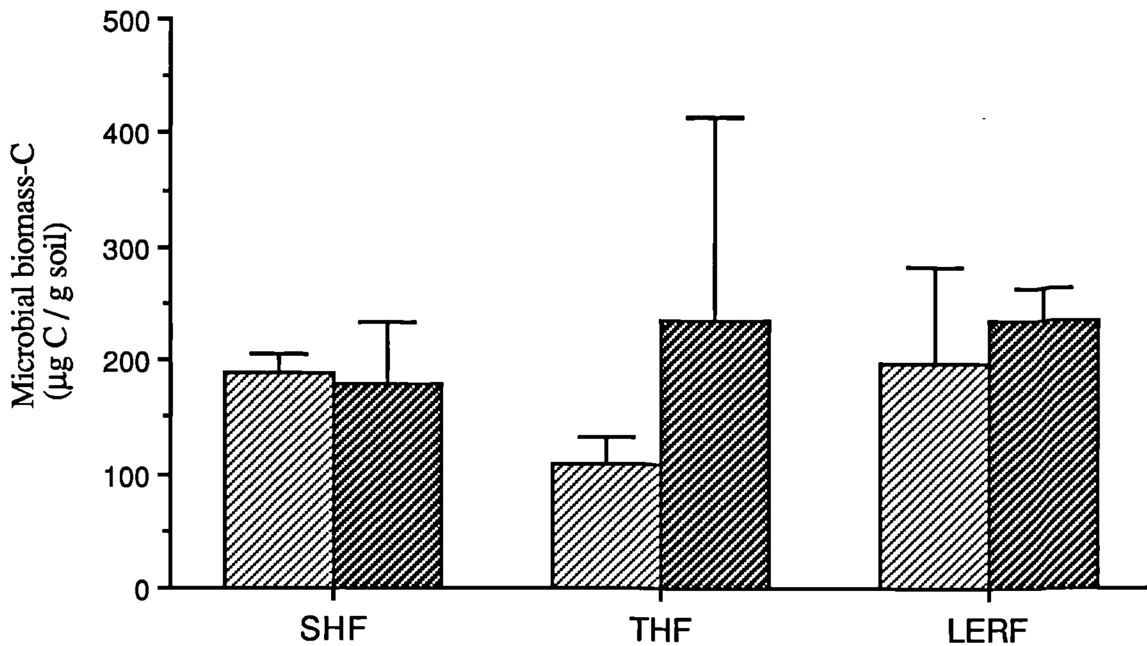


Fig. 3.2: Soil microbial biomass (µg C g^{-1} oven-dry soil 10 d^{-1}) in SHF, THF and LERF in the mineral soil and organic samples during the wet season. Values are means ± SD of the three replicate plots in each forest type.

respiration in both THF and LERF soils were identical (72 μg C g^{-1}) while the SHF soil showed a significantly ($p < 0.01$) lower value (27 μg C g^{-1}) for the soil respiration in this layer.

Microbial biomass measurements in both mineral and organic layers, from the SHF, THF and LERF plots are shown in Fig 3.2. There were no significant differences between layers in the soil samples from either SHF, THF, and LERF sites. In the THF soils, though an apparent higher microbial biomass in the organic samples were observed, there were great variability among the replicates. When comparing sites, the results were parallel to those obtained for soil respiration. The microbial biomass measurements in the mineral layer of both SHF and LERF soils showed virtually the same value (ca. 190 μg C g^{-1} soil), which was significantly higher ($p < 0.001$) than the values recorded for the THF soils (107 μg C g^{-1} soil).

In view of the evident large difference in bulk density between the mineral and organic samples a rough estimate of bulk density was made from LOI (Jeffrey 1970) and Table 3.2 compares the soil respiration and microbial biomass as measured per weight and per volume of soil. The differences between the mineral and organic samples are in the same order when the results are expressed on a mass or volume basis.

Microbial biomass and soil respiration between seasons

The measurements for seasonal comparisons were made using only mineral soil samples (Fig. 3.3). The samples from the SHF and LERF sites displayed

Table 3.2: Soil respiration and soil microbial biomass in the mineral and organic samples are shown on a mass (µg C g^{-1} 10^{-d}) and volume (µg C cm^{-3} 10^{-d}) basis of soil in the SHF, THF and LERF. Values are means ± SD of the three replicate plots in each forest type.

Forest types		Soil respiration		Microbial biomass	
		µg C g^{-1}	µg C cm^{-3}	µg C g^{-1}	µg C cm^{-3}
SHF	mineral	59.2 ± 16.3	35.3 ± 19.3	189 ± 15.6	111 ± 61.4
	organic	26.6 ± 4.7	18.1 ± 8.50	180 ± 54.3	124 ± 52.6
THF	mineral	36.5 ± 3.4	22.2 ± 10.6	108 ± 24.8	65.7 ± 35.9
	organic	73.8 ± 37.4	28.4 ± 13.9	236 ± 180	125 ± 54.0
LERF	mineral	56.3 ± 16.8	28.4 ± 11.6	198 ± 84.1	98.3 ± 42.1
	organic	70.6 ± 5.3	33.8± 10.2	235 ± 29.4	121 ± 32.7

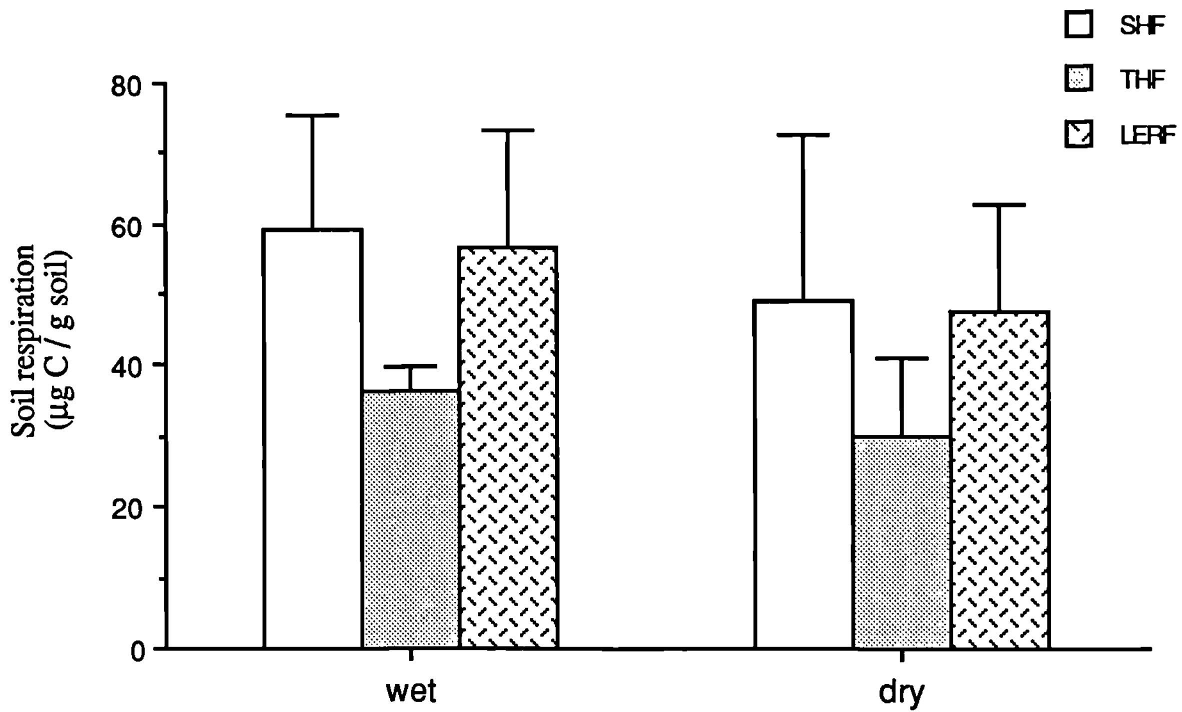


Fig 3.3. Soil respiration (µg C g^{-1} 10 oven-dry soil d^{-1}) in the mineral soil samples from SHF, THF and LERF in the wet and dry seasons. Values are means ± SD of the three replicate plots in each forest type.

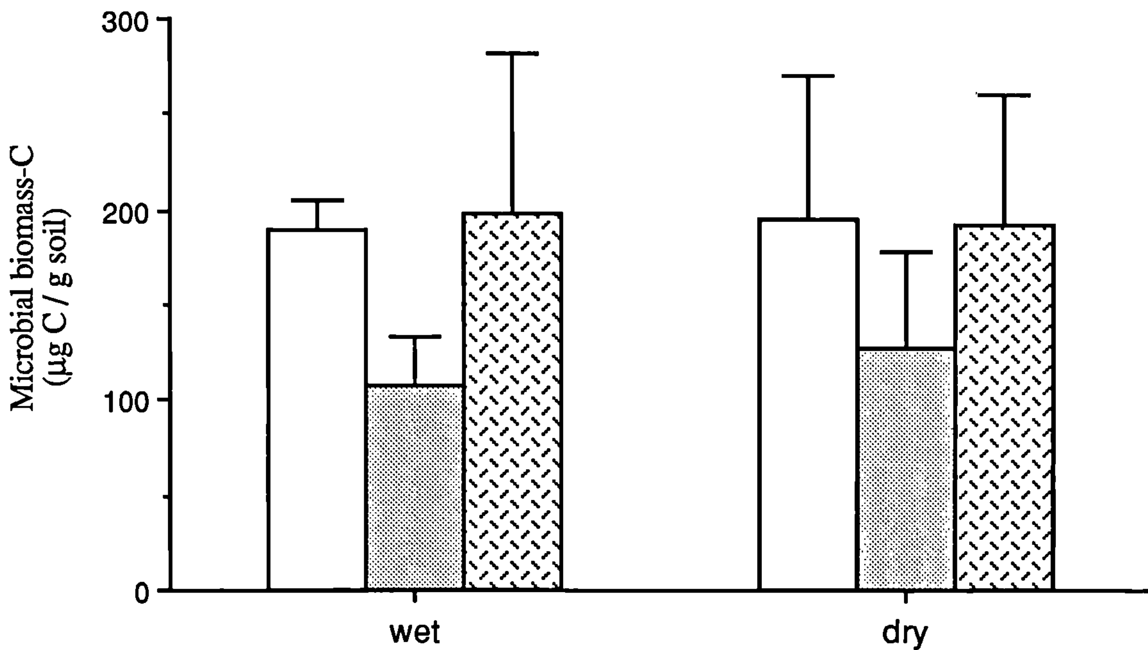


Fig. 3.4: Soil microbial biomass (µg C g^{-1} oven-dry soil 10 d^{-1}) in the mineral soil samples from SHF, THF and LERF in the wet and dry seasons. Values are means ± SD of the three replicate plots in each forest type.

very similar seasonal patterns for soil respiration. In both there was a trend (though not statistically significant) for higher respiration in the wet season than in the dry season. Soil samples from THF sites showed significantly lower ($p < 0.001$) soil respiration than the other forest types but no seasonal effect was observed (Fig. 3.3). Soil microbial biomass measurements from either the SHF, THF, and LERF sites showed no seasonal differences, and had very similar values for both wet and dry seasons (Fig. 3.4).

Regression analyses of both soil respiration and microbial biomass with LOI, soil pH and soil moisture were carried out to explain differences in the measurements. Although these soil factors were significantly different in the two seasons, neither soil respiration nor microbial biomass variations were explained by them. Soil pH (Table 3.3) was significantly higher ($p < 0.001$) in the LERF samples (pH_{KCl} 3.5) than in the other forest types (SHF, pH_{KCl} 2.5; THF, pH_{KCl} 3.0). However no significant difference was found between THF and LERF measurements for soil moisture and LOI. In the SHF and THF, soil pH ($p < 0.05$), soil moisture ($p < 0.01$) and LOI ($p < 0.001$) were significantly higher in the wet than in the dry season. In the LERF, however, soil pH ($p < 0.01$) and soil moisture ($p < 0.001$) but not LOI were significantly higher in the wet season (Table 3.3).

Table 3.3: The loss-on-ignition (LOI), pH_{KCl} and soil moisture in mineral soil samples collected during the wet and dry seasons from SHF, THF and LERF. LOI and soil moisture are expressed as a percentage of oven-dry soil. Values are means ± SD the three replicate plots in each forest type.

Forest type	**Season**	**LOI (%)**	**pH_{KCl} (log units)**	**Soil moisture (%)**
SHF	wet	0.23 ± 0.05	2.70 ± 0.30	6.41 ± 4.00
	dry	0.17 ± 0.08	2.40 ± 0.30	3.00 ± 2.30
THF	wet	0.19 ± 0.03	3.70 ± 1.30	6.50 ± 1.90
	dry	0.14 ± 0.02	2.40 ± 0.40	2.00 ± 0.90
LERF	wet	0.27 ± 0.07	3.90 ± 0.80	15.8 ± 2.80
	dry	0.25 ± 0.06	3.10 ± 0.20	8.80 ± 2.60

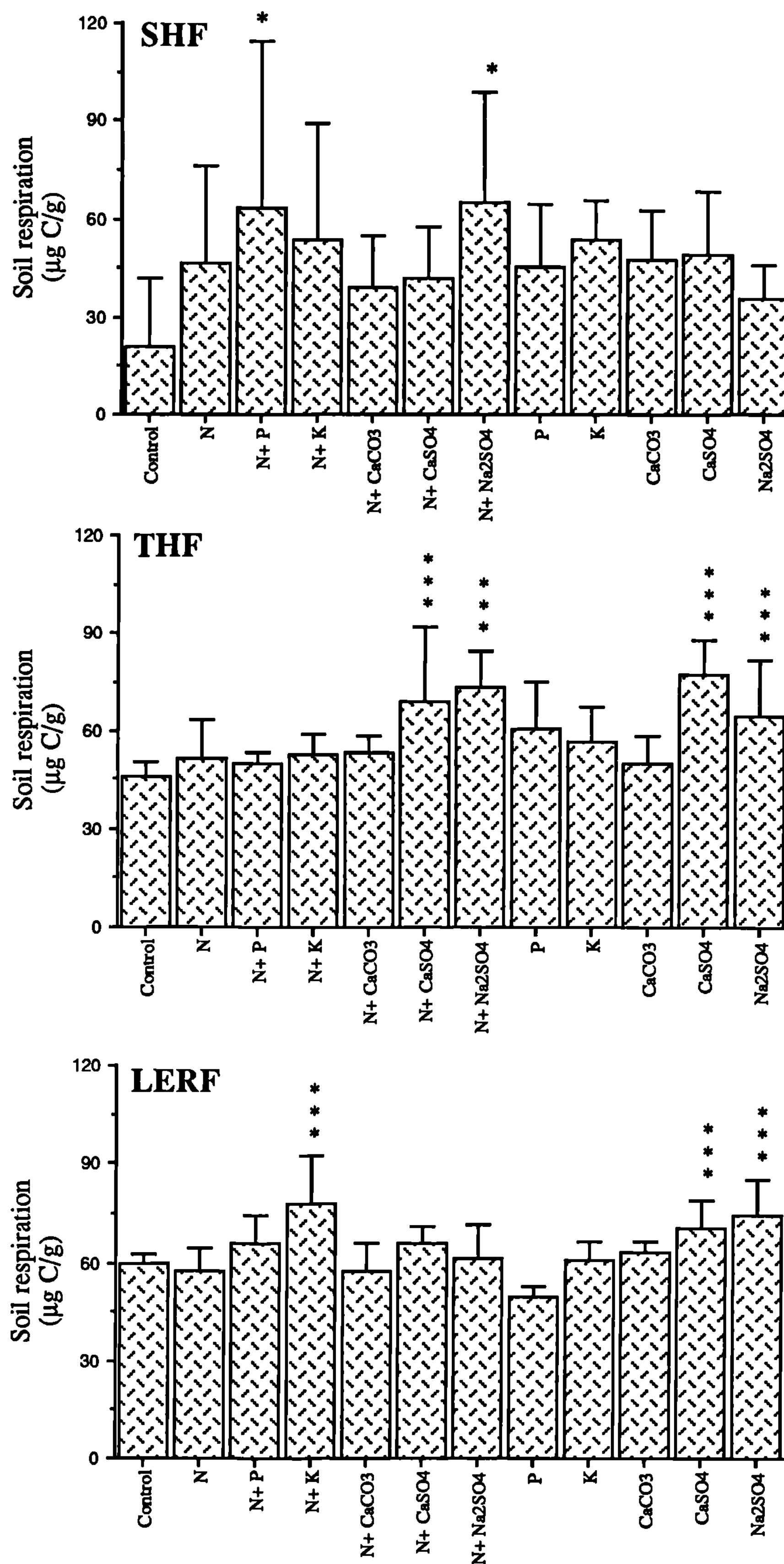


Fig. 3.5: Soil respiration (µg C g^{-1} oven-dry soil 10 d^{-1}) under different nutrient addition treatments (explained in the text) in SHF, THF and LERF. Values are means ± SD (n=3). Significance levels for ANOVA with Dunnet's test for comparisons with the control are: * < 0.05 ** < 0.01 *** < 0.001.

Effects of added nutrients on soil respiration in the laboratory

In general, SHF produced the lowest ($p < 0.001$) mean values for soil respiration in response to the nutrient additions compared with the other forest types (Fig 3.5). In the SHF, plot no. 1 showed significantly higher ($p < 0.001$) soil respiration on the addition of nutrients than plots 2 and 3 (Appendix 1.1). In SHF, there was great variability among the replicates, probably as a consequence of having samples from the open and closed sites, and only two out of the twelve treatments significantly changed ($p < 0.05$) the soil respiration rate. The combination of urea with dihydrogen sodium phosphate, and urea with sodium sulphate caused a doubling of the rate (Fig. 3.5a). The soil samples from the three replicate plots from the THF were significantly different ($p < 0.001$) for soil respiration response to nutrient addition. Plot no. 4 showed the highest, and plot no. 6 the lowest mean values (Appendix 1.1). In general, the addition of sulphur, either as calcium sulphate and sodium sulphate applied alone or in combination with nitrogen, significantly increased ($p < 0.001$) soil respiration, producing mean values more than 50% higher than the control (Fig. 3.5b). Adding calcium carbonate to the soil showed no stimulatory effect on the soil respiration in the THF nor did nitrogen, potassium or any of the combinations of nitrogen with phosphorus or potassium. In the LERF, plot no. 8 showed significantly lower ($p < 0.001$) soil respiration than plots no. 7 and 9 in response to the application of nutrients (Appendix 1.1). In general, soil respiration in the LERF plots was significantly higher ($p < 0.001$) with the additions of nitrogen with potassium, sodium sulphate, and calcium sulphate (Fig. 3.5c). The

Table 3.4: Mineral soil pH_{H20} (log units) in the nutrient addition treatments at 60 d and 180 d in SHF, THF and LERF. Values are means ± SD of measurements made within two replicate sub-plots within each of two plots in each forest type and hence n=6.

	Forest types					
	SHF		**THF**		**LERF**	
Treatment	60 d	180 d	60 d	180 d	60 d	180 d
N	3.1 ± 0.3	2.8 ± 0.3	2.6 ± 0.1	2.2 ± 0.2	3.6 ± 0.2	3.0 ± 0.2
P	3.1 ± 0.3	na	2.6 ± 0.1	na	3.6 ± 0.1	na
K	2.9 ± 0.1	na	2.6 ± 0.1	na	3.5 ± 0.2	na
NPK + $CaCO_3$	6.7 ± 0.1	7.5 ± 0.5	2.7 ± 0.3	2.3 ± 0.4	3.3 ± 0.3	3.0 ± 0.4
$CaCl_2$	2.9 ± 0.4	na	2.3 ± 0.2	na	3.3 ± 0.2	na
$CaCO_3$	6.8 ± 1.0	6.9 ± 0.9	2.4 ± 0.2	2.6 ± 0.6	2.9 ± 0.7	3.2 ± 0.4
Control	3.3 ± 0.4	3.1 ± 0.4	2.6 ± 0.1	2.3 ± 0.1	3.3 ± 0.3	2.9 ± 0.3

na = not analyzed.

Table 3.5. Soil respiration (μg C g^{-1} oven-dry soil) in the nutrient addition treatments at 60 d and 180 d in SHF, THF and LERF. Values are means ± SD of measurements made within two replicate sub-plots within each of two plots in each forest type and hence n=6.

	Forest types					
	SHF		**THF**		**LERF**	
Treatment	60 d	180 d	60 d	180 d	60 d	180 d
N	56.0 ± 62.8	20.0 ± 5.4	91.4 ± 80.0	101 ± 55.0	52.1 ± 38.4	111 ± 9.2
P	20.1 ± 10.0	na	83.7 ± 29.8	na	57.3 ± 49.8	na
K	68.9 ± 41.6	na	60.9 ± 37.3	na	52.1 ± 27.7	na
NPK + $CaCO_3$	104 ± 38.4	66.6 ± 35.6	93.3 ± 53.0	110 ± 31.0	86.5 ± 20.7	103 ± 32.3
$CaCl_2$	70.1 ± 50.5	na	91.1 ± 57.8	na	69.5 ± 42.4	na
$CaCO_3$	41.9 ± 12.7	100 ± 8.4	105.5 ± 62.0	128 ± 50.5	92.3 ± 56.0	135 ± 102
Control	30.5 ± 15.0	57.1 ± 51.0	48.5 ± 27.6	128 ± 32.9	66.5 ± 15.0	90.4 ± 53.4

na = not analyzed

response of soil respiration to the nutrient additions was very distinct. Only the addition of nitrogen with sodium sulphate caused soil respiration higher than the controls in both SHF and THF. Among THF and LERF, however, higher soil respiration occurred with both sodium and calcium sulphate. Among SHF and LERF soils, no similarities in their soil respiration response to the nutrient additions were observed.

Effect of added nutrients on soil respiration in the field

Only in the SHF the application of calcium carbonate increased soil pH (Table 3.4). At both the 60 d and 180 d samplings soil pH was significantly higher (all $p < 0.001$) than the control in the treatments which received either only calcium carbonate or the mixture of nitrogen, phosphorus, potassium and calcium carbonate (Table 3.4). In the THF and LERF however addition of calcium carbonate did not increase soil pH (Table 3.4) and it must be assumed that the total acidity in these soils was so high that insufficient calcium carbonate was added to have an effect. The soil respiration results were very variable and only in the SHF at the 60 d sampling was the rate of respiration significantly higher ($p < 0.05$) in the treatment with the mixture of nutrients. In the THF and LERF no significant differences were found among treatments at both sampling times (60 d and 180 d) (Table 3.5).

DISCUSSION

Comparison between wet and dry seasons

Seasonal changes in soil moisture control microbial population dynamics (Diaz-Raviña *et al.* 1988 & Singh *et al.* 1989). It has been demonstrated for lowland evergreen forests on oxisols that microbial biomass shows a pulsed behaviour with fluctuations in its values related to the nutrient availability and soil moisture (Grimaldi *et al.* 1992; Luizão *et al.* 1992; Lodge *et al.* 1994). Surprisingly, no seasonal variation was detected in either the microbial population or its respiratory activity in the SHF, THF, and LERF. There are two possible reasons for this: that the two sampling times did not reflect the actual seasonal variation; or the soil organic layer protected the microorganisms against desiccation. The rainfall in 1992 was unusual and the differences between the usually very wet April and the very dry September were not so high (Fig 2.3). On the other hand, as reported by Van Veen *et al.* (1987), organic matter has a capacity to buffer the microbial biomass against wetting-drying cycles in which drought may prevent microbial build-up. In SHF, where patches of exposed bare sand are a common feature, no protection by organic matter was expected. However the random selection had placed most of the sample points in sites with dense vegetation and organic soils. A different, situation, can be expected when the mineral soil is at the soil surface and subject to wide fluctuations in temperature and moisture (Ross 1993).

Soil respiration in the field is known to be closely linked with soil temperature and moisture. Under laboratory conditions, however, where soil

samples had their water content readjusted, the relationship between basal respiration and soil moisture was masked. The high soil respiration obtained in SHF soils, drier and poorer in nutrients than those under THF and LERF may be attributed to favourable moisture conditions, during the laboratory incubations (Insam 1990). With optimum conditions, the population of microorganisms expand and in so doing, immobilizes the nutrients.

Comparison between mineral and organic samples

In soil from SHF plots, the microbes have a similar biomass in the mineral and organic samples but are not equally active in both. Soil respiration was significantly higher in the mineral layer suggesting that the SHF microbes only mineralize readily decomposable substrate that percolate to the mineral layer. In the THF and LERF, the organic layers were better developed than in the SHF and in both, no significant differences were observed for both microbial biomass and soil respiration. The trend for higher microbial population in the organic samples of THF helps to explain why the measurements of seasonal effects, which were made only on the mineral soil always showed the lowest results for both microbial biomass and soil respiration in that forest. Microorganisms build up their population through their ability to decompose and utilize substrate around them. The fact that in the organic samples of THF there are many recalcitrant compounds such as lignin, chitin, humic acid, and fulvic acid, suggests that its microbial population is more specialized. The similarity in the microbial biomass measurements between the mineral and organic samples in the LERF plots

suggests that a more diverse microbial population, able to decompose a range of substrate is distributed down the whole soil profile.

Effect of laboratory nutrient additions

In the laboratory nutrient addition experiment (Fig. 3.5), two nutrient combinations caused a significant increase in SHF soil respiration: urea with sodium phosphate, and urea with sodium sulphate. It seems that nitrogen is limiting microbial activities in the SHF. Phosphorus and sulphur, in turn, seem to be important when enough nitrogen is supplied. In the THF, sulphur especially when combined with nitrogen, seemed to be the nutrient which stimulated the activity of the microorganisms whereas in the LERF, sulphur additions, and the combination of nitrogen with potassium, stimulated microbial activity. The increase in microbial activity caused by sulphur was unexpected. Enhanced microbial activity could be caused by the sulphur or by the added calcium or sodium (Persson *et al.* 1989). However, the latter is unlikely to be the case since sodium is at most a micronutrient while calcium did not produce so positive a response when added as calcium carbonate. Over 95% of the forest topsoil sulphur is organically bound (David *et al.* 1982). Earlier studies had suggested that the decomposition of carbon compounds was dependent on the available sulphur, *e.g.* Stewart *et al.* (1966) for soil glucose and cellulose decomposition. Stotzky & Norman (1961) also suggested that the initial concentration of essential inorganic nutrients, such as sulphates, determines the rate of utilization of added substrate. More recent studies have also shown that although addition of sulphate alone did

not increase soil respiration rates, higher rates were obtained with the addition of cellulose to soils initially amended with cellulose plus sulphate (Saggar *et al.* 1981). These results indicate that under certain conditions (*e.g.* adequate supply of other nutrients) sulphur may limit microbial decomposition. Unfortunately, there was no sulphur treatment in the field experiments.

High soil acidity has often been considered a strong microbial inhibitor. When acidity is reduced by liming, a more diverse decomposer community may establish, allowing a more efficient substrate utilization (Insam 1990). Contrary to that expectation, though calcium carbonate addition increased soil pH in the SHF its effect on soil respiration at the 60 d sampling did not last until the next (180 d) measurement. In THF and LERF neither soil pH nor soil respiration were influenced by calcium carbonate. Persson *et al.* (1990) also found no significant difference in soil respiration between mineral soils which received calcium carbonate and the controls. In both THF and LERF, however no trend for a neutralizing effect was observed which suggests that there is so much acidity in these soils that the calcium carbonate added was insufficient to alter the pH.

Chapter IV. Microbial Transformations of Nitrogen in the SHF, THF, and LERF soils.

INTRODUCTION

The availability of nitrogen, phosphorus, and potassium is of vital importance because they are the major plant mineral nutrients. Of the three, nitrogen is the most susceptible to microbial transformations. It is a key building block of protein molecules and is an indispensable component of all protoplasm (Alexander 1961). In terrestrial ecosystems nitrogen is mainly found in its organic form, which must be mineralized before it can be taken up by plants (Rosswall 1982). The mineralization includes a number of biological processes: organic nitrogen residues are microbially decomposed with the release of ammonium ions (ammonification), which can then be oxidized by microorganisms to nitrate ions (nitrification). The mineral forms of nitrogen are utilized by microorganisms (immobilization). The major portion of the pool of mineral nitrogen that is not immobilized is absorbed and assimilated by plants during their growth. Thus, soil mineral nitrogen represents a small and transitory pool in terms of the total nitrogen stock of any ecosystem. The major forms of mineral nitrogen (ammonium and nitrate) usually account for less than 2% of the total soil nitrogen content of soils (Haynes 1986). In contrast, a comparison of mineral soil nitrogen with microbial biomass nitrogen in tropical soils (Srivastava & Singh 1991) indicates that there can be around 20-40 times more easily mineralizable

nitrogen stored in microbial biomass tissues than exists in the soil. The microbial biomass represents a large pool of potentially plant-available nutrients, and there is frequently a greater amount of nitrogen and phosphorus in the microbial biomass than in the growing crop or in grazing animals (Jenkinson 1988; Brookes *et al.* 1985).

Rates of nitrogen transformations are affected by a number of chemical and physical properties of soils: high acidity (Alexander 1961), limiting elements (Mahler & Harder 1984; Olson & Reiners 1993), toxic metals (Reiners *et al.* 1985; Cronan 1980) and polyphenolic compounds (Liang & Tabatabai 1978; Rice 1979). The application of ammonium-based fertilizers slowly lowers the soil pH (Mahler & Harder 1984), which in turn can reduce nitrification rates and nitrogen mineralization (Clay *et al.* 1990). Many acid soils accumulate ammonium ions rather than nitrate ions suggesting that ammonium is retained on the cationic exchange complexes or possibly that these soils contain fewer nitrifying bacteria in comparison with neutral and alkaline soils (Alexander 1961). Under some conditions lime has been observed to stimulate nitrogen mineralization (Fu *et al.* 1987), while under others no stimulation has been found (Dancer *et al.* 1973). Historically it has been thought that soil acidity restricted the rate of nitrogen mineralization, and that liming increased it (Alexander 1961; Haynes 1986). This theory was supported by research which showed that liming soil in lysimeters reduced organic carbon compared with unlimed soil (Jolivet & Helias 1953). However Clay & Clapp (1990) showed that soil acidity by itself did not reduce nitrogen mineralization. For instance, they suggested that when ammonium-based

fertilizers, which release H^+ and hence lower soil pH, were applied to an acid soil, a chain of events could occur: (1) low soil pH reduced nitrification of applied fertilizers, (2) ammonium concentrations remained high for a long time, and (3) nitrogen mineralization was reduced by ammonia gas toxicity. Nyborg & Hoyt (1978) had previously reported that soil acidity alone did not restrict nitrogen mineralization. However, following the application of lime to acid soils, they observed a temporary stimulation in mineralization of soil nitrogen.

It is clear that rapid alteration of soil reaction by adding acid or alkali changes the amount of nitrogen in the inorganic pool through chemical degradation of soil organic matter (Cornfield 1959; Keeney & Bremner 1964) and by altering the microbial composition of the soil (Waskman 1923). Dancer *et al.* (1973) using an incubation technique found that nitrate accumulation after 15 d was a function of soil pH and that a three-fold to five-fold increase in nitrification occurred with a soil pH increase from 4.7 to 6.5. They concluded that soil pH is an excellent indicator of soil nitrification capabilities and confirmed the pattern described by Morrill & Dawson (1967). The soils of the SHF, THF and LERF offered an opportunity to study the effect of low pH (< 4) on the microbial transformations of nitrogen. Thus, the objectives of the present study were to: (a) estimate the actual stock of mineral nitrogen and its availability in the soils of SHF, THF and LERF, and (b) to evaluate its relationship with the soil microbial biomass and carbon mineralization.

MATERIALS AND METHODS

Incubation method to estimate nitrogen transformation

Incubation methods were developed to obtain estimates for potentially available organic nutrients in soil in the laboratory or in field conditions. Soil aliquots are incubated in flasks or bags and the production of inorganic nitrogen (i.e. ammonium and nitrate) released over a unit of time is measured (Keeney 1982). The results are often called 'mineralization rates' but this seems inappropriate because it does not take into account the implicit immobilization of nutrients occurring during the process. The expressions 'net release' or 'net mineralization' are better and the latter will be used hereafter.

Soil sampling

The nitrogen studies were carried out on the same samples used for the microbial biomass studies described in Chapter III. After incubation, the carbon dioxide produced was measured for microbial biomass and activity estimates and the remaining soil was extracted for mineral nitrogen.

Initial mineral nitrogen pool

Mineral nitrogen was extracted from each 25-g subsample at the start of the incubation (initial mineral nitrogen), and after 10 d (incubated mineral nitrogen) with 50-ml 2 M KCl (Bremner 1965) using a horizontal shaker for 30 min. Extracts were allowed to settle overnight at 5 °C and then vacuum

filtered. Ammonium ion concentrations were determined colorimetrically by flow injection, using a modification of the indophenol blue method (Gine *et al.* 1980). Nitrate ions concentrations were determined by a similar technique using a modification of the cadmium reduction method (Henriksen & Selmer-Ølsen 1970). Initial mineral nitrogen was the sum of initial nitrogen in ammonium and nitrate ions (NH_4-N + NO_3-N).

Nitrogen transformation rates

Net mineralization was calculated by subtracting the initial NH_4-N + NO_3-N from the NH_4-N + NO_3-N in the incubated soil samples. Net nitrification was calculated by subtracting the initial NO_3-N concentrations from those in the incubated soil (Keeney 1982).

Microbial biomass nitrogen

Microbial biomass nitrogen is estimated by the fumigation-incubation technique in an analogous way to microbial carbon (Jenkinson & Ladd 1981). The fumigation kills the microorganisms and the cell contents are released. These contents decompose when new or survivor organisms recolonise the soil and are detected as increased CO_2 production (see Chapter III) and greater nitrogen mineralization in fumigated soil (Ayanaba *et al.* 1976; Shen *et al.* 1984). Biomass-nitrogen was calculated as the flush of nitrogen mineralization, measured as ammonium ions over a 10-d incubation after fumigation (Ayanaba *et al.* 1976). Ammonium ions were extracted from the fumigated soil samples and determined following the procedure described

above. Analogous to microbial biomass carbon a correction factor (K_N) must be applied to convert the nitrogen flush to biomass-nitrogen. Although the conversion factor is well established for estimating microbial carbon (K_C) following a defined fumigation procedure (Anderson & Domsch 1978; Jenkinson & Ladd 1981), that for estimating microbial nitrogen (K_N) is less so. The K_N, the fraction of microbial biomass-nitrogen mineralized to inorganic nitrogen during the 10 d after fumigation, has been found to have a much wider range than K_C, depending on the type of microorganisms and the properties of the soil (Shen *et al.* 1984; Voroney & Paul 1984). Moreover, net mineralization rates, during the incubation of fumigated samples, depend on the microbial C:N ratio. Consequently, data on microbial biomass-nitrogen obtained by the fumigation-incubation technique have to be interpreted with caution. Nevertheless, Jenkinson (1988) proposed a K_N= 0.57 which would suit most soils with a low C:N ratio, and this value is used in this study. Microbial biomass nitrogen is calculated as:

$$Bio\text{-}N = \frac{(NH_4\text{-}N)_{fum}}{K_N}$$

where Bio-N is the microbial biomass nitrogen; $(NH_4\text{-}N)_{fum}$ is the amount of ammonium ions produced in the fumigated samples during the incubation, and K_N is the correction factor of 0.57.

Nutrients added in the laboratory on nitrogen-mineralization and nitrification.

The same bulked organic soil samples described in Chapter III were used to study possible nutrient resource limitation on the mineral nitrogen pool and on net mineralization and nitrification rates.

Initial mineral nitrogen was extracted from 5-g subsamples with 25-ml 1 M KCl. For the incubation, 50-g subsamples of each bulked sample were weighed into 250-ml screw-cap flasks and randomly allocated to one of the twelve treatments mentioned in Chapter III. At the end of the 10-d incubation, three separate 5-g subsamples were taken from each flask and their mineral nitrogen was extracted as described earlier. Initial mineral nitrogen and net mineralization and nitrification were then calculated as shown above.

Nutrients added in the field on soil mineral nitrogen pool.

This study was carried out on the plots fertilized by Luizão (1995) and discussed in Chapter III. For each nutrient addition treatment, two samples with the equivalent of 25 g of oven-dry soil were extracted for initial mineral nitrogen, using the procedure described earlier in this chapter.

Statistical analysis

Results were subjected to analyses of variance, with all data transformed to $\log_{10n}(x + 1)$ to homogenize the variance inherent in measuring chemical variables (Zar 1974). Also, regression analyses of the initial mineral nitrogen,

net nitrification and net mineralization rates with soil pH, soil moisture and LOI (loss-on-ignition), were carried out to explain differences in the measurements. Means of different treatments were compared by a one-way analysis of variance followed by Dunnet's test. All analyses were made using the statistical software MINITAB.

RESULTS

Initial mineral nitrogen between seasons

There were less than 3 μg N g^{-1} of mineral nitrogen (75% NH_4-N, 25% NO_3-N) in the SHF soil in both the wet and dry seasons (Fig. 4.1). Although initial mineral nitrogen did not significantly differ between the two seasons, nitrate concentrations were significantly lower ($p < 0.001$) in the wet season. In THF, ammonium concentrations were higher ($p < 0.001$) in the wet season whereas those of the nitrate were higher ($p < 0.01$) in the dry season. NH_4-N was 90% of the mineral nitrogen in the wet season and about 70% in the dry season (Fig. 4.1). In the LERF, NH_4-N was 80% of the initial mineral nitrogen in the wet season and 32% in the dry season (Fig. 4.1). In this soil, the sum of the NH_4-N and NO_3-N was not statistically different between seasons, but when analyzed separately, a significant difference was observed. NO_3-N was lower ($p < 0.001$) while NH_4-N was higher ($p < 0.05$) in the wet season.

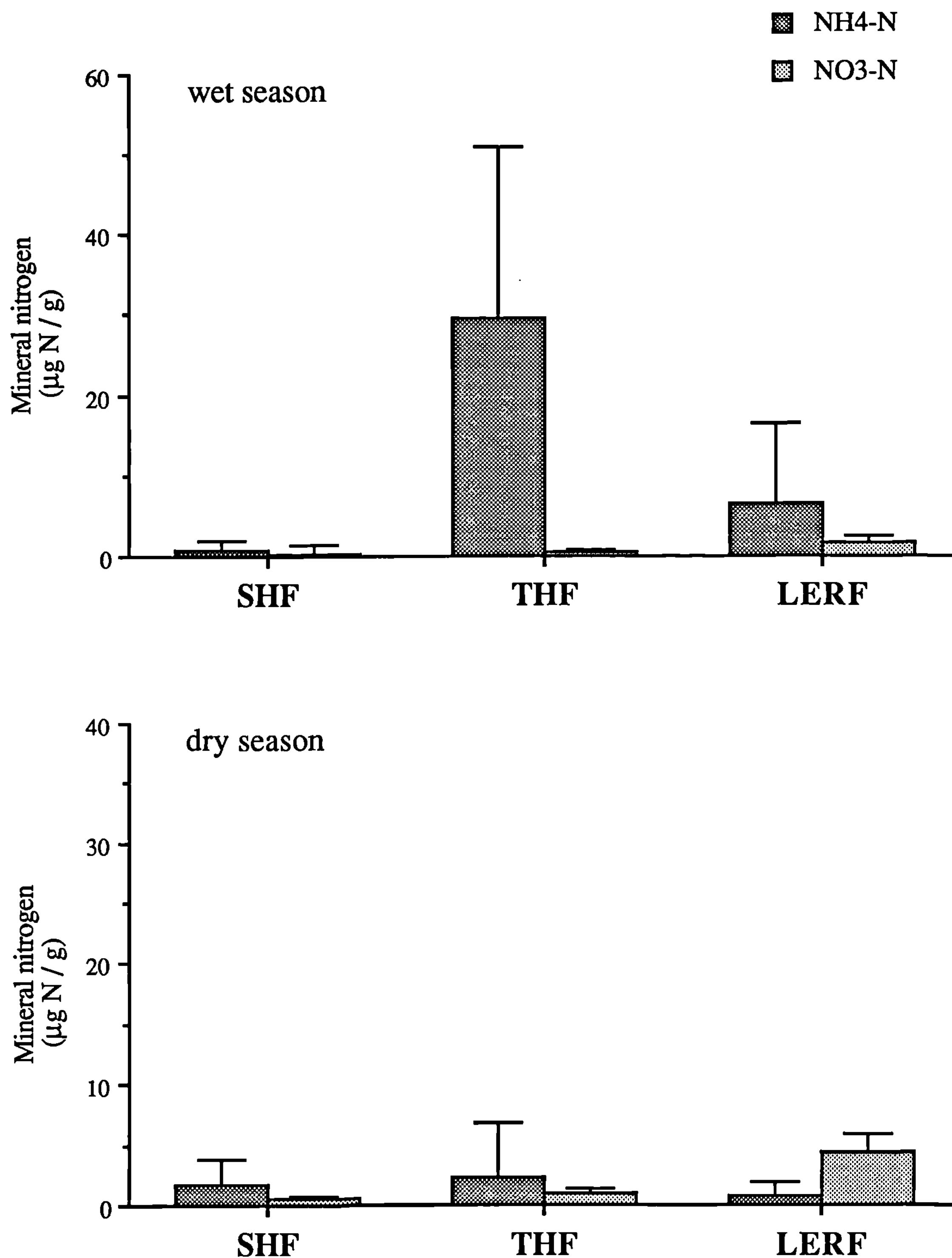


Fig. 4.1: Initial mineral nitrogen concentrations (µg N g^{-1} oven-dry soil 10 d^{-1}) in SHF, THF and LERF in the wet and dry season. Values are means ± SD of the three replicate plots per forest type.

Initial mineral nitrogen in the mineral soil and organic samples

Mean inorganic nitrogen was significantly higher in the organic layers for both SHF ($p < 0.001$) and LERF ($p < 0.05$) sites, when compared with the mineral layer (Fig. 4.2). Also in the organic layer, NH_4-N was the major component of mineral nitrogen in all forest types, but only in the SHF was the concentration significantly higher ($p < 0.001$) in the organic layer than in the mineral soil. NO_3-N was not significantly different between layers in either SHF or LERF. In the THF sites, however, mineral nitrogen concentrations in the organic samples were significantly lower ($p<0.001$) than in those from the mineral layer, which showed great variability within plots. Although always in low concentrations, NO_3-N was significantly lower ($p<0.001$) in the THF mineral soil samples than in the organic samples. Analogous to the microbial biomass and activity measurements in Chapter III, mineral nitrogen was also estimated per volume of soil as shown in Table 4.1. The differences between samples showed the same order as when the results were expressed on a mass basis.

Net mineralization rates

High and similar net mineralization rates were observed in both seasons for the samples from SHF plots, but in the dry season there was great variation among the replicates (Fig. 4.3a). On the other hand, samples from THF soils, though showing great variability, had a net mineralization that was significantly ($p < 0.001$) different between the two seasons. In the wet season, high net immobilization of nitrogen was recorded whereas in the dry season

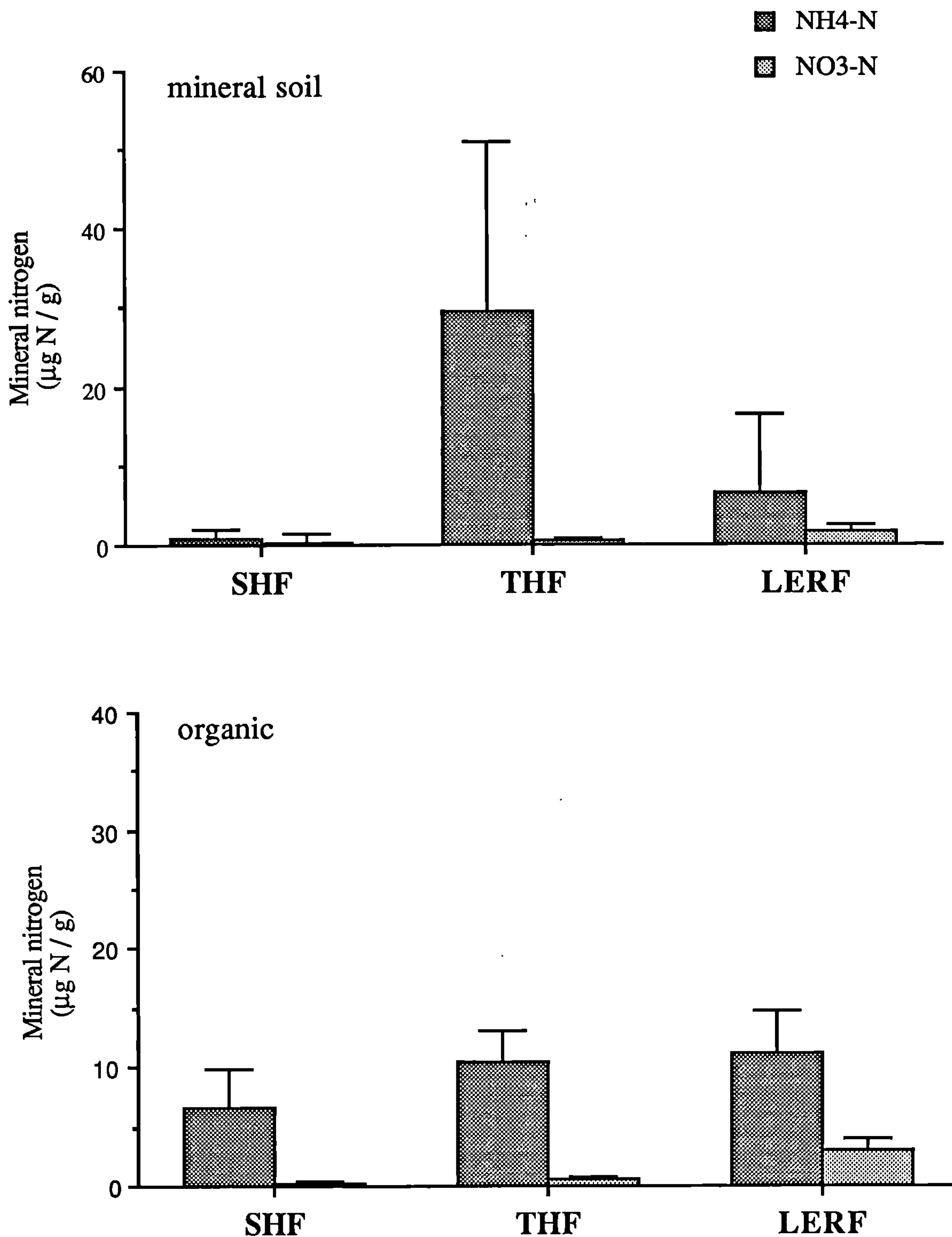


Fig. 4.2: Initial mineral nitrogen concentrations (µg N g^{-1} oven-dry soil 10 d^{-1}) in the mineral soil and organic samples of the SHF, THF and LERF (wet season). Values are means ± SD of the three replicate plots per forest type.

Table 4.1: Initial mineral nitrogen in the mineral and organic samples on a mass (μg N g^{-1} 10 d^{-1}) and a volume (μg N cm^{-3} 10 d^{-1}) basis for soil in the SHF, THF and LERF. Values are means ± SD of the three replicate plots per forest type.

Forest types		Initial NH_4-N		Initial NO_3-N	
		μg N g^{-1}	μg N cm^{-3}	μg N g^{-1}	μg N cm^{-3}
SHF	mineral	0.83 ± 1.26	0.48 ± 0.70	0.31 ± 0.10	0.18 ± 0.06
	organic	6.71 ± 3.06	4.85 ± 2.18	0.25 ± 0.09	0.18 ± 0.06
THF	mineral	29.6 ± 21.4	17.9 ± 12.4	0.51 ± 0.42	0.32 ± 0.27
	organic	10.4 ± 2.60	4.89 ± 2.02	0.50 ± 0.29	0.23 ± 0.16
LERF	mineral	6.57 ± 9.95	3.44 ± 5.17	1.60 ± 0.88	0.82 ± 0.45
	organic	11.2 ± 3.55	5.50 ± 2.07	3.05 ± 0.95	1.44 ± 0.35

there was a small net release of nitrogen in the soil. In the LERF sites, there was a trend for higher net mineralization in the wet season but there was much variability and it was not statistically significant.

Net nitrification

In general, although showing very low nitrate release, net nitrification was significantly higher ($p < 0.001$) in LERF than in SHF and THF, which showed similar and negligible rates. In both SHF and THF, there were significant differences ($p < 0.001$) between the net nitrification occurring in the wet season and the net release of nitrate in the dry season (Fig. 4.3b). In the LERF, the net nitrification in the wet season (4.7 µg N g^{-1} soil) was significantly higher than in the dry season (Fig. 4.3b). Regression analyses for all forest types together showed that either soil pH_{KCl} and soil moisture separately or soil pH and LOI combined explained the seasonal variation of initial soil mineral nitrogen ($p < 0.05$).

Microbial biomass nitrogen

For both seasons combined, mean microbial-biomass nitrogen was significantly higher ($p < 0.001$) in SHF compared with THF and LERF. Mean values of microbial-biomass nitrogen, with all forest types analyzed together, was significantly higher in the wet season than in the dry season. However when forest types were separated, only in the THF, was the biomass nitrogen significantly higher ($p < 0.05$) in the wet season (Fig. 4.3c). Though both SHF and LERF showed trends for higher biomass nitrogen in the wet than in the

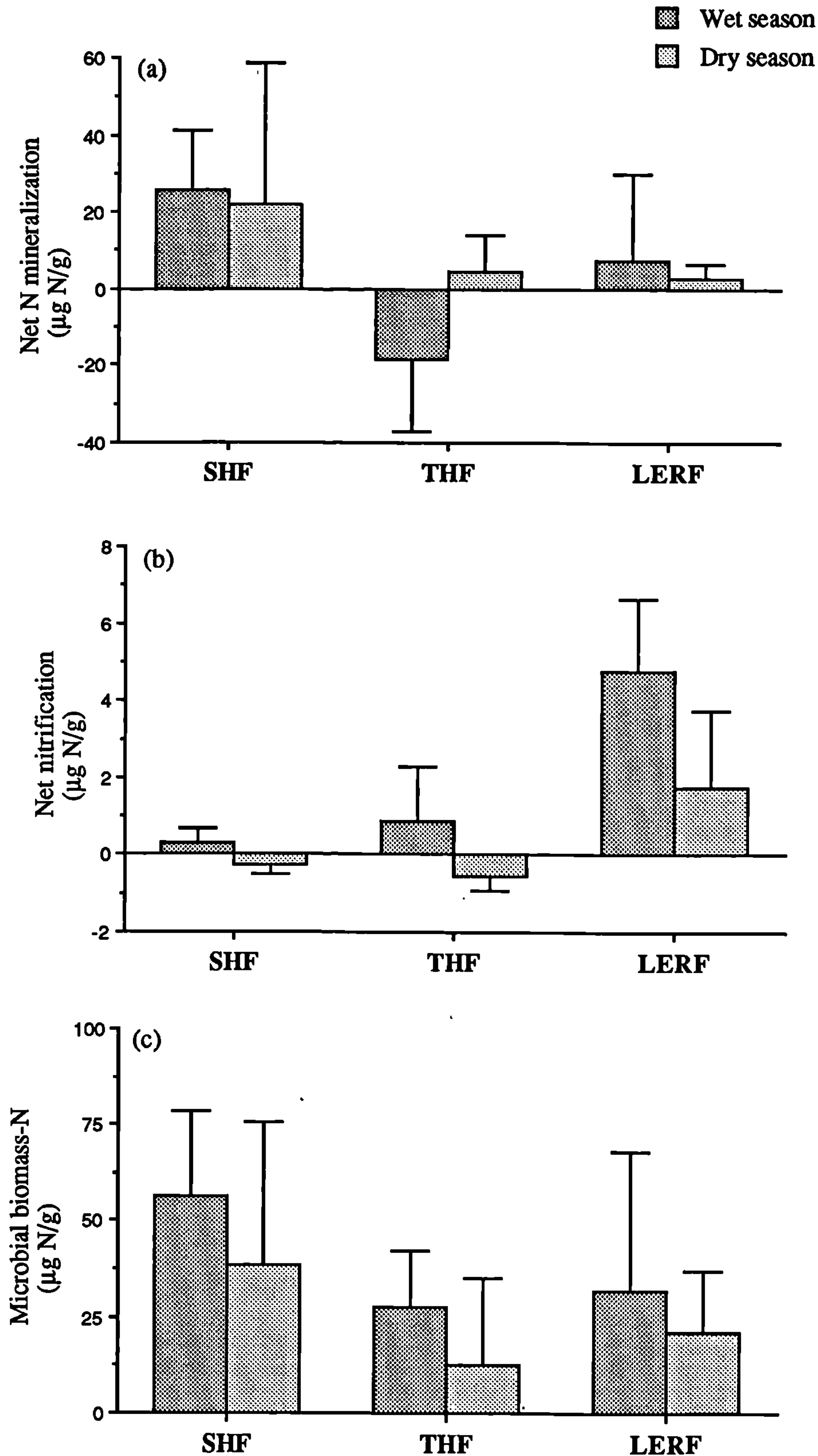


Fig. 4.3: (a) Net nitrogen mineralization (µg N g^{-1} oven-dry soil 10 d^{-1}), (b) net nitrification and (c) microbial biomass nitrogen, during the wet and dry season in SHF, THF and LERF. Values are means ± SD of the three replicate plots per forest type.

dry season, there was great variability for the SHF in the dry and LERF in the wet season and the differences between seasons were not significant.

Soil properties and nitrogen transformations and soil biomass-nitrogen.

In the SHF, some of the soil mineral nitrogen variation was explained by the difference in the soil moisture between wet and dry seasons ($p < 0.05$) (Table 3.3). However, when soil moisture, pH and LOI were combined the effect disappeared. Nevertheless, the interaction of the same three variables accounted for 52.2% ($p < 0.001$) of the variation of the initial nitrate concentrations in the soil. Although net nitrogen mineralization rates were not explained for either soil moisture, pH and LOI, soil moisture alone accounted for 58.4% ($p < 0.001$) of net nitrification variation, and the interaction of soil moisture, soil pH and LOI for 64% ($p < 0.001$). In the THF, contrasting with SHF, pH, soil moisture and LOI combined, accounted for 51% ($p < 0.001$) of the total mineral nitrogen variation. For variation between seasons, net nitrogen mineralization was best explained (31.3%) ($p < 0.001$) by the combined effect of soil pH, soil moisture and LOI. For net nitrification variations, however, though they were significantly affected by the interaction of these three variables ($p < 0.001$), soil moisture alone accounted for 48% ($p < 0.001$) of the variation. In the LERF, LOI and soil pH combined explained 76.8% ($p < 0.001$) of the variation of ammonium concentrations in the soil while for nitrate concentrations, the same variables accounted for only 21%. Similar to the SHF, net nitrogen mineralization variations were

not explained by soil pH, moisture and LOI, but their interaction explained 40% of the net nitrification variations ($p < 0.01$). In SHF, soil moisture accounted for 12% of the microbial-biomass nitrogen variations. No relationship was found for microbial-biomass nitrogen with either soil pH or LOI. In THF, LOI explained 21.5% ($p < 0.01$) of the variations. No other soil characteristic showed any relationship with microbial-biomass nitrogen. In LERF, biomass nitrogen variations were best explained (31%) ($p < 0.001$) by the interactive effect of LOI, soil moisture and soil pH.

Effects of nutrient additions under laboratory conditions.

Net nitrogen mineralization

All forest types showed no significant differences between the replicate plots in net nitrogen mineralization response to the nutrient additions (Appendix 2.1). In the SHF the addition of nitrogen in all of its combinations, after subtracting the nitrogen added, induced higher net nitrogen immobilization (Fig. 4.4a). Significant ($p < 0.001$) immobilization (from 4.47 $\mu g\ N\ g^{-1}\ 10\ d^{-1}$ in the control) were caused by (with the values of net mineralization in $\mu g\ N\ g^{-1}\ 10\ d^{-1}$ in parentheses): nitrogen (-69.0), nitrogen with sodium sulphate (-91.7); nitrogen with potassium (-86.8); nitrogen with calcium carbonate (-74.3), nitrogen with calcium sulphate (-76.5) and nitrogen with phosphorus (-76.6) (Fig 4.4a). For the THF, again the additions of nitrogen and its combinations caused high net immobilization ($p < 0.001$) of nitrogen in the soil during the 10-d incubation from 7.0 $\mu g\ N\ g^{-1}\ 10\ d^{-1}$ in the control to: -55.8 with nitrogen;

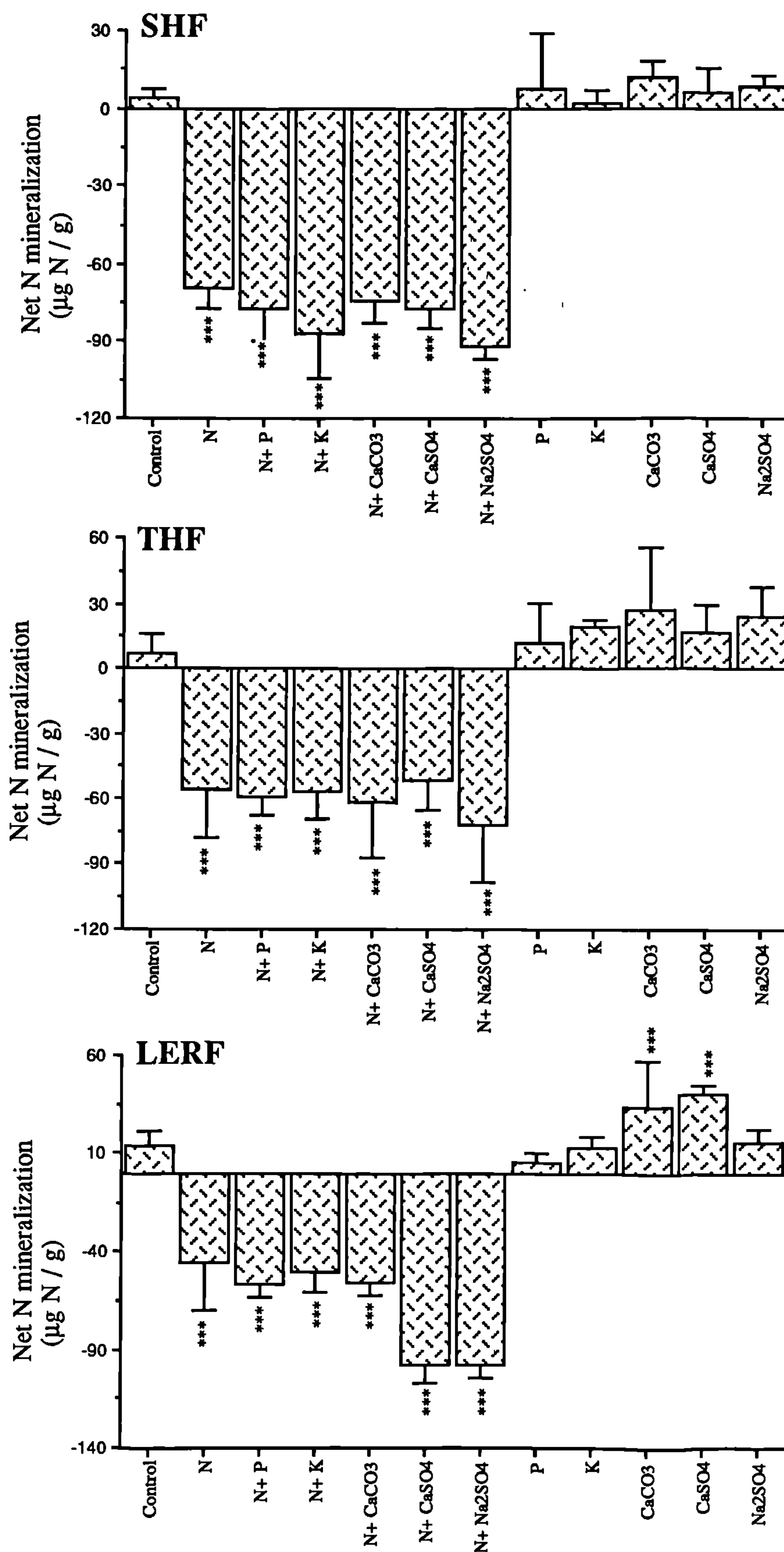


Fig. 4.4: Net nitrogen mineralization (µg N g^{-1} oven-dry soil 10 d^{-1}) under different nutrient addition treatments in the SHF, THF and LERF. Values are means ± SD (n=9). Significance levels for ANOVA with Dunnet's test for comparisons with the control are: * <0.05 ** <0.01 ***<0.001.

-71.5 with nitrogen and sodium sulphate; -61.6 with nitrogen and calcium carbonate; -59.1 with nitrogen and phosphorus; -56.2 with nitrogen and potassium, and -51.6 with nitrogen and calcium sulphate (Fig. 4.4b). In the LERF samples, six out of the twelve treatments significantly caused ($p < 0.001$) net immobilization rates from 14.5 µg N g^{-1} 10 d^{-1} in the control to: -45.5 with nitrogen; -97.8 with nitrogen and calcium sulphate; -97.6 with nitrogen and sodium sulphate, -49.9 with nitrogen and potassium; -55.1 with nitrogen and calcium carbonate; -56.3 with nitrogen and phosphorus whereas two treatments caused ($p < 0.001$) net mineralization, 39.9 with calcium sulphate and 33.7 with calcium carbonate (Fig. 4.4c).

Net nitrification

No significant differences in the net nitrification rates were found between plots for any forest type (Appendix 2.2). The SHF samples all had low concentrations of mineral nitrogen, and none out of the twelve treatments affected net nitrification rates (Fig. 4.5a). For the THF samples only the combination of nitrogen with phosphorus significantly increased ($p < 0.001$) net nitrification in the soil during the 10-d incubation (Fig. 4.5b). Phosphorus applied alone caused great variability in the net nitrification response. In the LERF samples, net nitrification rates were significantly different (all $p < 0.001$) from the control (8.36 µg N g^{-1} 10 d^{-1}) for seven out of the twelve treatments; net nitrification was increased by the additions of nitrogen (11.8 µg N g^{-1} 10 d^{-1}), phosphorus (12.6) and calcium carbonate (15.1) and net nitrification was inhibited by the additions of potassium (2.54), sodium

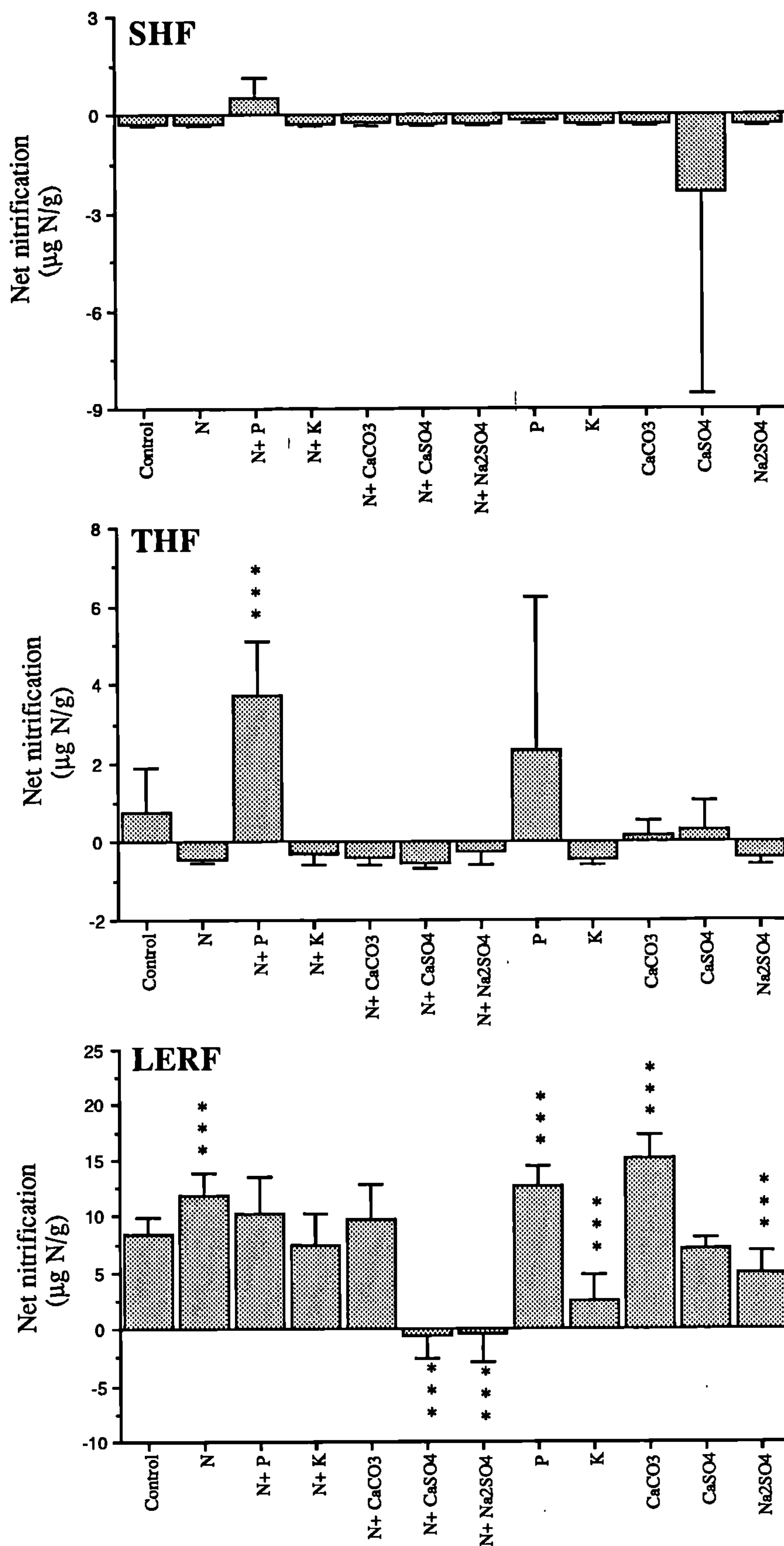


Fig. 4.5: Net nitrification (μg N g^{-1} oven-dry soil 10 d^{-1}) under different nutrient addition treatments in the SHF, THF and LERF. Values are means ± SD (n=9). Significance levels for ANOVA with Dunnet's test for comparisons with the control are: *< 0.05 **< 0.01 ***< 0.001.

sulphate (4.93) and the combinations of nitrogen and calcium sulphate (-0.59) and nitrogen and sodium sulphate (-0.50) (Fig. 4.5c).

DISCUSSION

As expected for acid forest soils, ammonium ions were the dominant inorganic form of the nitrogen in SHF, THF and LERF but as shown in Fig. 4.1 both ammonium and nitrate ions are always at low concentrations. Luizão *et al.* (1995) also found low mineral nitrogen supply in another Amazonian lowland evergreen forest on sandy soils, on Maracá Island. Table 4.2 compares the rates of net mineralization and nitrification for several Amazonian forests. Though net nitrogen mineralization in the SHF, THF, and LERF was in the lower range for tropical forests, the mineralization values were similar to other studies (Table 4.2). The results obtained in this study agree with the conclusions of Vitousek & Sanford (1986) that low rates of nitrogen mineralization are found in tropical forests on spodosols. Net nitrification in the SHF and THF are the lowest values recorded (Fig 4.3b). Herrera & Jordan (1981) working in a heath forest (Tall *Caatinga)* similar to the THF also found very low nitrification rates which they attributed to the low soil pH and the high concentration of tannins in the root mat. According to Hankinson & Schmidt (1984), nitrification in highly acid environments must result from the activities of one or more of the following: (1) classic autotrophic nitrifiers active despite their acid sensitivity; (2) acid-tolerant autotrophic nitrifiers; (3) heterotrophic nitrifiers. So far, no acid-tolerant autotrophic nitrifier has been identified, and no heterotrophic

nitrifier has been shown to function in other than in pure culture conditions. Chemosynthetic autotrophic nitrifiers capable of nitrification, however, have been isolated from certain acid soils (Belser & Schdmit 1987). Jansson (1958) demonstrated that mixed heterotrophic populations were successful in competing with nitrifying bacteria for limiting amounts of ammonium. According to Rosswall (1982), nitrifying populations, with their high Michaelis-Menten constants (K_m) and thus with relatively low affinities for ammonium ions, only used ammonium not needed by heterotrophic bacteria. Although no attempt was made in the present study to isolate and identify the soil microorganisms, the results indicate that among the bacteria, the heterotrophes were the dominant group and that they used only ammonium ions as a nitrogen source and almost completely inhibited the nitrifying bacteria by competition. Nevertheless, in LERF, with less acid soil than SHF and THF, nitrifiers seemed to succeed among other microorganisms and a relatively higher net nitrification was measured in both seasons. The differences in nitrogen transformations between forest types may reflect differences in nutrient inputs but feedbacks within the soil-plant-microorganism cycle could reinforce the pattern (Vitousek & Matson 1988). For instance, in the LERF, as already shown in Chapter III, the microbial population seems to be more diverse, and might include, besides heterotrophic fungi, both ammonifying and nitrifying bacteria. It seems that the combination of a higher soil pH, rapid mineralization of dead fine roots from the root mat and the residual forest floor litter fuelled nitrification. This agrees with the conclusion of Verhagen *et al.* (1992) who found that nitrifying

Table 4.2: Comparison of net nitrogen mineralization and nitrification (µg N g^{-1} unit $time^{-1}$) in soils from other forests in Amazonia.

Location	Forest type	Sample depth (cm)	Net mineralization	Net nitrification
Reserva da[1] Campina, Manaus	SHF on spodosol	0-10	24.0 (10.5 - 58.8)	0.01 (-0.5 - 0.6)
	THF on spodosol	0-10	-6.7 (-20.0 - 14.2)	0.1 (-0.9 - 2.2)
	LERF on ultisol	0-10	5.1 (-15 - 30.0)	3.2 (-0.3 - 6.6)
Reserva Ducke[2], Manaus	LERF on oxisol	0-15	19.0 ± 3.0	22.0 ± 28.0
	THF on spodosol	0-15	8.0 ± 1.2	6.0 ± 1.3
FUCADA[3], Manaus	LERF on oxisol	0-5	5.8 (-100 - 92)	6.1 (-6 - 54)
Maracá Island[4], Roraima	LERF on ultisol	0-10	11.3 ± 6.7	10.3 ± 5.8
San Carlos[5], Venezuela	LERF on oxisol	0-10	13.9 ± 3.99	14.9 ± 3.90
	LERF on ultisol	0-10	1.81 ± 1.87	3.5 ± 1.57

[1] this study, mean and range of two sampling times with a 10-d incubation. Samples taken from 0-10 cm depth in the mineral horizon below the organic horizon.

[2] Vitousek & Matson (1988), mean ± SE of one sampling time with a 10-d incubation.

[3] Luizão *et al.* (1992), mean and ranges of an annual cycle with a 10-d incubation.

[4] Marrs *et al.* (1991), mean ± SD of four seasonal samplings with a 30-d incubation.

[5] Montagnini & Buschbacher (1989), mean ± SD of one sampling time with a 30-d incubation.

bacteria can survive unfavourable circumstances (e.g. competitive heterotrophic microorganisms) as inactive, but viable, cells. However, in times of favourable C:N ratios which induce net mineralization, nitrifiers become active and then the formation of nitrate may begin again (Roberston & Vitousek 1982) because of the lack of a carbon source for the heterotrophic bacteria (Verhagen *et al.* 1992). In this study, however, the C:N ratio was measured only once, in the 5 April 1992 sampling, when all the mineral soils showed C:N ratios below 20, which favoured net mineralization. The mineral nitrogen availability in the soil is highly seasonal. In all forest types, there were more ammonium ions during the wet season while, the opposite was found for nitrate ions. The regression coefficients found between most of the nitrogen measurements and soil moisture and loss-on-ignition show that nutrient (labile organic matter) and water availability play important roles in the transformations of nitrogen in these soils, as Luizão *et al.* (1992) showed for a nearby lowland forest on an oxisol. Marrs *et al.* (1991) working on their sandy soil site on Maracá Island, found low mineralization in the dry season and suggested that seasonal differences were not directly related to the water shortage but to the reduced availability of nitrogen or a change in C:N ratio associated with water availability.

SHF was the forest type with the highest net nitrogen mineralization and the highest microbial-biomass nitrogen. Mineral forms of nitrogen are utilized by microorganisms (immobilized), particularly during decomposition of organic residues with a low nitrogen content. The mineral nitrogen that is not immobilized by soil microorganisms will be available for plant root uptake

which seems to be the case for SHF soils. However, very little net nitrogen mineralization was observed in both THF and LERF suggesting that most of the inorganic nitrogen has been retained within the microbial biomass. The relatively high biomass nitrogen measured in all forest types shows that most of the mineralized nitrogen was immediately immobilized in the microbial tissues, potentially an important nutrient conservation mechanism. Taking into account that indigenous soil microorganisms are better competitors for nutrients than plant roots and their known preference for ammonium ions (Jansson 1958; Alexander 1961; Verhagen *et al.* 1992) these results are not surprising. Thus, microbial biomass can serve as a potential source of mineralizable nitrogen for plants. Bonde *et al.* (1988) observed that microbial biomass nitrogen accounted for 55-89% of mineralized nitrogen in 40 weeks. In conclusion, it seems clear that as a consequence of slow decomposition rates, slow nitrogen release (see Chapter V) and greater nitrogen immobilization by decomposers, nitrogen availability to plants is dependent on the microbial biomass.

Addition of nutrients

In all forest types, all treatments which included nitrogen resulted in high net immobilization of the nitrogen. Immobilization of mineral nitrogen is a consequence of increased activity of the microbial biomass. Generally, as a result of the increased activity the microbial biomass also increases and more mineral nitrogen is immobilized (Lang & Beese 1985). Considering that even the calcium carbonate addition, at least in the amount used in this study,

was not enough to increase the soil pH in the THF and LERF, it seems that soil pH is one of the factors severely limiting nitrogen mineralization in these soils. However, since in the SHF soils, a significant pH increase in the soils were not followed by enhanced net nitrogen mineralization shows that low pH is not the only factor influencing the nitrogen transformation.

Usually application of both calcium carbonate and calcium sulphate allows a separation of the effects of increased pH from those of increased calcium. However, when a positive response was also observed with sodium sulphate, as it was the case in the LERF soils, then there is a possibility that sulphur can be limiting. As already observed for soil microbial activity (see Chapter III), also for nitrogen mineralization addition of sulphate alone enhanced the rate of the process in the LERF soils. Interestingly, all sulphate treatments significantly inhibited nitrification in the LERF. Also only in the LERF, application of calcium carbonate alone was effective in enhancing net nitrogen mineralization. The effect of liming (calcium additions) on the fate of ammonium ions has been related to the C:N ratio (Andersson & Persson 1988; Persson *et al.* 1989, 1990). However, several studies have indicated that the effect of liming on nitrogen mineralization can also be negative in forest soils with a low C:N ratio (Lang & Beese 1985; Ibron & Runge 1989; Ilmer & Schinner 1991). All forest soils in this study had a low (< 20) C:N ratio (Chapter II), but the enhanced nitrogen mineralization observed in the LERF cannot be attributed to liming since the calcium carbonate addition increased soil pH in the SHF but not in the THF and LERF. Additionally, there are strong indications that liming of forest soils stimulates bacterial

activity more than that of fungi (Griffin 1985). Thus, the general lack of an increase in net nitrogen mineralization in both the SHF and THF when only calcium carbonate was applied may be explained by an increase in bacterial relative to fungal decomposition. Furthermore, liming may give rise to an increased degradation of polyphenols because their toxic effect is counteracted by calcium (Carlyle 1986). Polyphenol compounds are found in all forest types, but are especially abundant in the SHF and THF (Anderson & St John 1981), and do not contain nitrogen; consequently their degradation coincides with nitrogen immobilization. Enhanced nitrogen mineralization in the limed treatments does not necessarily mean a pH limitation and this was evident from the calcium carbonate application in the THF and LERF where there was no increase in pH. Nyborg & Hoyt (1978) reported that liming forty acid surface-soils (pH 4.0-5.6) roughly doubled nitrogen mineralization during a 28-d incubation. However the effect of lime ceased 1 or 2 years after the application, perhaps because all the $CaCO_3$ had dissolved. In the present study nitrogen was added as urea, a compound common in the soil as a product of the breakdown of nucleic acids and also of the excretions of large animals (Alexander 1961). Thus, as an intermediate in microbial metabolism, urea applied to the soil is very readily hydrolysed, and much of it is transformed to ammonium ions and immobilised in a few days. No significant changes in soil pH were observed in the urea treatments.

Nitrifier populations are low in these soils as can be seen from the nitrification rates with nutrient additions. Net nitrate production can only increase when there is ammonium available for nitrifying microorganisms.

Therefore, the extent to which nitrate production and leaching may increase depends on whether liming stimulates mineralization or immobilization of ammonium. Since nitrogen mineralization was inhibited by many of the added nutrients, no ammonium was left for the nitrification process. In both SHF and THF net nitrification was inhibited (although not significantly) by nearly all nutrients applied. Even the addition of urea did not increase nitrification. Olson & Reiners (1983) found in their study that a low-rate of nitrification in the forest floor and a response to nitrapyrin (an inhibitor of nitrifier activity) indicated that nitrifier populations were present but somehow unable to respond to an ample ammonium ion supply. Their data showed that relatively insoluble polyphenols, which can inhibit nitrification, were concentrated near the soil surface. Allelochemicals including tannins, phenols and volatile terpenoids released from leaves and leaf litter are capable of inhibiting nitrogen mineralization, and in particular nitrification, in a wide variety of forest soils (Lohdi & Killingbeck 1980, Baldwin *et al.* 1983, White 1988). Studies have suggested that labile inhibitors of nitrification may be responsible for delays in nitrate production (Vitousek & Matson 1985) or its complete inhibition (White 1986). Rice & Pancholy (1972, 1973a, b) suggested that nitrification, during vegetation succession, was progressively inhibited by chemicals produced by plant tissues. It is curious that polyphenols with a presumably general protein binding effect, should inhibit nitrification but not mineralization. It is possible that bacteria (which oxidize ammonium ions) are more sensitive to polyphenols than are fungi which may be the principal mineralizing organisms in these acid soils.

Chapter V. Fine Root Growth, Nutrient Release, and Colonization by Litter Animals, in Decomposing Leaf-Litter in SHF, THF, and LERF.

INTRODUCTION

Roots are not only important to plants, for nutrient and water absorption, but also to the soil biota, as carbon and mineral sources. Living and apparently healthy roots exude a wide range of soluble organic substances including sugar, amino acids, and organic acids which attract the microbial population (Rovira 1969). Studies in rhizotrons following the sequence of colonization at a growing root tip have shown sloughed root cap cells, exudates and exfoliates, which induce microbial activity and subsequent faunal feeding and mineralization (Coleman 1985). Thus, soil and litter fauna as well as soil and litter microbe populations have a role in the recycling of nutrients locked in dead organic matter (Anderson 1975; Blair *et al.* 1992) since they help to break up plant remains.

The density of some faunal groups can be very high (Petersen & Luxton 1982) and as shown by the results of several faunal exclusion studies, they can have a great influence on decomposition (Edwards & Heath 1963). Fragmentation of organic matter, channelling, and mixing of soil components are key roles in which soil fauna stimulate microbial activity, and enhance

the rate of decomposition (Crossley 1977; Swift *et al.* 1979). Also, material which has passed through the gut of soil animals is more readily attacked by the microorganisms and the rate of mineralization is increased (Lavelle 1984). Where fast nutrient mineralization rates are observed, rapid capture is necessary to prevent losses from the system through leaching. There are many biological mechanisms which may prevent loss of nutrients. Surface root mats are allegedly widespread in Amazonia (Herrera *et al.* 1978) and are often held to be an adaptation for nutrient conservation (Jordan 1989) owing to the rapid nutrient uptake by the active feeding roots (Stark & Jordan 1978). Thompson *et al.* (1992), however found no root mat in the sandy Maracá forest soils and suggested that factors other than nutrient-poor soil are responsible for its occurrence.

Cuevas & Medina (1988) tested the effect of roots on the rate of the decomposition of leaves of three native tree species of a *Tierra firme* (lowland evergreen rain forest) on an oxisol and found that roots reduced the turnover of calcium and magnesium but had no effect on the rate of release of potassium. It might be expected that under heath forest on coarse sands, where the roots are mostly among the soil organic matter, prevention of root entry into litter bags would delay the weight loss of the material and contribute to an accumulation of nutrients released during its decomposition. In addition the absence of roots might be expected to affect the colonization of the decomposing leaves by litter animals.

The aim of this chapter is to test these hypotheses by assessing the influence of fine roots on the weight loss, nutrient uptake from leaf litter, and faunal colonization in the SHF, THF and LERF.

MATERIALS AND METHODS

Litter-bag technique

The litter-bag technique (Bocock & Gilbert 1957) was used to assess the influence of fine roots on decomposition. The technique consists of enclosing plant material, of known weight and nutrient concentrations in mesh bags and placing them in the field to allow decomposition to take place. At assigned time intervals, a number of litter bags is retrieved and their weight and chemical nutrients are measured in order to follow the rate of decomposition and nutrient release of the enclosed litter.

Methods

Leaves of *Clitoria racemosa* Benth. (Leguminosae), a species widely used in decomposition studies in the area (Luizão & Schubart 1987), and which has a moderate rate of decomposition (F.J. Luizão & R.C.C. Luizão unpublished) were chosen for the study. Freshly fallen leaves were collected at INPA's campus and air-dried before use. Nylon bags of 1.5-mm mesh, measuring 20 cm x 22 cm with lateral holes of 10 mm for free access of litter mesofauna and macrofauna were used. Each bag contained about twelve leaves of *Clitoria* of known weight and chemistry.

Two experiments were made: (1) one at the onset of the rainy season (December 1991), and the other (2) at the onset of the dry season (August 1992). In each experiment there were twenty-four pairs of litter-bags placed randomly in each of the nine plots. The bags were placed on the litter layer, in positions which were cleared of other recently fallen leaves. For each pair, one of the bags was lifted every week and inverted to prevent the growth of roots into it. The control bags were left undisturbed. In each experiment the bags were retrieved after 30, 60, 120, 180, 270 and 360 d. At each retrieval four pairs of litter bags were removed from each plot.

It was observed that leaves in the bags which were lifted weekly were, unexpectedly, decomposing more rapidly than the undisturbed ones. It was thought that the inverting of the bags and hence the exposing both sides of them to leaching and desiccation might render the leaves more easily attacked by the soil biota. To test this hypothesis a third experiment was set up in the following wet season, starting in December 1992 and in one plot only in SHF, THF and LERF. This experiment also tested the effect of sun exposure on the litter decomposition at the SHF where there were many open areas. In the LERF and THF plots, sixteen pairs of litter-bags were randomly placed on the forest floor while the SHF plot was sub-divided into open and closed sites and sixteen pairs of litter-bags were randomly distributed in each. Both litter-bags of the pair were lifted up weekly, but one was replaced in the same position while the other was inverted before replacing. Retrievals were made after 30, 90, 180 and 270 d. At each retrieval four pairs of litter-bags were removed from each plot.

For all experiments, the litter-bags were immediately brought to the laboratory after retrieval, each bag was opened and any fine roots and detritus, adhering to both the litter and the roots, were removed. The leaves and roots were oven-dried at 60 °C for 24 h to determine their dry weight, and ground before chemical analysis. In experiment 1 the oven drying was delayed until after a soil faunal extraction.

Chemical analysis

Chemical analyses were made in the laboratory of the Centro de Energia Nuclear na Agricultura (CENA), Piracicaba, São Paulo. Each ground sample was digested in two ways: 0.20 g was digested in concentrated sulphuric acid and hydrogen peroxide, with a selenium catalyst (Allen *et al.* 1974) for nitrogen analysis; and 0.75 g by nitro-perchloric digestion, for the other elements. Total nitrogen concentrations were determined using a Technicon Autoanalyser (Reis *et al.* 1980). Potassium was determined by an atomic absorption spectrophotometer Perkin-Elmer Model 306 (Zagatto *et al.* 1979). Phosphorus, calcium, magnesium, manganese, aluminium, boron, copper, iron, and zinc were determined by a Jarrel-Ash Model 975 atomic emission spectrometer with induced plasma (Krug *et al.* 1979) Organic carbon was determined by dry oxidation in a Carmograph P12 Wosthoff (Carbon Biological Oxidizer).

Litter animal extraction

The faunal extraction was made only with samples from experiment 1 which was started in the wet season. Immediately after opening the bags the litter fauna were extracted by a Berlese-Tullgren funnel system (MacFadyen 1963) In this method, an electric lamp bulb (25 W) positioned 20 cm above the samples was used as a heat source to drive the animals into a collecting jar with dilute formaldehyde solution. This method is good for loose litter samples. After extraction, the animals were stored in 70% ethanol, identified to broad taxonomic groups (Wallwork 1976), and counted. Soil animal density was expressed as number of individuals per litter bag rather than per unit mass of root (because there were many bags with no root) nor per weight of material (because where fine roots penetrated litter bags they created an extra mass of material).

Data analyses

Data on rates of nutrient release were based on the initial content (remaining dry weight multiplied by the concentration of the element at the time of retrieval) of each element in the decomposing leaves. Statistical analyses were made on the concentrations and contents of the elements. The data were transformed either using $\log_{10}$ or arcsin transformations (Zar 1974) before running the analyses using the Minitab (1993) software. Statistical differences between treatments were tested using a one-way analysis of variance.

RESULTS

Litter decomposition

Root biomass

In the litter bags with roots, root mass was significantly ($p < 0.001$) higher in the LERF and smaller ($p < 0.001$) in the SHF in experiments 1 and 2 (Fig. 5.1). Roots were never observed in the SHF plot 2 and were always higher in plot 1 in both experiment 1 ($p < 0.001$) and experiment 2 ($p < 0.01$) (Table 5.1). In the lifted bags in all forest types root biomass was always negligible (Fig. 5.2).

Leaf litter dry mass

In all forest types and for experiments 1 and 2, no significant differences in the rates of litter decomposition were observed between the bags with and without roots (Fig. 5.3). Despite the fact that rates of decomposition in experiment 2 were lower in SHF and THF compared with the LERF, their residual masses within each forest type were always independent of the root presence. In experiment 3, the two ways for excluding roots (the bags simply lifted or lifted and reversed) showed no significant differences in rates of decomposition (Fig. 5.4).

Element concentrations and contents of the leaves in experiment 1

In SHF the presence of roots significantly influenced the concentrations of only two elements, aluminium ($p < 0.05$) and potassium ($p < 0.001$) which

Table 5.1: Root mass (g bag^{-1}) in the bags with roots at each retrieval time in each plot of the SHF in the experiments 1 and 2. Values are means ± SD of four litter bags retrieved per plot.

	Plot 1		Plot 2		Plot 3	
Experiment	1	2	1	2	1	2
30 d	0.03 ± 0.04	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
60 d	0.02 ± 0.03	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
120 d	0.05 ± 0.06	0.03 ± 0.05	0.01 ± 0.02	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
180 d	0.09 ± 0.02	0.07 ± 0.05	0.0 ± 0.0	0.03 ± 0.04	0.05 ± 0.07	0.01 ± 0.02
270 d	0.11 ± 0.06	0.06 ± 0.04	0.0 ± 0.0	0.01 ± 0.03	0.0 ± 0.0	0.02 ± 0.03
360 d	0.26 ± 0.21	0.19 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.04	0.07 ± 0.10

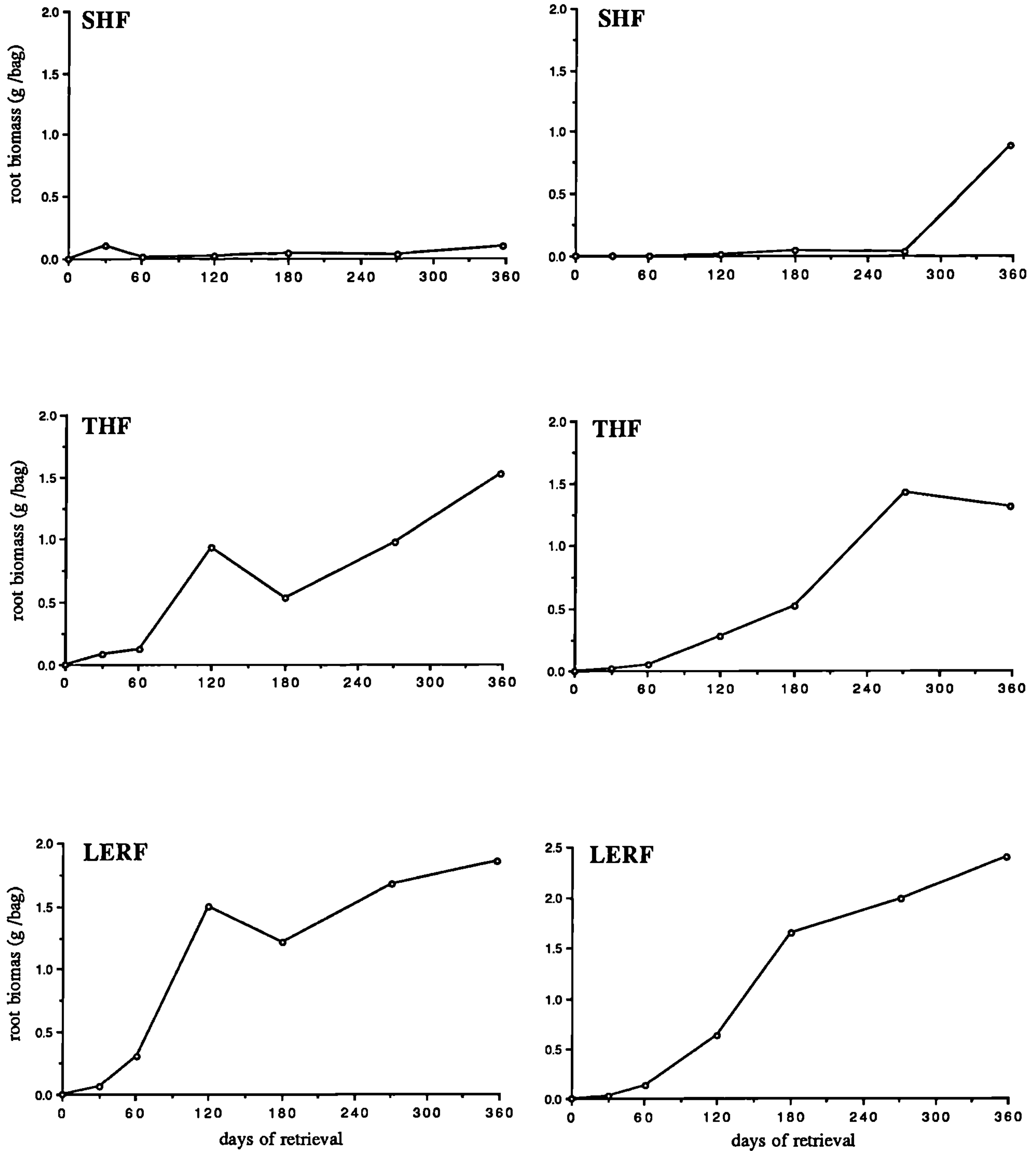


Fig. 5.1: Mean root biomass (g bag^{-1}) in bags with roots in experiments 1 and 2. Each of the three replicate plots in the SHF, THF and LERF had four bags retrieved each time.

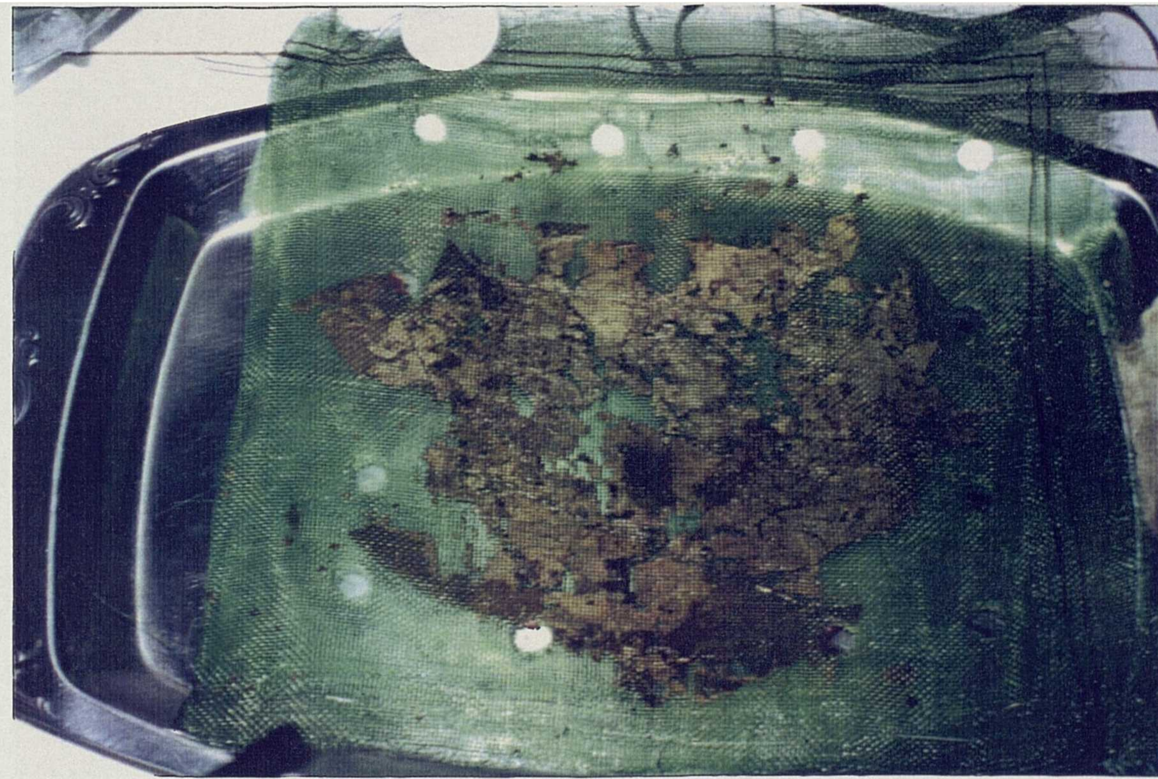


Fig. 5.2: Appearance of the litter bags containing decomposing leaves after 180 d in the treatments (a) with roots and, (b) without roots.

showed higher concentrations with roots and the differences were significantly (both $p < 0.001$) related with plot 1, which had higher root biomass. Fig. 5.5 shows the percentage initial content of the elements in the SHF for which neither elements had their rate of release significantly influenced by roots. In the THF, two of the macronutrients, phosphorus ($p < 0.05$) and potassium ($p < 0.001$) showed higher concentrations, and boron ($p < 0.05$), calcium and magnesium (both $p < 0.001$), lower concentrations in the bags with roots. From the rate of content release in the THF (Fig. 5.6) potassium ($p < 0.05$) showed significantly slower and boron ($p < 0.01$) significantly faster release in the bags with roots. In the LERF, in parallel to SHF, aluminium ($p < 0.01$) and potassium ($p < 0.001$) showed higher concentrations and boron, calcium, magnesium, manganese and zinc showed (all $p < 0.001$) lower concentrations in the bags with roots. Rates of content release in the LERF (Fig. 5.7) show that aluminium ($p < 0.001$) were slower and, calcium ($p < 0.05$), magnesium (0.05), boron ($p < 0.001$), manganese ($p < 0.001$) and zinc ($p < 0.001$) were released faster in the bags with roots.

Element content of the leaves in experiment 2

In the SHF, the presence of roots significantly influenced the concentrations of eight out of the eleven elements analysed. Aluminium ($p < 0.01$), calcium ($p < 0.001$), iron, magnesium, manganese, potassium and phosphorus (all $p < 0.05$) showed higher concentrations in the bags with roots. Only zinc ($p < 0.001$) showed a lower concentration in the bags with roots. In the SHF

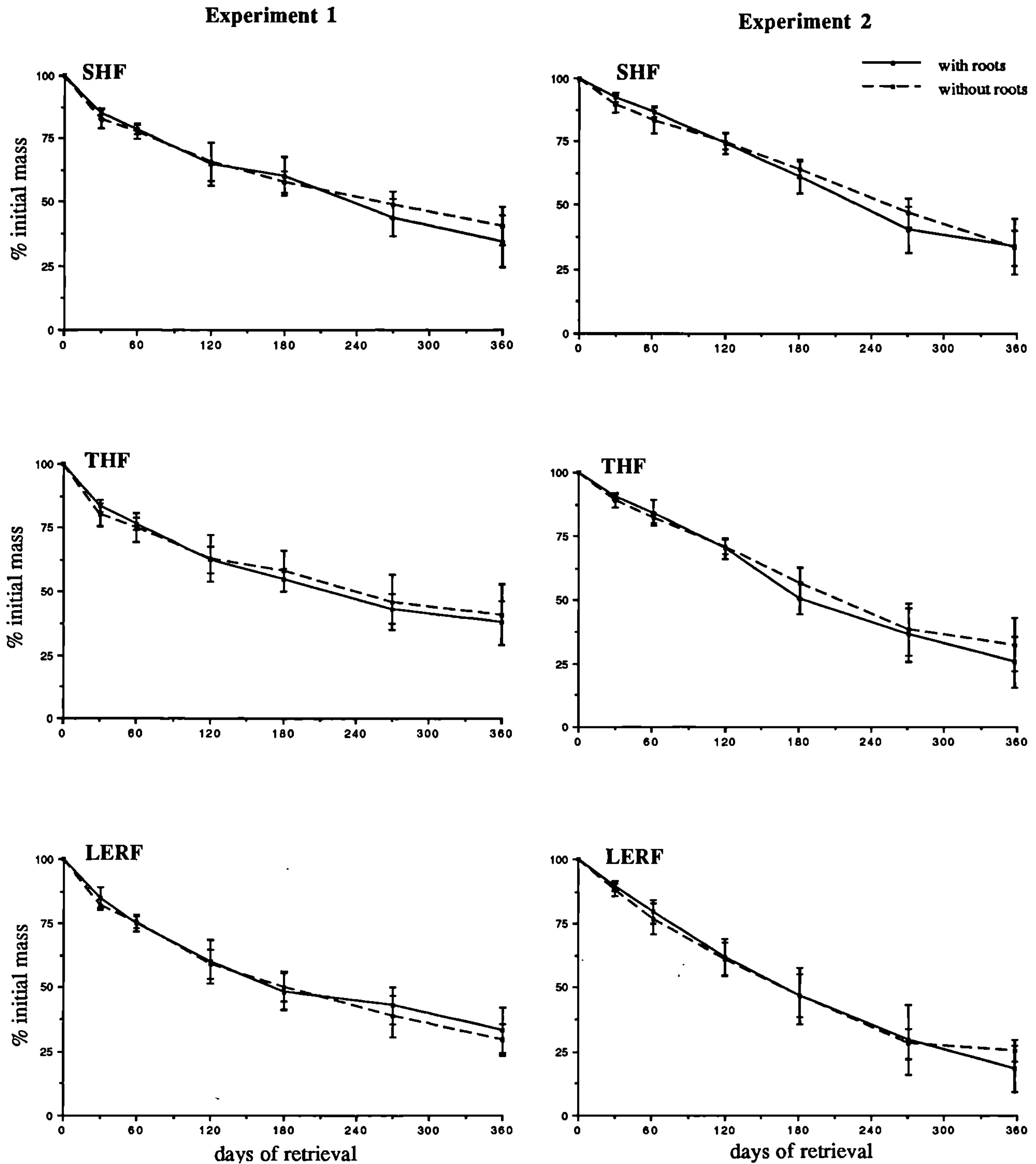


Fig. 5.3. Mean percentage of initial mass of the decomposing leaves in the treatments with and without roots for the experiments 1 and 2. Values are means ± SD. Each of the three replicate plots in the SHF, THF and LERF had four bags retrieved each time and hence n=12.

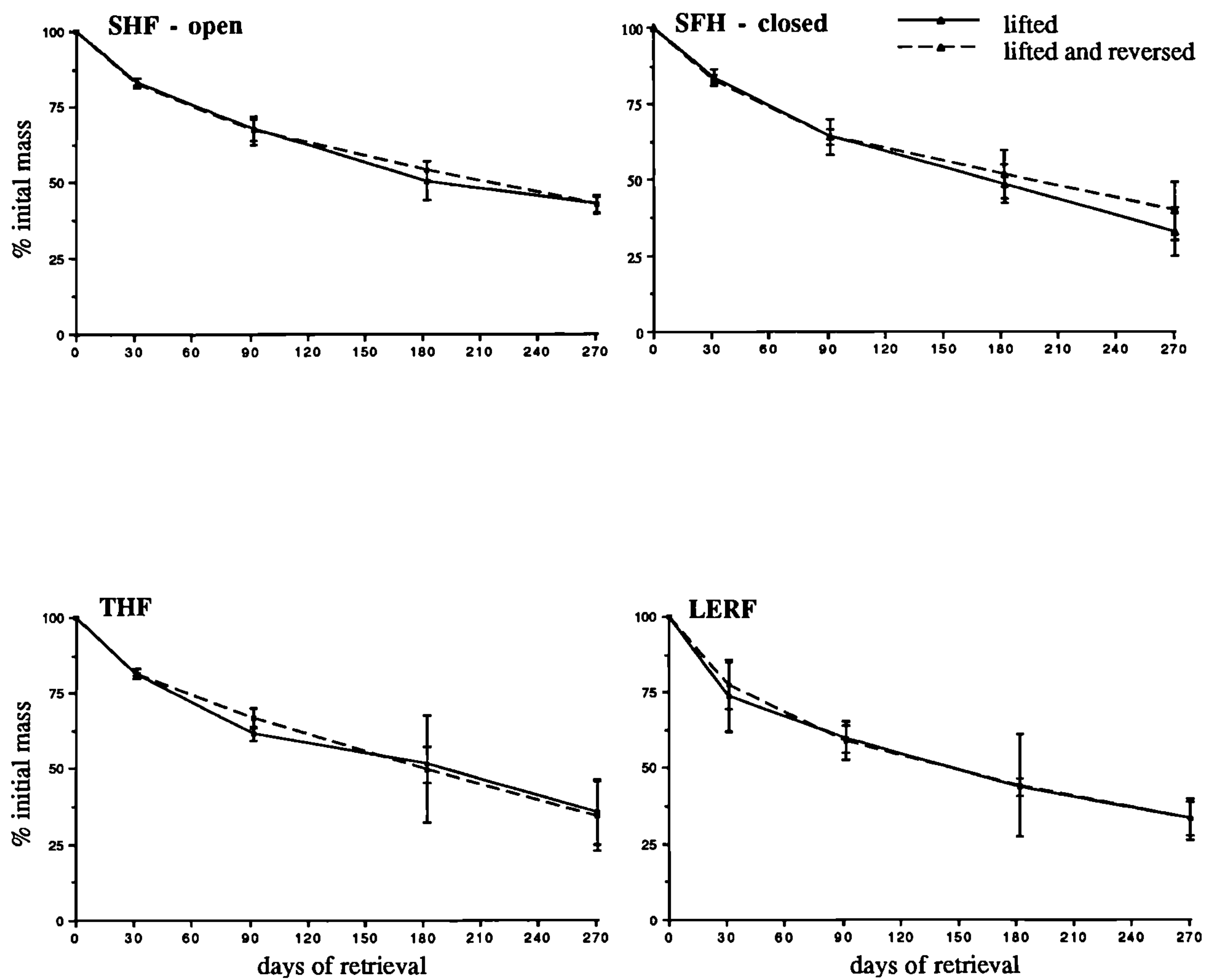


Fig. 5.4: Percentage of initial mass of the decomposing leaves in the treatments lifted and lifted and reversed in the open and closed vegetated sites of SHF, THF, and LERF. Values are means ± SD. Each site within the SHF plot and the THF and LERF had four bags retrieved each time and hence n=4.

though there was significantly more roots in plot 1, the two-way (root mass and plots) analysis of variance showed that only two elements, calcium and aluminium had their concentrations significantly (both $p < 0.01$) related with the higher root biomass in plot 1. In the SHF, only the concentrations of nitrogen, boron and copper were not affected by the roots. The rates of element release in Fig 5.8 shows that in the SHF, aluminium ($p < 0.01$), calcium ($p < 0.05$) and manganese ($p < 0.05$) were released faster while zinc ($p < 0.01$) was released slower in the bags with roots. In the THF, aluminium and iron (both $p < 0.01$) showed higher concentrations in the bags with roots, while boron ($p < 0.01$), manganese and zinc (both $p < 0.001$) showed lower concentrations in the bags with roots. All other elements showed no differences in concentrations between treatments. In the THF, the presence of roots significantly influenced the rates of release of three elements (Fig 5.9); aluminium ($p < 0.001$) and iron ($p < 0.05$) were released slower and zinc was more quickly released in the bags with roots. In the LERF, only calcium ($p < 0.01$) and magnesium ($p < 0.05$) of the macronutrients showed significantly lower concentrations in the presence of roots. In parallel with both SHF and THF, aluminium and iron (both $p < 0.01$) showed higher concentrations in the bags with roots. In fact, in all three forest types both aluminium and iron showed accumulation up to 180 d with concentrations higher than initially, independent of the presence or absence of roots. However the concentrations of both elements were higher in the presence of roots. On the other hand, boron, manganese (both $p < 0.01$), and zinc ($p < 0.001$) showed lower concentrations in the bags with roots than without.

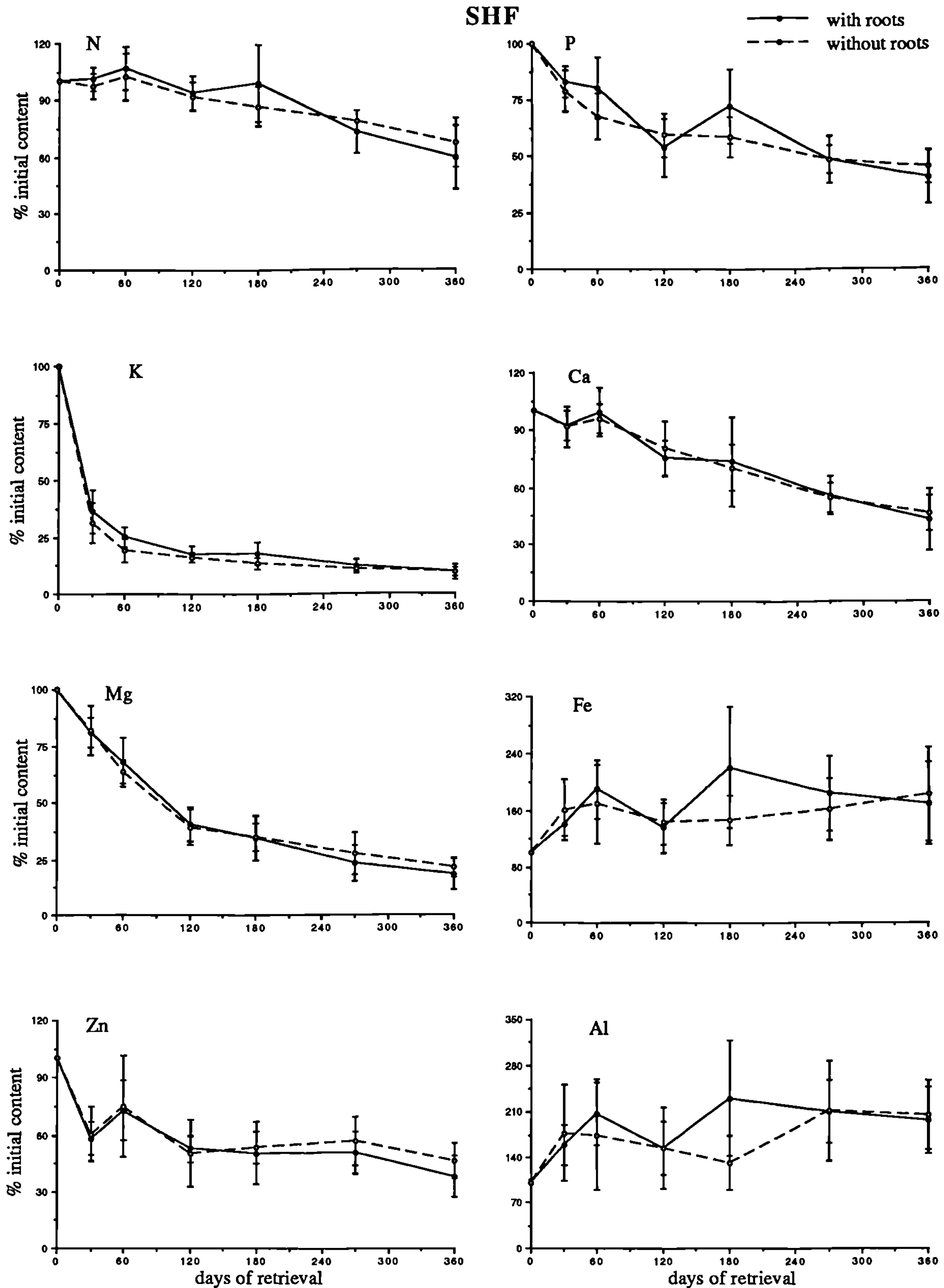


Fig. 5.5: Percentage of the initial content of mineral elements in the decomposing leaves with and without roots in the litter bags from the SHF during the experiment 1. Values are means ± SD. Each of the three replicate plots in the SHF had four bags retrieved each time.

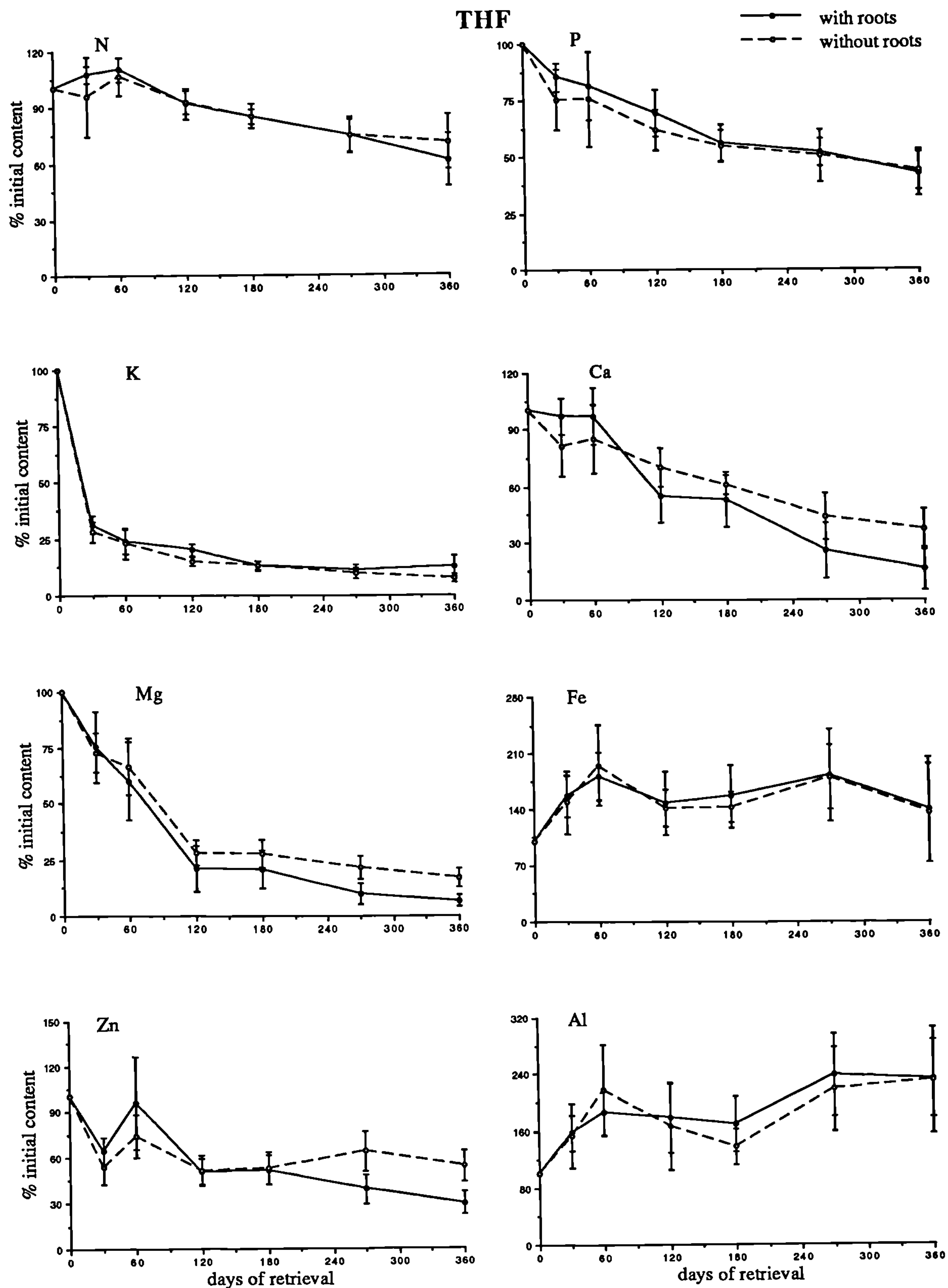


Fig. 5.6: Percentage of the initial content of mineral elements in the decomposing leaves with and without roots in the litter bags from the THF during the experiment 1. Values are means ± SD. Each of the three replicate plots in the THF had four bags retrieved each time and hence n=12.

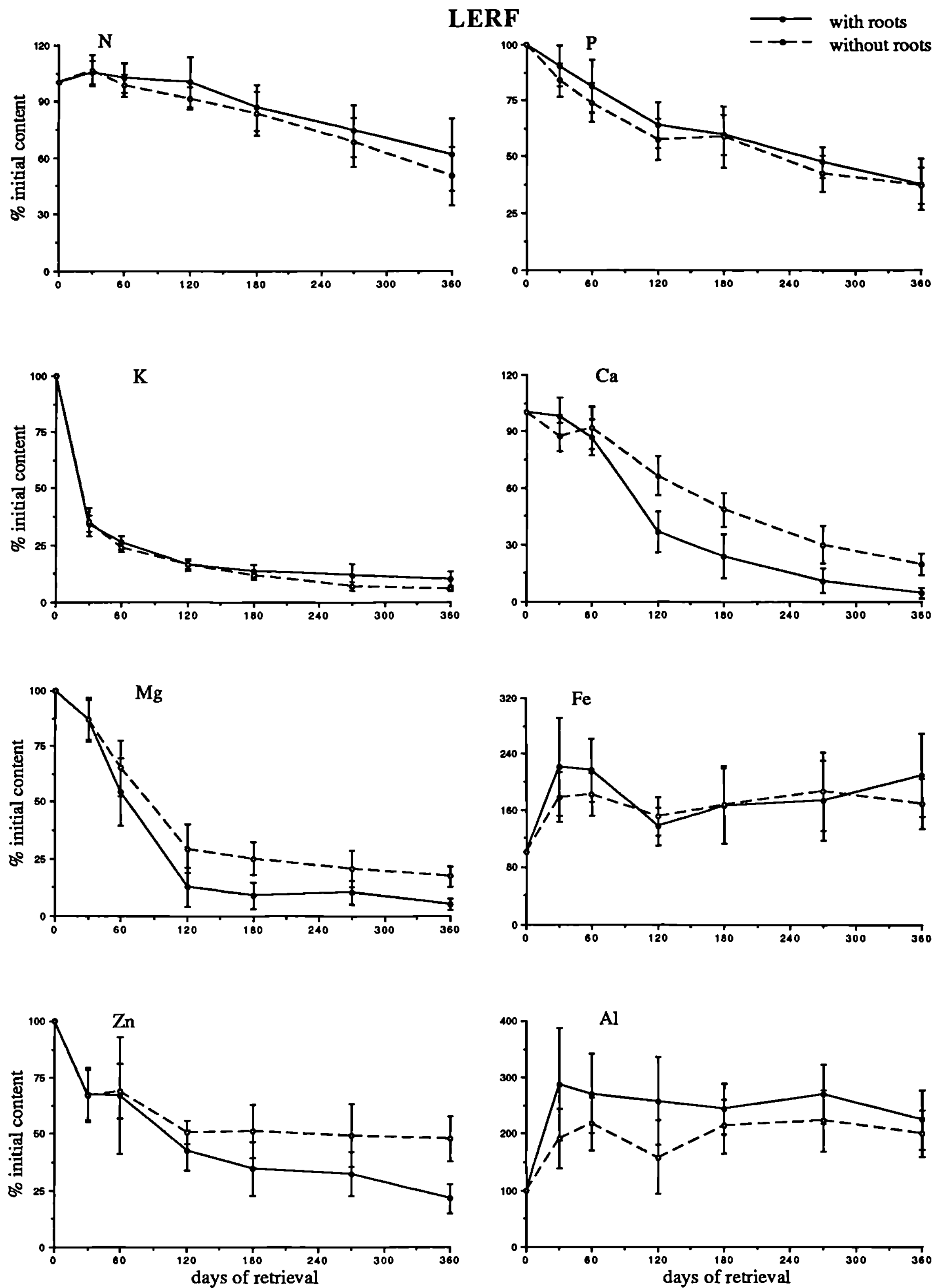


Fig. 5.7: Percentage of the initial content of mineral elements in the decomposing leaves with and without roots in the litter bags from the LERF during the experiment 1. Values are means ± SD. Each of the three replicate plots in the LERF had four bags retrieved each time and hence n=12.

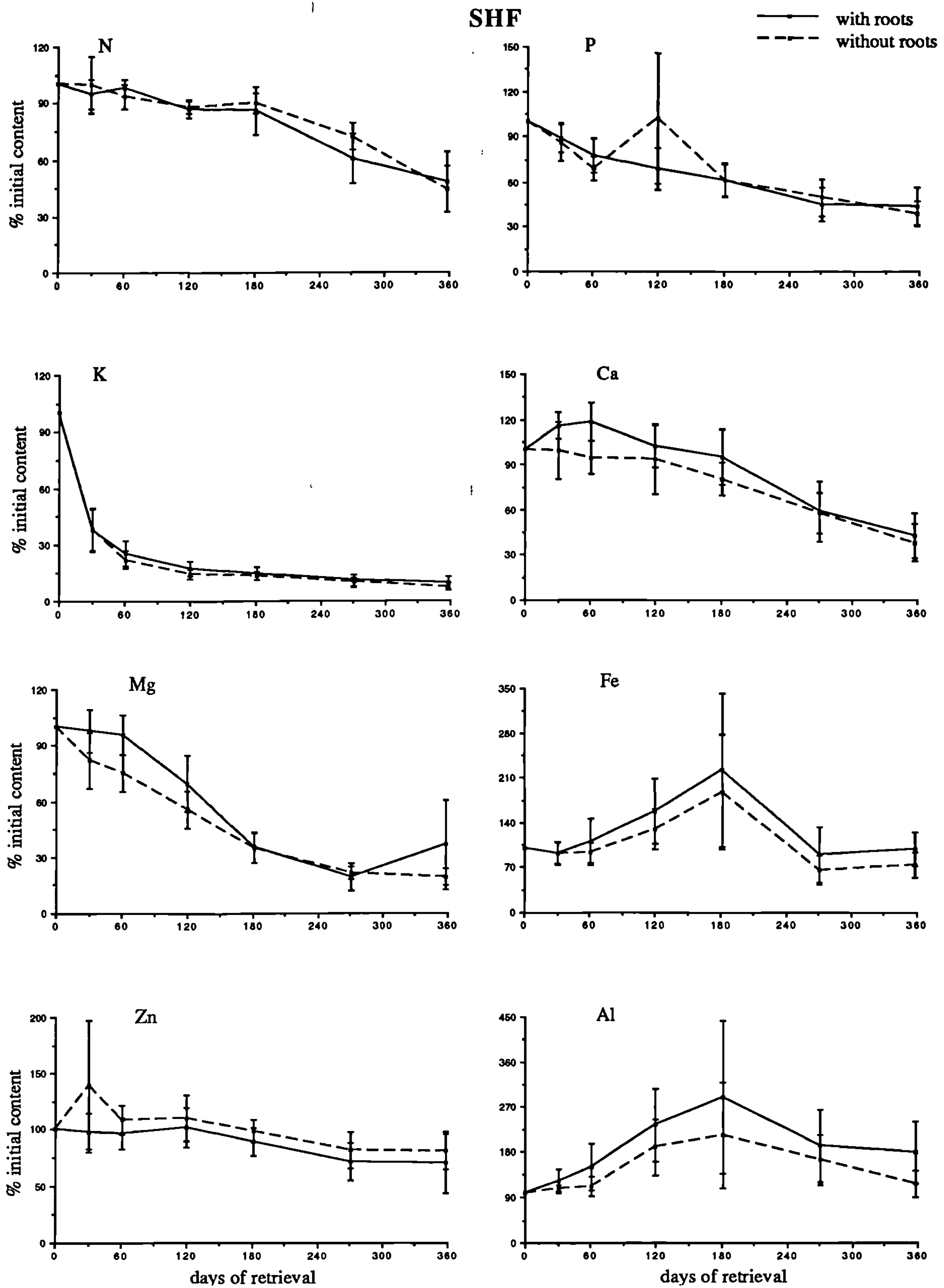


Fig. 5.8: Percentage of the initial content of mineral elements in the decomposing leaves with and without roots in the litter bags from the SHF in the experiment 2. Values are means ± SD. Each of the three replicate plots in the SHF had four bags retrieved each time and hence n=12.

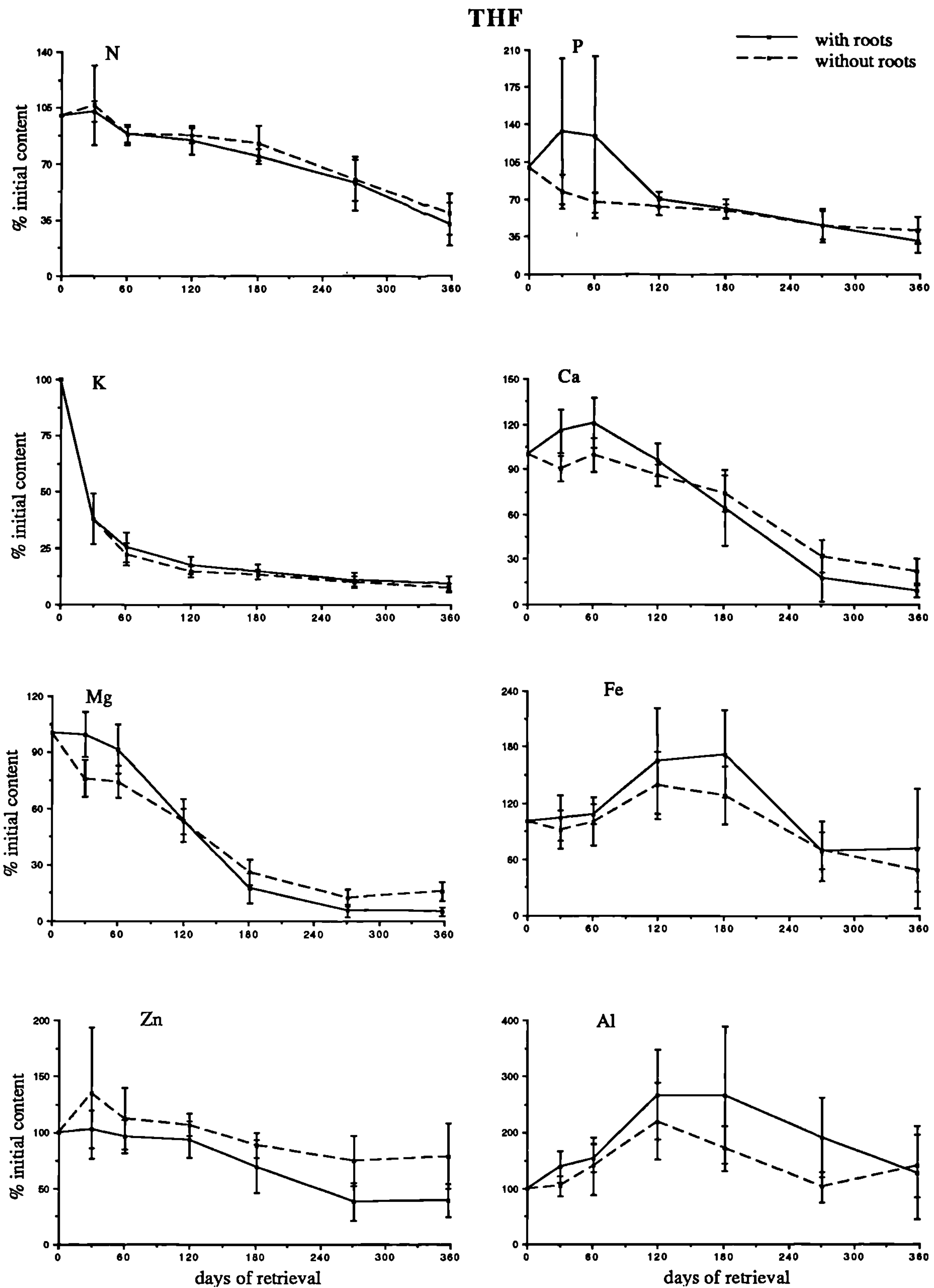


Fig. 5.9: Percentage of the initial content of mineral elements in the decomposing leaves with and without roots in the litter bags from the THF during the experiment 2. Values are means ± SD. Each of the three replicate plots in the THF had four bags retrieved each time and hence n=12.

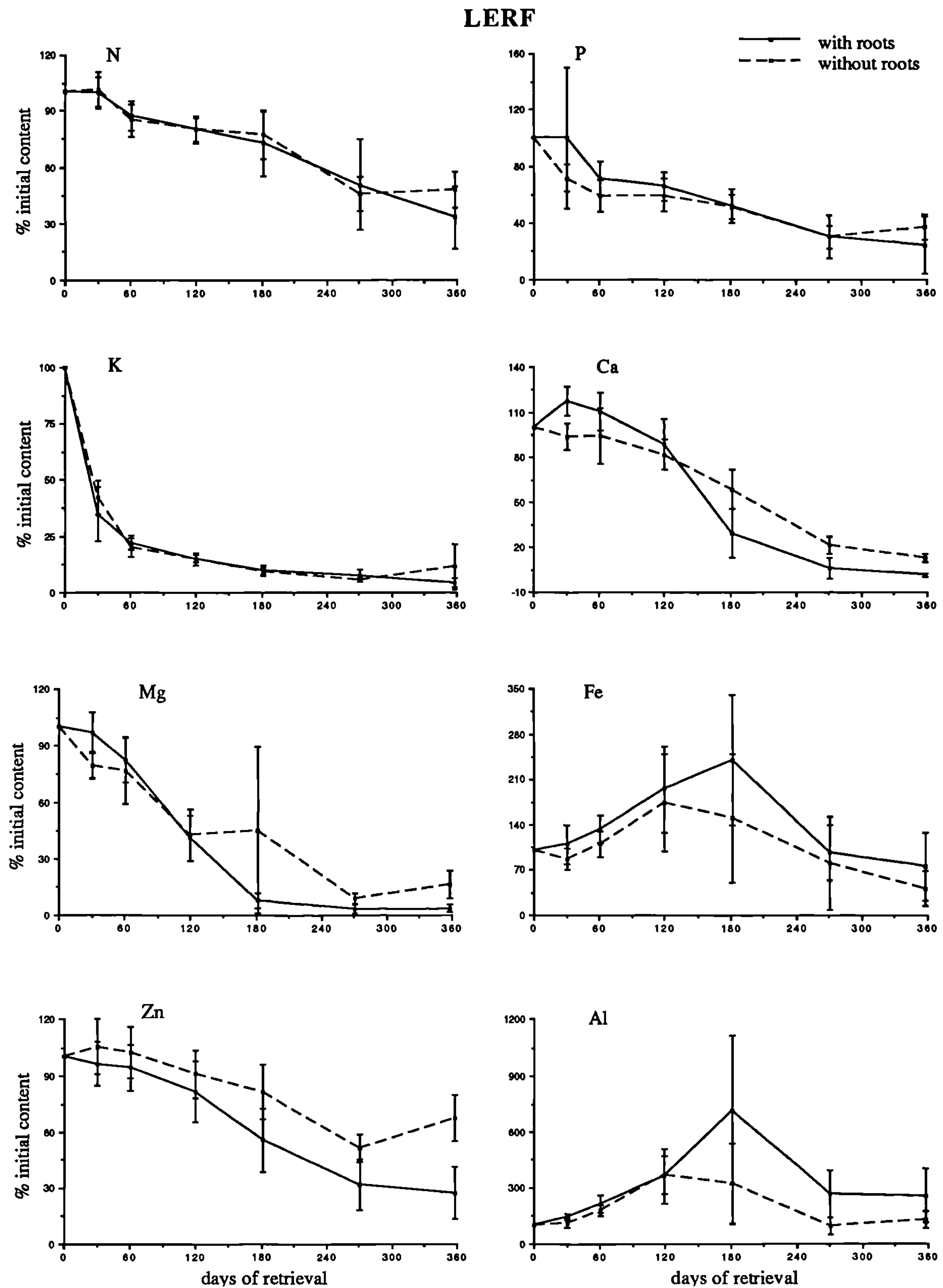


Fig. 5.10: Percentage of the initial content of mineral elements in the decomposing leaves with and without roots in the litter bags from the LERF during the experiment 2. Values are means ± SD. Each of the three replicate plots in the LERF had four bags retrieved each time and hence n=12.

From the rates of element release (Fig. 5.10) in the LERF, aluminium ($p < 0.05$) showed slower, while manganese ($p < 0.05$) and zinc ($p < 0.001$) showed faster release of their initial content in the bags with roots.

Element content of the leaves in experiment 3

For all forest types and elements no significant differences in rates of release were observed between the two root-excluding treatments (Appendices 3.1-3.4).

Litter animals

Composition of litter animal communities into the litter bags

A total of forty-one taxonomic groups of litter animals were found in the bags (Appendices 4.1-4.6). Fig. 5.11. shows the composition pattern of the nine most abundant animals in each treatment and forest type as means of all retrieval times to simplify comparisons between treatments and all forest types. All the less abundant taxa (including Lepidoptera, Homoptera juveniles, Hemiptera juveniles, Diptera adults, Chilopoda and Enchytraeidae) were pooled together and represented as 'other' in both the Appendices and Fig. 5.11. In SHF, nine out of the twenty-one main taxonomic groups which were present in the bags with roots, were absent from the bags without roots: Homoptera juveniles, Coleoptera adults, Diplura, Opiliones, Protura, Earthworms, Pauropoda, Symphyla, and Isoptera (Appendix 4.1-4.2). Of these, the first five groups group which were present in all the bags with

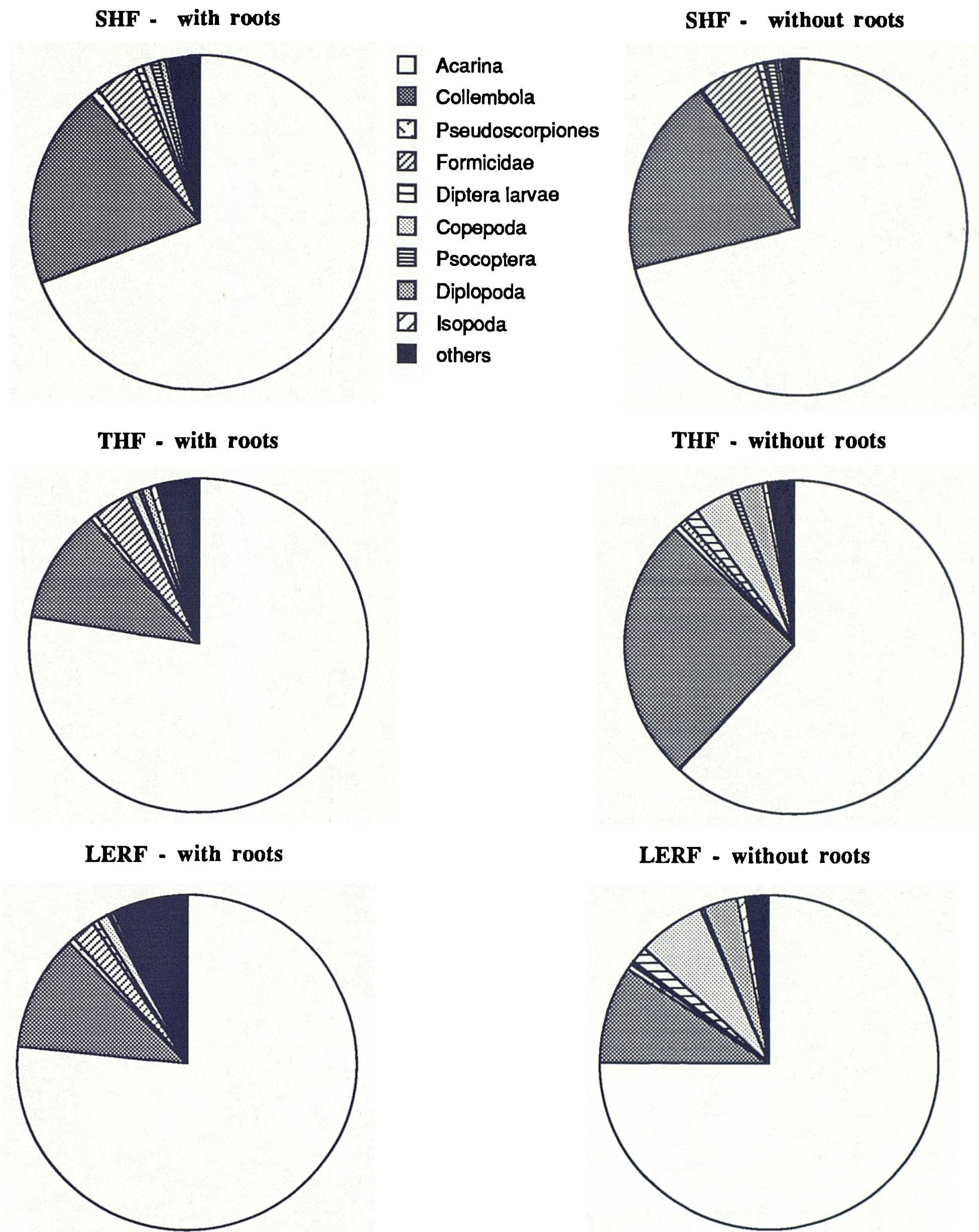


Fig. 5.11: Mean percentage composition (densities of animals) of the animal communities in the decomposing leaves of *Clitoria racemosa* enclosed in litter bags over the 1-year period of the experiment, in the SHF, THF, and LERF. In the SHF n=51 (bags with roots), n=19 (bags without roots); in the THF n=51 (with), n=24 (without); and in the LERF n=54 (with), n=26 (without).

roots were never present in the bags without roots in THF (Appendices 4.3-4.4). Protura were the only group which was present in the bags with roots and absent from all the bags without roots in SHF, THF and LERF (Appendices 4.1-4.6)

In all three forest types the bags with roots showed a similar composition pattern for the total litter animals (Fig. 5.11). In the SHF no difference between treatments was found for either the number of groups or total and individual densities of the taxonomic groups. In all forest types, independent of treatment, Acari were by far the most abundant group. Overall in the THF and LERF, both the number of taxonomic groups and the total density of animals were higher (all $p < 0.001$) in the bags with roots. In both THF and LERF there were significant (both $p < 0.001$) more Acari in the bags with roots than in those without. Collembola, also independent of treatment, was the second most abundant group in all forest types but only in the LERF a significantly ($p < 0.001$) higher number of Collembola were found in the bags with roots. In the THF, besides Acari and Collembola, the most numerous groups, Pseudoscorpiones were the only other group which showed significantly ($p < 0.05$) higher density in the bags with roots. In the LERF, however, three other groups showed significant differences between treatments: Pseudoscorpiones ($p < 0.001$) and Formicidae ($p < 0.05$) showed higher and Diplopopda ($p < 0.01$) showed a lower density in the bags with roots (Fig. 5.11).

Temporal distribution of the litter animals

Over the year of the experiment, the SHF and THF were similar, (Figs 5.12 and 5.13). Up to 120 d (all during the rainy season) the density of individuals increased and the density of animals was the same in bags with and without roots. At 180 d (at the beginning of the dry season), a large decrease in litter animal density (which fell to nearly zero in most groups) was observed in both treatments. However, from then on (dry season), animal density generally increased in the bags with roots, but the numbers in the bags without roots remained low (Figs. 5.12 and 5.13). Exceptions to these patterns were the Psocoptera in the SHF, which showed their maximum density in the mid dry-season (270 d) in the bags without roots (Fig. 5.12) and, Copepoda and Dipteran larvae in the THF, where Copepoda density was highest in the bags without roots after 60 d and slightly higher afterwards (Fig. 5.13). Dipteran larvae, in turn, were always higher at 270 d, and later, in the bags without roots. LERF showed no clear pattern of temporal variation. Although litter animals in the bags with roots usually showed an increase in density up to 120 d, this was not always the case for the animals in the bags without roots (Fig. 5.14). Also the beginning of the dry season (180 d) in the LERF did not affect the litter animals as markedly as in the SHF and the THF. In fact, a decreasing effect of the dry of season was frequently observed in all forest types for the animals in the bags with roots. In the LERF, the density of some groups, such as Copepoda, Dipteran larvae and especially Diplopoda was higher in the bags without roots (Fig. 5.14).

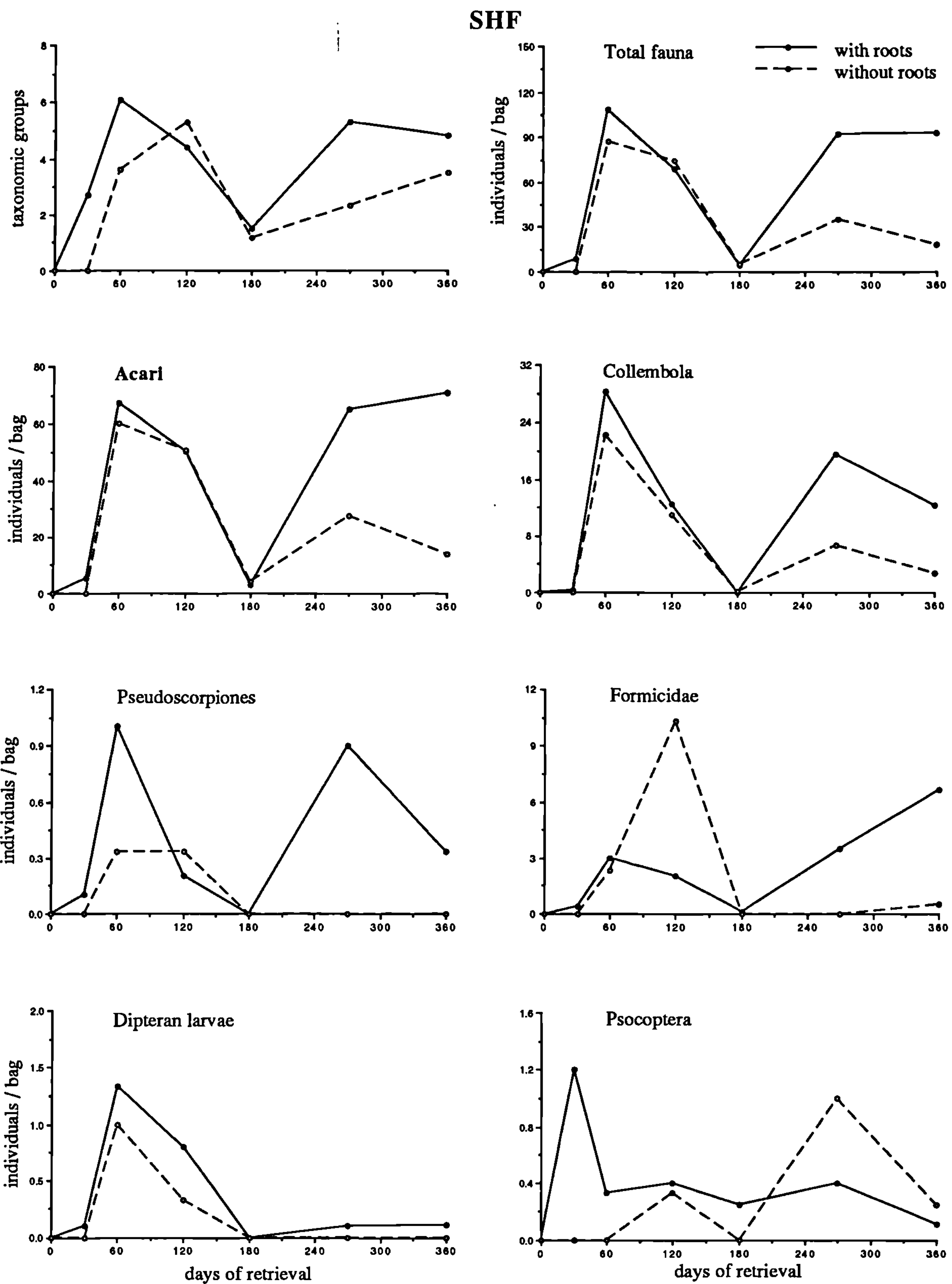


Fig. 5.12: Mean numbers of taxonomic groups, total faunal density (number of individuals per bag) and densities of the main taxonomic groups for each retrieval in the bags with roots and in the bags without roots in the SHF. Each of the three replicate plots had three bags retrieved each time and hence n=9.

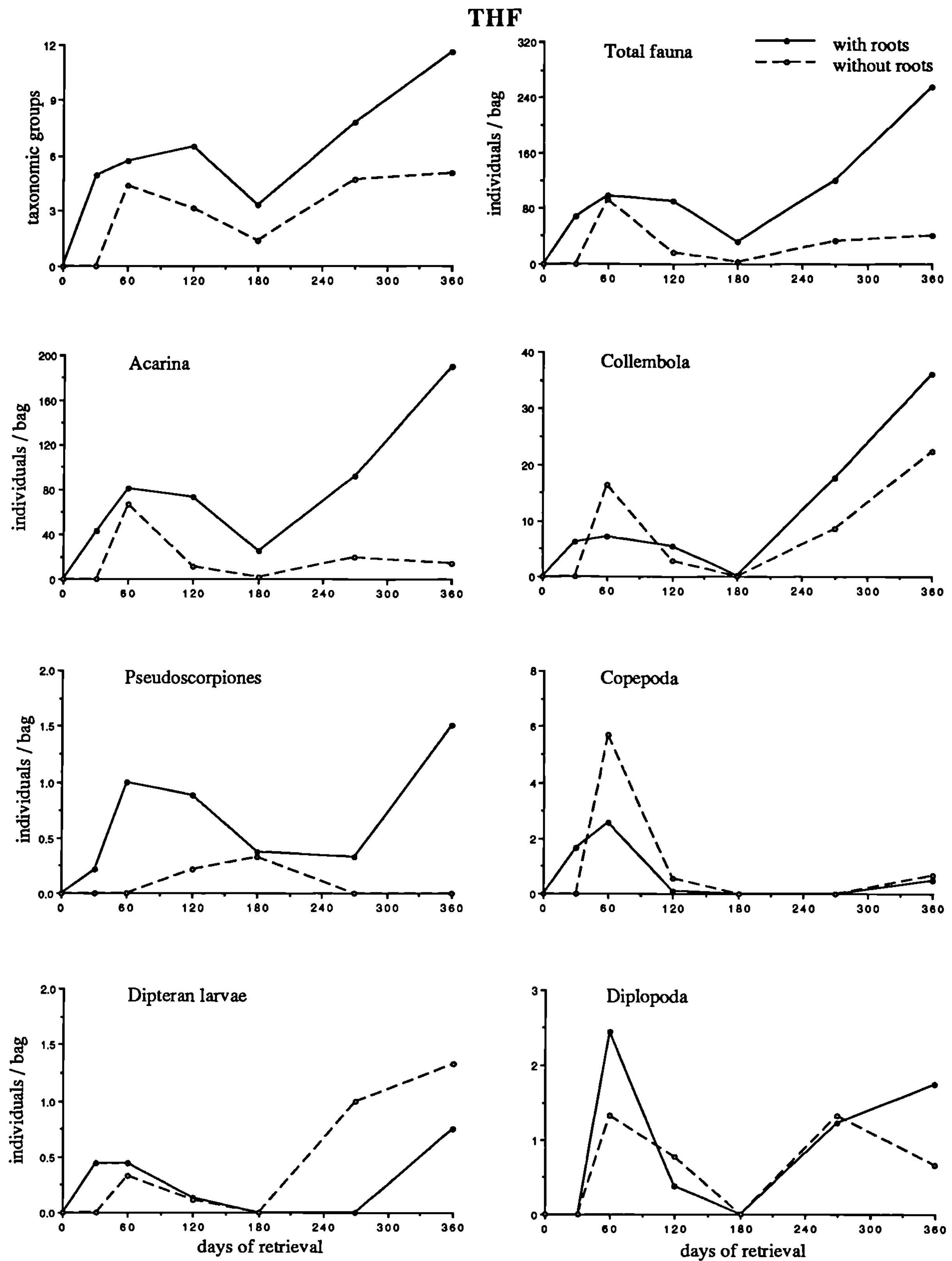


Fig. 5.13: Mean numbers of taxonomic groups, total faunal density (number of individuals per bag) and densities of the main taxonomic groups for each retrieval in the bags with roots and in the bags without roots in the THF. Each of the three replicate plots had three bags retrieved each time and hence n=9.

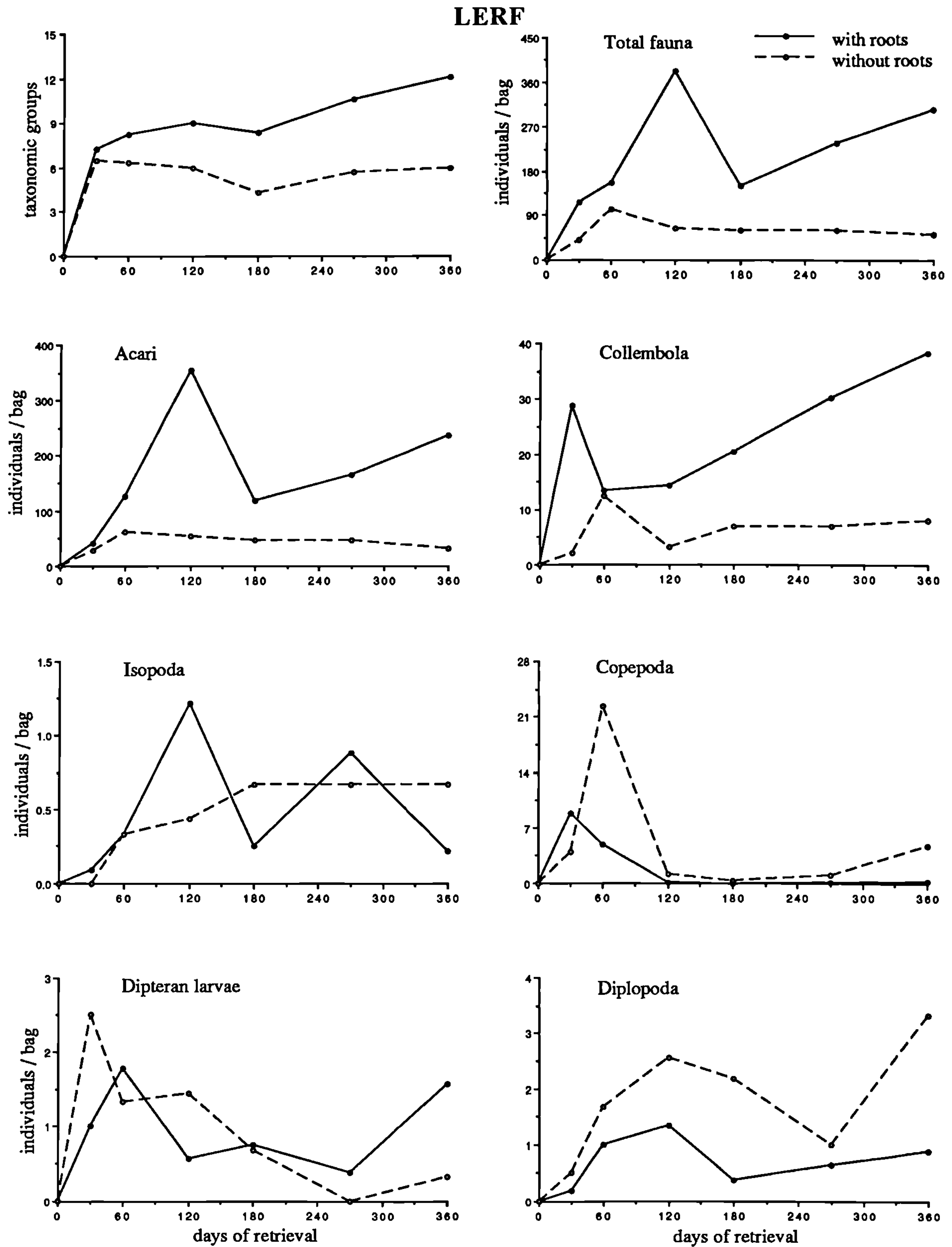


Fig. 5.14: Mean numbers of taxonomic groups, total faunal density (number of individuals per bag) and densities of the main taxonomic groups for each retrieval in the bags with roots and in the bags without roots in the LERF. Each of the three replicate plots had three bags retrieved each time and hence n=9.

DISCUSSION

The influence of roots on decomposition

Independently of the roots, the rates of leaf-litter decomposition for *Clitoria racemosa* on spodosols found in this study were lower than in the LERF, and much lower than former studies with the same species in forests on oxisols (Luizão & Schubart 1987). This fits in the general view that on spodosols, nutrient dynamics are slower (Vitousek & Sanford 1986; Medina & Cuevas 1989). The leaves apparently had to undergo some decomposition before fine roots became attached to them and that stage was achieved earlier in the wet season experiment. In the dry season experiment, roots only started to develop when the rains began. A similar effect was observed by Klinge & Herrera (1978) for a Tall *Caatinga* (≡ THF) in Venezuela.

The results obtained in the experiment 3 where inverting the bags had no significant effect on litter mass nor rates of release of elements shows that the treatment used in experiments 1 and 2 was suitable to assess root influence on decomposition.

In experiment 2 in SHF, where only a small mass of roots was produced in the litter bags, eight elements showed differences in their rate of release between treatments. It is noteworthy that only calcium and aluminium of the eight elements showed differences in release rate which were clearly related to the presence of roots, since in plot 1 root growth was higher than in the other two plots of the SHF. The other six elements showed similar release rates to those measured in plots 2 and 3. The other elements were probably

more influenced by the seasonal effects acting differently in the two treatments. Since it was the dry season in the first half of experiment 2, leaching was not strong and there was a possibility of a higher input of contaminant residues from the litterfall (Luizão & Schubart 1987). The leaves in the undisturbed bags (with roots) were more likely to accumulate elements than the bags that were lifted and inverted weekly. Of the six elements which showed differences between treatments in SHF, five had a slower rate of release in the bags with roots. In experiment 1, where leaching was likely, only aluminium and potassium accumulated in the bags with roots. Thus, it is concluded that for *Clitoria* leaves placed in the more open SHF there might have been some effect of lifting the bags, especially in the drier period.

A feature common to all forest types in experiment 1 (dry season) was the significant contribution of roots to the accumulation of iron and aluminium, which reached higher than initial concentrations. Recently, Lodge *et al.* (1994) suggested that in acid soils of tropical forests where microbial biomass is dominated by fungi, a recycling of nutrients from partially decomposed litter into fresh litter occurs, because of the susceptibility of the litter fungi to drying and also because nutrients can be recycled and stored at high concentrations by fungi. Since most of the roots in the SHF and in the root mat of the THF and LERF were highly mycorrhizal with either vesicular-arbuscular or ectomycorrhizal associations (Chapter VII), they may have been responsible for the accumulation of some nutrients. Lodge *et al.*'s (1994)

suggestion was mainly concerned with macronutrients, but seems here to apply to aluminium and iron.

In experiment 2 (dry season), aluminium and iron persisted and accumulated in the bags with roots in the SHF and the LERF. Potassium was the only element that was generally released slower, in all forest types, in the bags with roots.

Root mat development has been viewed as an important mechanism to enhance nutrient conservation in Amazonian forest ecosystems on poor soils where the roots absorb nutrients released from decomposing organic matter (Medina *et al.* 1978). However, in this study, neither nitrogen nor phosphorus were released faster where roots were present. In fact, nitrogen showed no significant differences between any of the treatments or forest types. The only instance where phosphorus release was different between treatments was in experiment 2 in the THF, where it showed a slower, rather than faster release in the presence of roots. In this study, faster release possibly caused by root uptake, was observed for calcium, magnesium, boron, manganese and zinc in both the THF and LERF. This is in marked contrast with Cuevas & Medina (1988) who found that only nitrogen was apparently a limiting nutrient and hence was quickly taken up by the roots in the Tall *Caatinga* (≡ THF) forest whereas in a *Tierra Firme* forest (≡ LERF) roots from the mat adhered to the litter and promoted the release of calcium and magnesium. In conclusion, the alleged role of fine roots (and their fungal associates) in the efficient recovery of minerals during decomposition (St John 1983) is only partially confirmed. The results in this study showed that

fine roots in the litter bags contribute to the accumulation of some elements and the release of others. In the present study despite the impact roots had on element release or accumulation, no differences were found in the mass loss of the leaves.

Roots and litter animals

The abundance and frequency of some of the litter animals colonizing the decomposing leaves in the litter bags were different in the three forest types and in the two treatments. In contrast to the negligible influence of the roots in the leaf litter decomposition, it was expected that physical disturbance would have a greater influence on litter animals. Hence, the inherent difficulty in separating the effects of the absence of roots from those of disturbance, on animal abundance. It is noteworthy that in THF and LERF which produced more roots (Chapter VI), the bags without roots had many fewer litter animals than in SHF where few or even no roots were found. This suggests that root presence is causal for animal abundance. Some groups of animals are colonizers, others are decomposers and feeders on the roots growing attached to the leaf litter. St John *et al.* (1983) observed that when both roots and vesicular-arbuscular mycorrhizal hyphae (see Chapter VII) proliferate locally, as in litter bags, this event triggers the colonization of the soil biota. Thus, the presence of roots seems to increase the activity of some litter animals.

As shown by the temporal distribution of the litter animals, particularly the marked drop in densities at the 180-d retrieval (the start of the dry season)

their abundance was related to climatic factors. Fluctuations in the densities of some animals were synchronous suggesting a common factor influencing them, possibly related to stages of decomposition as well as climate. Similar results were found by St John *et al.* (1983) who observed similar patterns of abundance of soil organisms.

Overall, the densities of litter animals were higher in the LERF than in both THF and SHF and, all forest types had more animals in the bags with roots than those without. However, in the LERF, one group, the Diplopoda was exceptional. Diplopoda were more abundant in the bags without roots than with roots. The relatively higher frequency and density of Diplopoda, important litter decomposers, in the LERF could be related to the higher mass of humus on the forest floor (Petersen & Luxton 1982). The Diplopoda are geophagous (Coleman, 1985) and are not dependent on roots but they are highly dependent on moisture for their activities. Although moisture content in the litter-bags was not measured, it is well known that fine roots are good water absorbers (Persson 1980) and may compete with litter animals for water. Under these circumstances, animals which depend on high moisture, such as Diplopoda, may be excluded by roots.

The abundance of the total litter fauna was highly influenced by that of the two most abundant groups, Acari and Collembola, which were present in the large majority of the litter bags in both treatments at any time of the experiment. Collembola generally have small populations in tropical forests, but when a thick organic topsoil is present, densities can be much higher (Goffinet 1975). However in this study, Collembola always showed the

highest relative abundance in SHF, the most open and dry forest type. The opposite was found for Acari, which broadly agrees with reports that Acari are more numerous in forests than in non-wooded areas (Ribeiro & Schubart 1989). Collembola and Acarina generally live on litter surfaces and in air-filled soil micropores in the soil (Wallwork 1976). Most are non-specific feeders (Anderson 1975), but both the Oribatei mites, the predominant class of Acari in Central Amazonian forests (Ribeiro & Schubart 1989), and Collembola are primarily fungivorous, feeding on organic debris and associated fungi. Smaller species of Acari feed more selectively on fungi (Anderson 1975) which are usually associated with roots (see Chapter VII) and this explains the higher abundance and frequency of Acari in the treatment with roots. Formicidae were the third most abundant litter animal group in all forest types and had a significantly higher density in the bags with roots in LERF. Tropical ants usually are in great abundance and have a wide variety of food types and nest sites (Levings & Windsor 1982). They are considered as the dominant predators and competitors for resources in tropical lowland forests (Olson 1994). Their low frequency and abundance in the treatments without roots in the THF and especially in the LERF suggests that somehow this group is affected by the absence of roots, possibly the absence of a prey which depends on roots.

Pseudoscorpiones, a group of predators of litter animals, were always, among the five most frequent groups in the bags with roots and in those without roots they were among the ten most frequent groups. These observations agree with studies showing relatively high numbers of Pseudoscorpiones

present in forests (Adis 1988; Blair *et al.* 1992). As a typical non-specialized predator of the litter fauna, the densities of Pseudoscorpiones followed closely the pattern recorded for the total litter fauna.

In conclusion, roots seems to have an indirect influence on litter animals through stimulation of microbial activity on decomposing leaves by adding assimilable substrates (Lavelle *et al.* 1993), and hence attracting litter animals (many fungivorous or bacterivorous).

Chapter VI. Fine Root Growth in SHF, THF and LERF.

INTRODUCTION

The amount, form and distribution of nutrients in the soil influence root growth and its distribution. The roots in turn provide a direct input of organic matter and mineral nutrients through exudation and death (Singh *et al.* 1989). Fine roots make up most of the length of a root system (Persson 1980) and their importance for nutrient cycling outweighs their relatively small contribution to the total root biomass (Sanford 1989). Fine roots have been held to represent a large and dynamic portion of the below-ground biomass and nutrient capital and a significant part of net primary production in many forests (McClaugherty *et al.* 1982). In contrast, however, Green (1992) found that fine root turnover was less important than the above-ground small litter production in supplying organic matter to the soil in the lowland evergreen forest at Danum, Malaysia.

Gower (1987) hypothesised that fine root biomass is governed by the mineral nutrient that is limiting in a forest ecosystem, particularly phosphorus or calcium or both in many tropical forests. In Amazonia, Cuevas & Medina (1988) testing that hypothesis, demonstrated that the roots in a lowland evergreen tropical rain forest on an oxisol proliferated vigorously in an inert growth medium placed on the root mat in response to additions of calcium or phosphorus. In a heath forest (Tall *Caatinga* ≡ THF) on a spodosol the

roots in the same treatment responded added nitrogen. They found no difference with added nitrogen in root growth in the inert growth medium when it was placed in the soil.

Heath forest spodosols are frequently of coarse texture, poorly buffered and highly acidic (Tables 2.7 and 2.8; Klinge 1965; Bravard & Righi 1991) and are usually considered to be among the most nutrient-poor soils. However, accumulating evidence has been shown that when the organic layers were considered in the soil analyses of heath forests in south-east Asia they have either a similar or even higher concentration of nutrients than the soils of neighbouring lowland evergreen rain forests with higher biomass (Proctor *et al.* 1983; Whitmore 1989). In this study, as shown in Table 2.7 for the SHF and 2.8 for the THF, though the mineral horizons are poor in nutrients, Table 2.10 shows that other forest soils elsewhere are even poorer. Although the mineral horizons, in the case of the sites under study were low in nutrients, the organic horizons above them had a much higher nutrient concentration.

Recent studies on nutrients in the sandy soil and litter on Maracá Island, northern Brazil, found no evidence that nutrients were limiting either plant growth in the forest nor seedling survival and growth in gaps. (Scott *et al.* 1992; Thompson *et al.* 1992). Though these forests experienced a long dry season, no drought effect was observed. Thus, since drought is not a consistent pattern it is unlikely to be the cause of heath forests. The most consistent difference between heath forest soils and other forest soils is the

soil acidity, with a pH of much less than 4.0 for most heath forest soils (Whitmore 1990).

Though the causal factors are not well established, heath forests are characterized by low stature and biomass, and accentuated sclerophylly (Klinge 1965; Lisboa 1975). These characteristics of the vegetation associated with a slower decomposition (Chapter V) lead to a deep accumulation of organic matter on the soil surface. A feature of that organic matter is the root mat, a well developed net of superficial roots which constitutes a substantial fraction of total root biomass (Klinge & Herrera 1978). The root mat is often believed to be one of the mechanisms for nutrient conservation owing to its apparent efficiency in extracting nutrients from decomposing leaves (Herrera *et al.* 1978). In the lowland evergreen rain forests on both oxisols or ultisols, the roots grow more intermixed with the litter layer (Stark & Jordan 1978). But, a root mat in the LERF soils is not a universal feature as demonstrated by Green (1992) and Thompson *et al.* (1992).

In this study, six different experimental treatments were carried out in the SHF, THF and LERF, aimed at testing the potential limiting effects of different nutrient additions and pH on fine root growth.

MATERIALS AND METHODS

Ingrowth bag technique

The ingrowth bag technique is based on the ability of roots, through their plasticity, to exploit episodes of mineral nutrient availability (Lund *et al.*

1970). The technique uses bags in which a root-free volume of soil or other medium, which has imbibed the solution under test, is placed in or on the soil to estimate fine root growth. This method is particularly useful in allowing within-site comparisons among treatments applied to the growth medium (Steen *et al.* 1991). Nevertheless it has been pointed out that a limitation of this technique is the effect on the root proliferation of the quality of the inert growth medium (usually vermiculite or perlite) onto which adsorbed nutrients supposedly remain available to plants (Cavelier 1989).

Methods

In this study, both medium-sized (3-6 mm particles) vermiculite and sand were used as growth media. The sand was collected from the open SHF site, because it was visibly free of roots and humus material, and used as a substrate for all the plots. Rather than the ingrowth cylinders used by Cuevas & Medina (1988), a nylon ingrowth bag of 12 cm x 12 cm and 2-mm mesh was used for this experiment. It was well known from previous studies (Luizão & Schubart 1987) that the 2-mm mesh size of the bags would not prevent root invasion (Fig 5.2). Fine roots were defined as ≤ 2 mm diameter, since in the 70 d of the experiment no roots larger than this were found in any ingrowth bag. Two substrates were used: sand and vermiculite. The bags with sand were placed on the soil surface (after removal of the litter layer); the bags with vermiculite were placed on the soil surface, and in the soil to a depth of 10 cm. There were six nutrient treatments in which the growth

media were imbibed in distilled water (controls) or 0.1 M solutions or suspensions of one of potassium chloride, calcium carbonate, sodium hydrogen phosphate, calcium chloride, or urea. After 24 h imbibition the growth media were placed in the nylon ingrowth bags.

Each plot was divided into four 25 m x 25 m quadrants in which four replicate soil bags (one per quadrant) of each nutrient treatment were randomly placed. In SHF, some of the randomly located positions were in places with bare sand. The bags were left undisturbed for 75 d (all in the rainy season) from 2 January 1993 to 15 May 1993. Then, all the bags were removed, after carefully cutting, at the bag surface, all roots that penetrated the mesh. The bags were taken to the laboratory where they were stored at -18 °C. During the root processing, each bag was thawed, shaved for any remaining outside roots, opened and then all the fine roots in the bags were separated from the soil by sieving and flotation. The residue was carefully checked for any root fragments. Roots were dried at 60 °C to constant weight and then weighed for dry mass.

RESULTS

Overall, there was great variability in the response to the nutrient addition experiment both within and between plots, and for most of the data the standard deviations were close to or even higher than the mean values. Nevertheless, as already observed in Chapter V, independently of either treatment or substrate, significantly less fine root growth was found in the ingrowth bags from the SHF sites ($p < 0.001$) than in those from the other

two forest types. Fine root production was significantly lower ($p < 0.001$) in the ingrowth bags with sand compared with those with vermiculite (Fig. 6.1). In the nutrient addition experiment as a whole, it was observed that the calcium carbonate and calcium chloride treatments caused higher (both, $p < 0.001$) production of fine roots than any other treatments, which in fact showed little or no effect on the root growth (Fig. 6.1). The three plots (nos 1-3) in the SHF showed a different response among each other. Plot no. 1 showed significantly ($p < 0.001$) higher mean production of fine roots than the other two, which had very similar mean values (Fig. 6.1a). In the SHF, the vermiculite bags placed on the soil had a significantly ($p < 0.001$) higher production of fine root growth than the others. Although showing great variability, calcium carbonate, calcium chloride and potassium chloride were the treatments which most increased (all $p < 0.05$) fine root growth in the SHF. In the THF (Fig. 6.1b) there were no differences between plots in the root growth response to the nutrient additions. In this forest type both vermiculite bag positions, either on the litter layer or within the soil, produced similar fine root growth which was significantly ($p < 0.001$) higher than in the samples with sand. Calcium additions were the only treatments which caused significantly higher fine root growth (both $p < 0.001$). In the LERF there were no differences among the three plots in their response to the nutrient addition. However, a significant difference was found for the substrates and bag positions (Fig. 6.1c). The vermiculite bags placed within the soil had a higher production of fine roots compared with the bags placed on the litter layer which in turn produced more fine roots ($p < 0.001$) than the

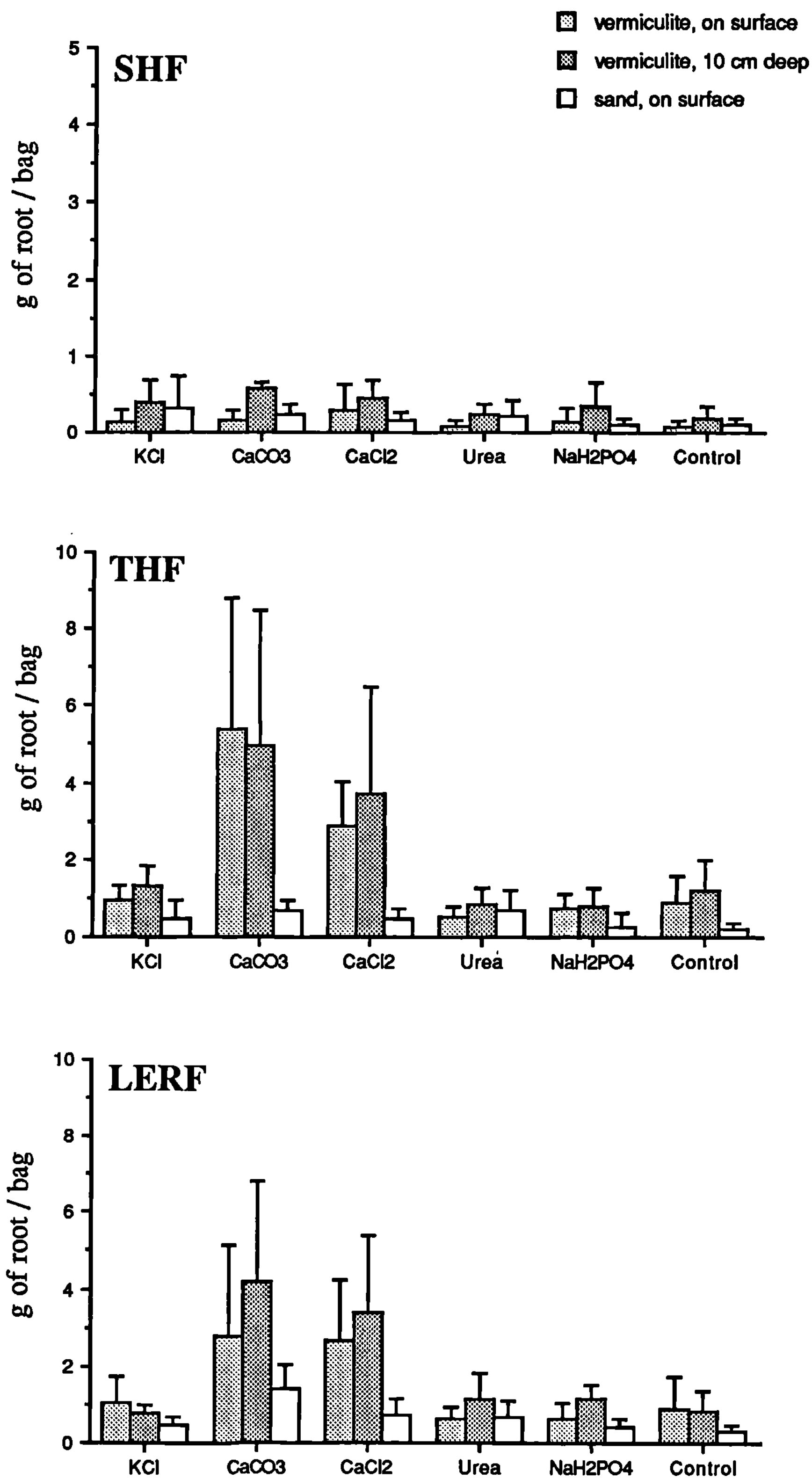


Fig. 6.1: Dry mass of roots per bag in the bags containing vermiculite on the soil surface, vermiculite at 10 cm depth in the soil and sand on the soil surface, under different nutrient additions. Bars are means ± SD of four bags in each experiment and treatment in each of the three replicate plots in the SHF, THF and LERF and hence n=12.

bag containing sand. In the LERF, calcium chloride and calcium carbonate at both depths in the vermiculite were the only treatments which significantly increased (both $p < 0.001$) fine root growth.

DISCUSSION

The high fine root production in THF, when compared with SHF is in contrast with Cuevas & Medina (1988) who found that fine root growth of 120 g m^{-2} yr in Tall *Caatinga* (≡ THF) was lower than the 235 g m^{-2} yr^{-2} in the Low *Bana* (a forest more stunted than the SHF). They credited the unexpected lower root growth in the Tall *Caatinga* (≡ THF) to the occurrence of flooding events which impaired the root production. The very low fine root production in the sand from the SHF may be due to the leaching of the added nutrients from it, since 1440 mm of rain fell during the experiment and, in January, the first month of the experiment, rainfall was an average of 130 mm per week. Calcium status of the SHF, THF and LERF soils was very low and the overall positive response (in all bag positions, media and forest types) to both calcium treatments suggests that fine root growth is restricted by both low soil pH and low calcium. The pH of soils under heath forests, is usually low, even by tropical standards and those of the SHF and THF were not exceptional (see Chapter II). The heath forests previously studied in central Amazonia have strongly acid soils (Klinge 1965; Lisboa 1975). In soils with aluminium ions, pH is controlled by a complex hydrated aluminium ion buffer system which sets a lower limit to pH, with values much below 4.0 (Fitter & Hay 1991). In the SHF where exchangeable aluminium was nearly

zero (Fig 2.7) no pH buffering was expected. Under these conditions, hydrogen ions, are likely to be at high concentrations and directly toxic for most plants (Fitter & Hay 1991). The lack of difference between the effect of calcium carbonate and calcium chloride addition on the root mass in the LERF site as opposed to the THF is likely to be a result of the initially higher soil pH and better calcium status of the LERF (Tables 2.7- 2.9). However, Luizão (1995) also working on the soils in the SHF, THF and LERF plots, testing possible nutrient limitation for seedling growth in both field and glasshouse, found a significant positive effect of calcium carbonate addition, but a significant negative effect of calcium chloride, in all. Although Luizão's (1995) results are difficult to square with those found in this study it is worth pointing out that increased fine root production does not necessarily imply increased plant growth (E.V.J. Tanner, personal communication). Plants might respond to fertilization by first increasing root production and then only after the root biomass reaches a level of equilibrium with the soil will they show any above-ground increase (Silver 1994). The differences in the results may also be related to the fact that most of the roots penetrating the bags originated from mature plants (not seedlings) which might respond differently to nutrient addition.

The results of this study contrast with that of Cuevas & Medina (1988), working in different types of rain forest (*Tierra firme*, Tall *Bana* and Low *Bana*) at San Carlos, Venezuela. Although their *Tierra firme* showed some similarities to the LERF and Tall *Bana* to the THF they found significant differences among treatments only when the bags were placed on the soil

surface and not when they were placed in the soil. In *Tierra firme*, root growth responded positively to potassium hydrogen phosphate and calcium chloride and, in Tall *Bana* fine root growth was stimulated by ammonium chloride addition only. Proctor (1992) pointed out that owing to the very poor root growth in the unfertilized treatments of Cuevas & Medina (1988) their study did not allow them to reach firm conclusions about the limiting nutrients in the *Tierra firme* and *Bana* forests. Their data lend no support to the view that nitrogen, phosphorus or potassium are limiting in lowland evergreen rain forests, which confirms the results of the present study.

The results obtained here agree with Whitmore (1990) whose conclusions were that heath forests in general are mainly limited by low pH. In the present study however, the pronounced effect of added calcium on fine root production in the SHF and THF emphasizes the importance of the low availability of calcium as a nutrient. Lack of response of fine roots to nitrogen, phosphorus and potassium addition is not a surprise since several recent tropical studies have shown similar results. Green (1992) found no effect of nitrogen, phosphorus and potassium on fine root growth in a tropical lowland rain forest in Danum, Malaysia, nor Pendry (1994) in a tropical lowland rain forest in Belalong, Brunei. However, J. Proctor (unpublished) working in an evergreen lowland rain forest and in heath forest in Barito Ulu, Indonesia, found that phosphorus significantly increased fine root growth in a dipterocarp forest, but not in the heath forest.

Chapter VII. Occurrence of Mycorrhizal Associations in SHF, THF, and LERF.

INTRODUCTION

Mycorrhizal associations are ubiquitous on tropical trees. Fungi associated with plant roots in mycorrhizal symbioses are mostly Basidiomycetes or Zygomycetes (Harley & Smith 1983). It has been suggested that 71% of tropical plants form vesicular-arbuscular mycorrhizas (VAM) (Sieverding 1991) and, although surveys have been very limited about 95% of tree species in natural tropical forest ecosystems are thought to be mycorrhizal exclusively with VAM fungi (Le Tacon *et al.* 1987). Mycorrhizas provide an intimate link between the soil environment and the functional nutrient absorbing system of the plant root. Mycorrhizal associations are thought to be beneficial to plants in several ways, including: improving transport and storage of nutrients, improving water diffusion from the soil, and providing protection against root pathogens (Read *et al.* 1985). Many believe their key role is in improving the acquisition of immobile ions, particularly phosphorus (Stribley & Read 1974; Tinker 1975; Herrera *et al.* 1978). Enhanced nutrient uptake is attributed to the extensive, well-distributed absorbing surface provided by mycelia which explore the soil beyond the root hair and its phosphorus depletion zones (Stribley & Read 1974; Tinker 1975). Others, however, argue that there is little evidence under field conditions of VAM functioning as a bridge between roots and soil phosphorus since most of the

results are based on experiments carried out under artificial conditions (Fitter 1986). It has been suggested that under field conditions the cost of the mycorrhizal association to the plant could outweigh the benefits (Fitter 1977; Koide & Elliott 1989). VAM do impose a carbon drain on the host. Fitter (1986) found that in one of his test sites the application of fungicide to kill VAM fungi increased plant nitrogen and phosphorus concentrations proportionally to the reduction in infection whereas in another site he observed both deleterious and beneficial effects apparently related to the soil water status. He concluded that VAM infection is only beneficial to plants under certain environmental conditions. Accordingly, as suggested by Allen & Allen (1986) it may be that mycorrhizas have advantageous effects on plants only during times of stress. In their study, effects of mycorrhizas on stomatal resistance were observed only during drought. In such a relationship, where the roots of the host plants and the mycorrhizal fungi do not always derive mutual benefit, the mycorrhizal fungi may often function as highly specialized parasites (Jackson & Mason 1984, Fitter 1986, Allen 1991). No doubt a better understanding of the carbon costs of the mycorrhizal association is necessary to fully assess the degree of its benefit to older, mature plants in natural ecosystems (Reid 1990).

Studies in Amazonian ecosystems have suggested that the presence of roots and hyphae in the organic layers or in the upper soil contributes to the efficient cycling of minerals (St John 1985). Three main factors influence the distribution of mycorrhizas in Amazonian forest soils: the plant species, the soil type and the depth of the roots. In the ultisols of Central Amazonia,

there is little or no accumulation of humus at the soil surface. Most fine roots are found in the upper part of the mineral soil and mycorrhizal infection of all kinds is less intense than in the organic layers of spodosols (St John 1985). Other distinctive types of soil supporting characteristic forests in Amazonia are the spodosols, some of which are seasonally flooded, often overlain by a layer of humus and leaf litter. Most of the fine roots form a distinct layer, sometimes termed a 'root mat'. In these forests almost all species are mycorrhizal; many of them ectomycorrhizal (ECM) (St John 1980). From a mycological point of view Singer & Araújo (1979) classified the forests in Central Amazonia as ectotrophic and anectotrophic. Ectotrophic forests *e.g.* heath forests on spodosols are dominated by ECM trees; anectotrophically mycorrhizal forests *e.g.* lowland evergreen rain forests on oxisols, have only scattered or no ectomycorrhiza. It has been suggested that ectomycorrhizal fungi inhibit litter-decomposing microorganisms, leading to litter, and later raw humus accumulation on the forest floor as a consequence of ectotroph dominance (Singer & Morello 1960; Singer 1984). Gadgil & Gadgil (1975) showed that for *Pinus radiata* forest there are higher rates of litter decomposition in the absence of ectomycorrhizal roots. Thus, leaf-inhabiting litter fungi in both dry and wet seasons in *Campinarana* (≡ THF) are fewer than in the forest on oxisols. Ectomycorrhizas are especially associated with common trees found in THF such as *Aldina heterophylla* (Leguminosae) and *Pradosia schomburgkiana* (Sapotaceae). Scleromorphism with toxic substances (Lisboa 1975) in the litter and ectomycorrhizal

predominance have been the main reasons given for the litter accumulation in *Campinarana* (≡ THF) and *Campina* (≡ SHF).

In forest systems with predominantly ectomycorrhizal fungi, they are mainly present in the roots developed in the surface horizons of the soil, especially in the humus layer (Harley & Smith 1983). The deepest roots in the soil, are rarely infected (St John 1983). According to the Janos (1983) model, VAM species are abundant in relatively fertile soils but ectomycorrhizal species become more competitive at low fertility. Nonetheless the VAM species of the great majority of tropical forest trees, which include many valuable species, are virtually unstudied (Mason & Wilson 1994).

The interest in mycorrhizal fungi has led to intensive research on the structure and function of the fungal community itself. Identifications of mycorrhizal spores across gradients and serial chronosequences have shown shifts with changes in plant species and changes in species composition and abundance of mycorrhizas between seasons and years (Allen & Allen 1990; Wilson *et al.* 1992). These changes may be important for community level processes because different species of fungi are known to confer different physiological responses on the same plant species, including different rates of growth, nutrient uptake, and water uptake (Vogt *et al.* 1982; Whittingham & Read 1982).

In general there is no association between spore numbers and infection development for individual fungal species since species of arbuscular mycorrhizal fungi differ in the numbers of spores produced and different hosts give different spore numbers (Abbot & Robson 1982). The relationships

between colonization of roots and spore formation, and between spore distribution and abundance and subsequent formation of the mycorrhizal association is uncertain (Abbot & Gazey 1994). Thus, generalizations about the importance of numbers of spores may be difficult to make since various species may produce different numbers of spores under similar conditions (Sylvia 1986).

The objective of the present study was to make a preliminary survey of the species of the VAM fungi occurring in the three forest types without taking into account spore abundance.

MATERIAL AND METHODS

Soil sampling

On 10 June 1994, the period of transition between the wet and dry seasons, samples of the top 20 cm of soil, which included all the organic layers of the profile, were collected in all three plots in each of SHF, THF and LERF. For each plot, three random points were selected and a square corer of 50 mm X 50 mm was used to collect the soil. The three samples were pooled together to make one composite sample for each plot, since only a spore identification exercise was undertaken.

Spore extraction and identification

Two extractions were made from each plot sample of about 100 g of soil. Spores were extracted using the sucrose centrifugation technique (Walker *et*

al. 1992). Briefly, the technique consists of wet sieving the soil with tap water through a sieve of 700 µm to remove roots and other organic debris. Everything passed in the first sieve was then filtered through a 45 µm sieve where the spores were trapped. The resulting mixture was centrifuged at 900 **g** (gravity) in water for 4 min after which the water in the tubes was poured out. The tubes were then filled with 48% sucrose solution and subjected to another centrifugation for 15 sec. The supernatant water/sucrose solution containing the spores was poured into small fine (45 µm) sieves, rinsed and placed into Petri dishes to be examined. In addition, roots left on the first sieve (700 µm) of each extraction were briefly examined for the presence or absence of ectomycorrhizas. However, owing to time restrictions, no further attempt was made to identify the species of fungi or the rate of infection in these 'first-sieve' roots.

Spores were suspended in water for an initial examination under a dissecting microscope. Illumination was by incident light from a quartz-iodine fibre-optic source. During the examination, dead spores were recognized by either being empty (lacking cytoplasm) or parasitized showing holes in the outer wall. Only spores that seemed to be alive and in good condition were selected for identification. Spore colour was described from freshly extracted spores which were suspended in water in plastic Petri dishes and illuminated by the same light source used for examining the spores. Colour was determined by comparison with a standard colour chart (Anon. 1969). Specimens were mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske & Tessier 1983) with and without Melzer's reagent (Morton 1986) for further study under a

compound microscope. All the identifications were done using the collection of spore descriptions provided by the Mycorrhiza Research Unit at the Forestry Commission (Roslin). The spore photomicrographs were taken using a Polaroid camera coupled to a Zeiss compound microscope. The films were colour Polaroid instant pictures with a print size of 8.5 cm x 10.8 cm.

RESULTS

Examination of the roots under the dissecting microscope revealed that from all plots there were roots infected with ectomycorrhizal fungi, most of them basidiomycetes. These fungi were of several species. One of them, *Cenococcum geophilum* Trappe, was very easy to recognize by the infected root tips which have the appearance of a burnt match and by the black sclerotia of various sizes which are conspicuous in the extracts (Trappe 1969). Also, *Amanita* sp. was frequently observed in the samples LERF plots.

As expected from field material, most of the extracted VAM spores were dead. A general view of the extracts showed that VAM spores were low in both numbers and diversity in all forest types, especially in the SHF and THF. No quantification of total species numbers was made however. Since samples from field material do not show developmental sequences of spores, proper identification to the species level was more difficult (Walker 1992). Nevertheless, a selection of micrographs from the specimens which allowed some identification are shown in the Figs 1 - 16. Although the spore types found will not represent the full range of species for each of the forest sites, they may give an indication of trends in diversity. The colour in the

illustrations are somewhat different from those of the actual specimens because of the photocopying. However, accurate colour descriptions are given in the text by reference to the standardized colour chart.

Among the spore types recovered from SHF soil (Figs 1 - 4) there were of two previously undescribed species (C. Walker personal communication) belonging to the genus *Scutellospora*. This genus is characterized by a swollen hyphal tip called a bulbous hyphal attachment (Nicolson & Gerdermann 1968). The spores germinate by means of a germination shield formed in an inner wall group of membranous or coriaceous walls. Many species in this group have ornamented walls (Koske & Walker 1985). Fig. 1 *Scutellospora* 'rugose' (provisional name) is an undescribed species which produces small pale ochraceous no. 6 (Anon. 1969) spores which are comparatively small for the genus (measuring 100-128 µm x 114-134 µm). The species resembles *Acaulospora* spp. in its spore size but the bulbous cell and the germination shield is typical of the genus *Scutellospora*. The spore has three wall groups (Walker 1983) with the outermost wall (called a unit wall) ornamented with rugosities. There is also a laminated structural wall as part of the outer wall group. In the intermediate group there is a membranous wall while the inner group shows an amorphous wall which turns purple with Melzer's reagent.

Fig. 2 shows another, apparently undescribed, species of *Scutellospora* 'smooth' (provisional name), characterized by a pale ochraceous no. 6 (Anon. 1969), hyaline, ovoid smooth spore, with a size range of 163-210 µm x 142-210 µm. In contrast with the *Scutellospora* sp. in Fig. 1, this species had no

ornamentation in the outer wall but this wall showed a positive Melzer's reaction.

Fig. 3 shows an apparently undescribed *Glomus* sp. belonging to the *G. maculosum* group (Walker 1992). This group of species which includes *Glomus fistulosum* Skou & Jakobsen, *Glomus claroideum* Schenck & Smith, and *Glomus maculosum* Miller & Walker is characterized by showing two groups of walls (Walker 1983), with the first group being two-layered, an outermost wall, usually evident in the young spores, which disappears when it matures. The opposite happens to the innermost flexible wall that becomes more obvious in mature spores. The three tiny ovoid spores recovered from SHF soil were in a loose cluster, with colour corresponding to ochre no. 9 (Anon. 1969) and size range from 55-63 µm x 41-47 µm. There were so few specimens of these spores that a proper characterization of the species was impossible.

Fig. 4 shows a different species of the *G. maculosum* group. The spore is lighter in colour than the one described in Fig. 3, corresponding to the pale ochraceous no. 6 (Anon. 1969), and is globose in shape and bigger in size, measuring 81-93 µm x 82-93 µm. At this higher magnification picture both the flexible inner wall and the breaking outermost wall are evident. In contrast with the *Glomus* sp. shown in Fig. 3, this species was the most frequent in SHF at the time of the collection, especially in plot 1, which had the highest vegetation cover.

Figs 5 - 8 show some of the spores recovered from THF. Fig. 5 shows the only spore of *Scutellospora tricalypta* (Herr. & Ferr.) Walkers & Sanders

recovered from THF soil and it is the first record of *S. tricalypta* since its original description from various sites in Cuba (Ferrer & Herrera 1981). The spore wall is composed of three easily separable walls characterized by the dark colour and ornamented appearance of the outer wall. The ornamentation is made of small yellowish spines. The specimen found in this study was lighter than those described by Ferrer & Herrera, being cream no. 3 (Anon. 1969) and measured 367 µm x 356 µm. Interestingly, one of the sites from where it was previously recovered (Ferrer & Herrera 1981) is described as "white sand with a lot of organic matter" which corresponds with the broad description of the THF.

Fig. 6 shows an undescribed *Scutellospora* sp. easily identified by its small germination shield. From the four specimens recovered from THF soils, three were ellipsoid in shape with a lateral attachment of the bulbous cell and hyaline, with the colour corresponding to sulphur yellow no. 55 (Anon. 1969). The transparency of the wall reveals the spore interior and makes the germination shield very obvious. The spores are very similar to one found in the *Cerrado* (wooded savanna) forest in Planaltina, Brazil and currently under study by M.J. Miranda & J.L. Spain (C. Walker, personal communication). Fig. 7 shows the fourth specimen of the *Scutellospora* sp. described in Fig. 6, which was globose in shape but showed the same characteristics already described in Fig. 6.

Fig. 8 shows an unusual type of secondary formation, auxiliary cells, possibly from a *Gigaspora* spp. These structures are occasionally formed within roots, but such an occurrence is rare. The doubt on the identity of the genus comes

from the fact that the auxiliary cells in the only two genera known to form them (*Scutellospora* and *Gigaspora*), are described as having knobby and spiny shapes, respectively. The cluster of cells found is this study is different. Unfortunately, the centrifugation used in the extraction is damaging to the big and fragile spores such as those of *Gigaspora*. In an attempt to avoid this damage several simpler extractions (shaking a small amount of soil into a glass of water and filtering it through a 45 μm sieve) were made, but no spores were found.

In addition to the spores, the soil extracts from all plots in THF, had very many small greenish bubbles of organic compounds, possibly polyphenols, released by the vegetation, suggesting a high concentration of these substances in THF.

Figs 9 - 16 show some of the spores recovered from LERF soil. Fig. 9 shows part of a spore-cluster of a sporocarp of *Glomus microcarpum* Tul. & Tul. This is a young sporocarp since the laminated wall of the spores is still thin with not many laminae. With age the wall becomes thicker and there are many visible laminae. Three sporocarps of this species were recovered from LERF soils. The main characteristic of the sporocarps is the lack of an erect, pointed hyphal aggregation on their surface (Gerdermann & Trappe 1974). The individual spores found in LERF were usually globose, measuring 57-60 μm x 57-60 μm with colour corresponding to the ochre no. 9 (Anon. 1969).

Fig. 10 shows *Acaulospora foveata* Trappe & Janos, clearly identified by its main characteristics, the thick walls and the pits of the outermost ornamented wall. The three specimens found in LERF soil measured 189-203

μm x 200-247 μm, with a colour corresponding to sienna no. 11 (Anon. 1969), which agreed with the description by Janos & Trappe (1982) of *A. foveata*, first recovered from tropical forest soils in Mexico, Costa Rica and Panama. Spores are globose to ellipsoid with sizes usually between 185-310 μm x 215-350 μm and yellowish-brown to light reddish brown in colour, and usually found without an attached hypha. *A. foveata* is readily distinguished from other species with a pitted surface by its larger pits and lack of distinctive reaction of the inner wall layer to Melzer's reagent (Janos & Trappe 1982). Fig. 11 shows a spore of *Acaulospora* sp., possibly *Acaulospora longula* Spain & Schenk, judging by its size (200 μm x 235 μm) and the reaction of the inner wall to Melzer's reagent. However, since this was the only specimen from LERF soil, no proper identification was possible.

Fig. 12 shows an unidentified spore. This specimen, shows some of the characteristics of the *Glomus* and *Acaulospora* genera. There is an inner wall, which although similar in some ways to the *G. maculosum* group (Walker 1992), is much thicker and more like *Acaulospora* spp. The spore base is apparently just vestigial hyphae similar to *Acaulospora nicolsoni* Walker, Reed & Sanders but also similar to some *Glomus* spp which have lost their subtending hyphae. There was no reaction to Melzer's reagent, a feature of *Glomus* spp.

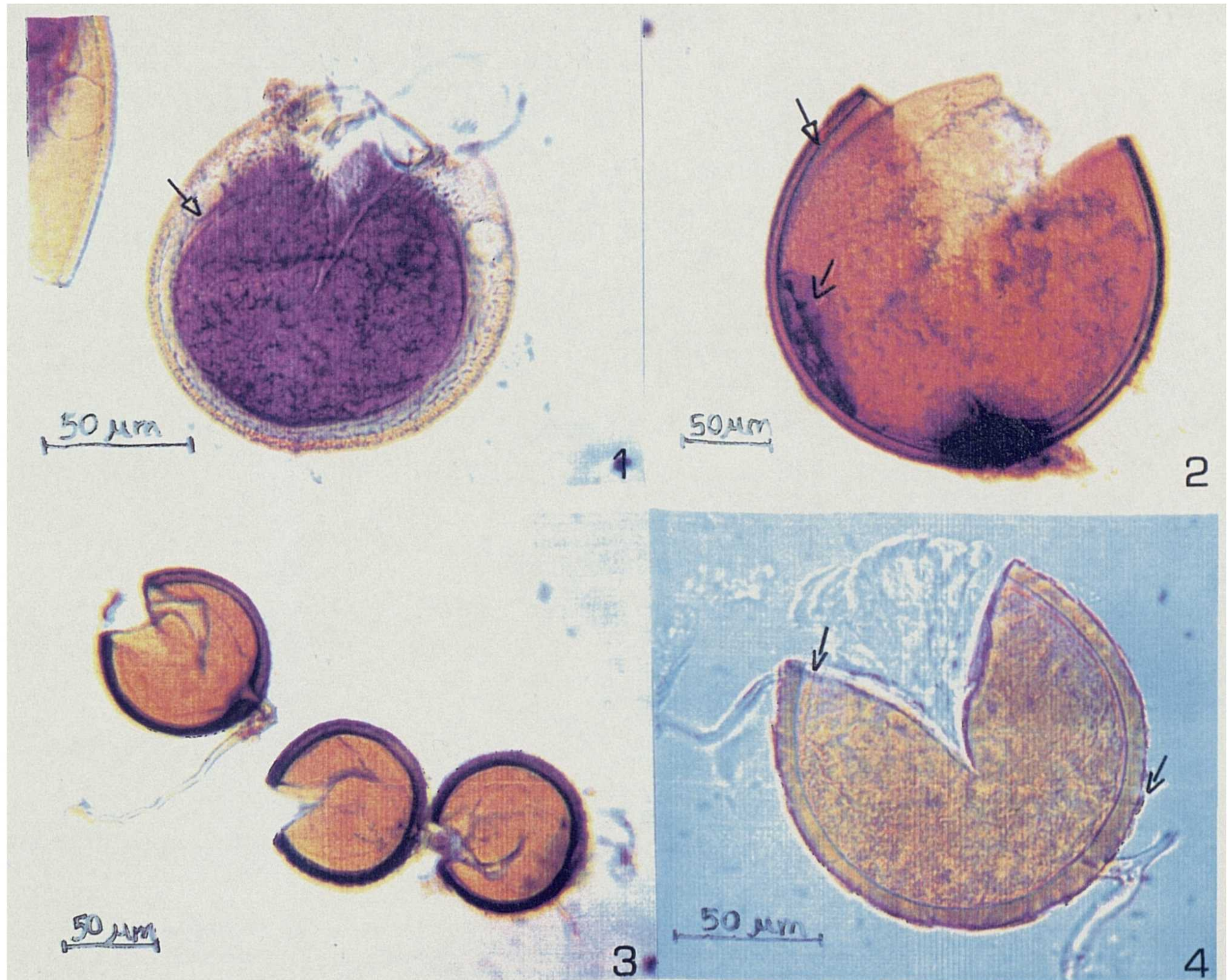
Fig. 13 shows a broken spore of *Glomus mosseae* (Nicol. & Gerd) Gerdemann & Trappe, a species characterized by a funnel-shaped base and a two-layered wall; the outer wall is hyaline and the inner wall laminate and coloured (Walker 1992). The specimens found in the LERF soil were ochraceous no.

8 (Anon. 1969), measuring 80-99 µm x 95-105 µm without including the hyphal attachment.

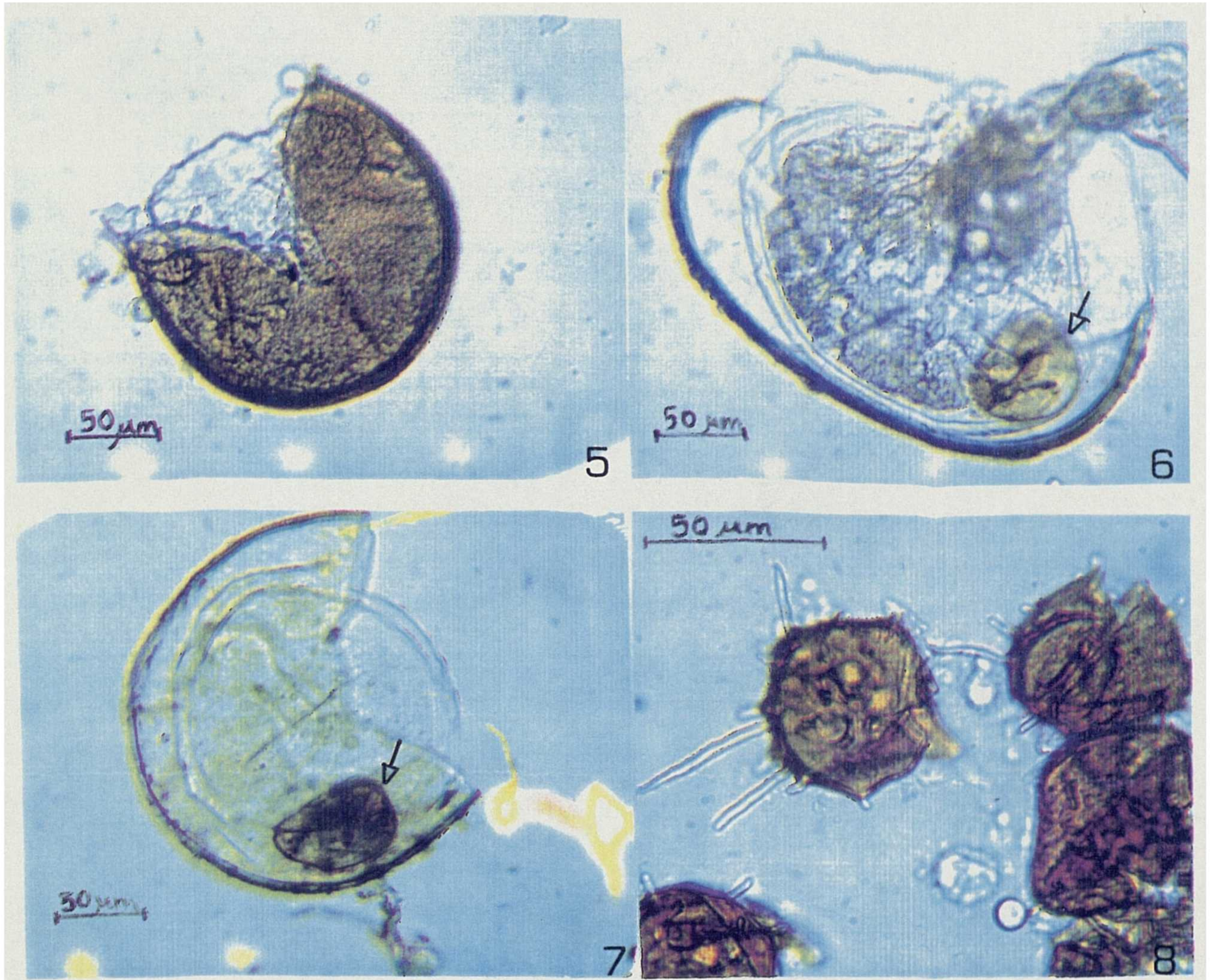
Fig. 14 shows an apparently undescribed *Glomus* sp. which shares some of the features of *G. mosseae*, a funnel-shaped base and two-layered wall, and some of the characteristics of *Glomus constrictum* Trappe with a constricted spore base on some specimens and a dark pigmentation to the main structural laminated wall. However, the spores of this species are smaller than either *G. mosseae* or *G. constrictum* and the pigmentation is intermediate between the two.

Fig. 15 shows, in higher magnification, a spore of *G. microcarpum*, taken from a spore-cluster other than the one shown in Fig. 9. The thickening of the wall at the spore base is a sign of spore maturity and a phase before the spore content separates from attached hyphae (Gerdemann & Trappe 1974).

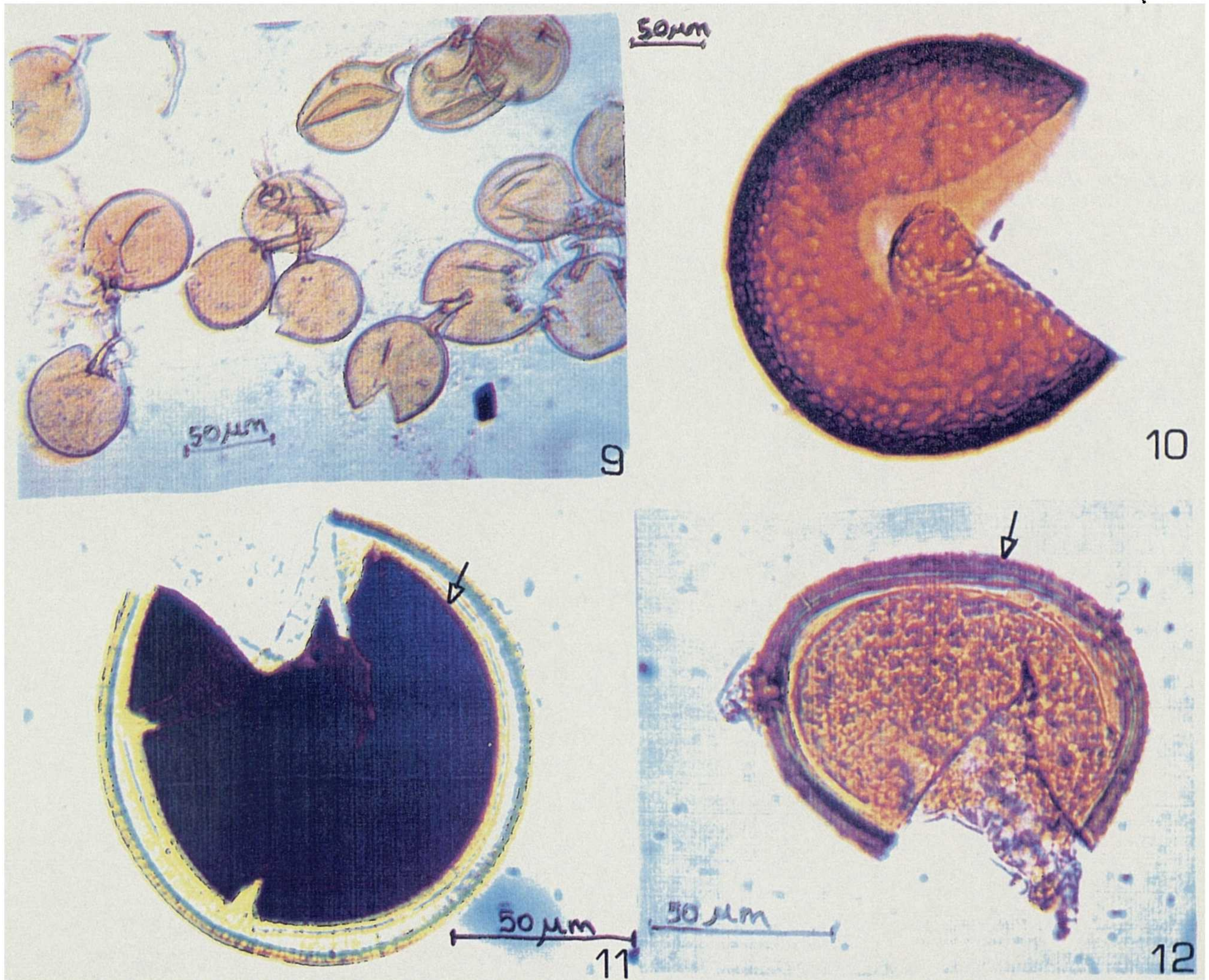
Fig. 16 shows individual spores from a sporocarp of *Sclerocystis rubiformis* Gerd. & Trappe. Individual spores were mostly ellipsoid to clavate and measured 180-235 µm x 157-172 µm in length including the hyphal attachment and colour corresponding to cinnamon no. 11 (Anon. 1969). The genus *Sclerocystis* was recently revised on the basis of comparative studies of spore ontogeny and sporocarp morphology (Wu 1993) and the characteristics found in many of the sporocarps recovered from LERF soils generally agreed with the description provided there. Sporocarps of *S. rubiformis* are usually globular with radially arranged chlamydospores (spores). Spores are arranged on a thick-walled central plexal cell which is connected with a broad hyphal stalk.



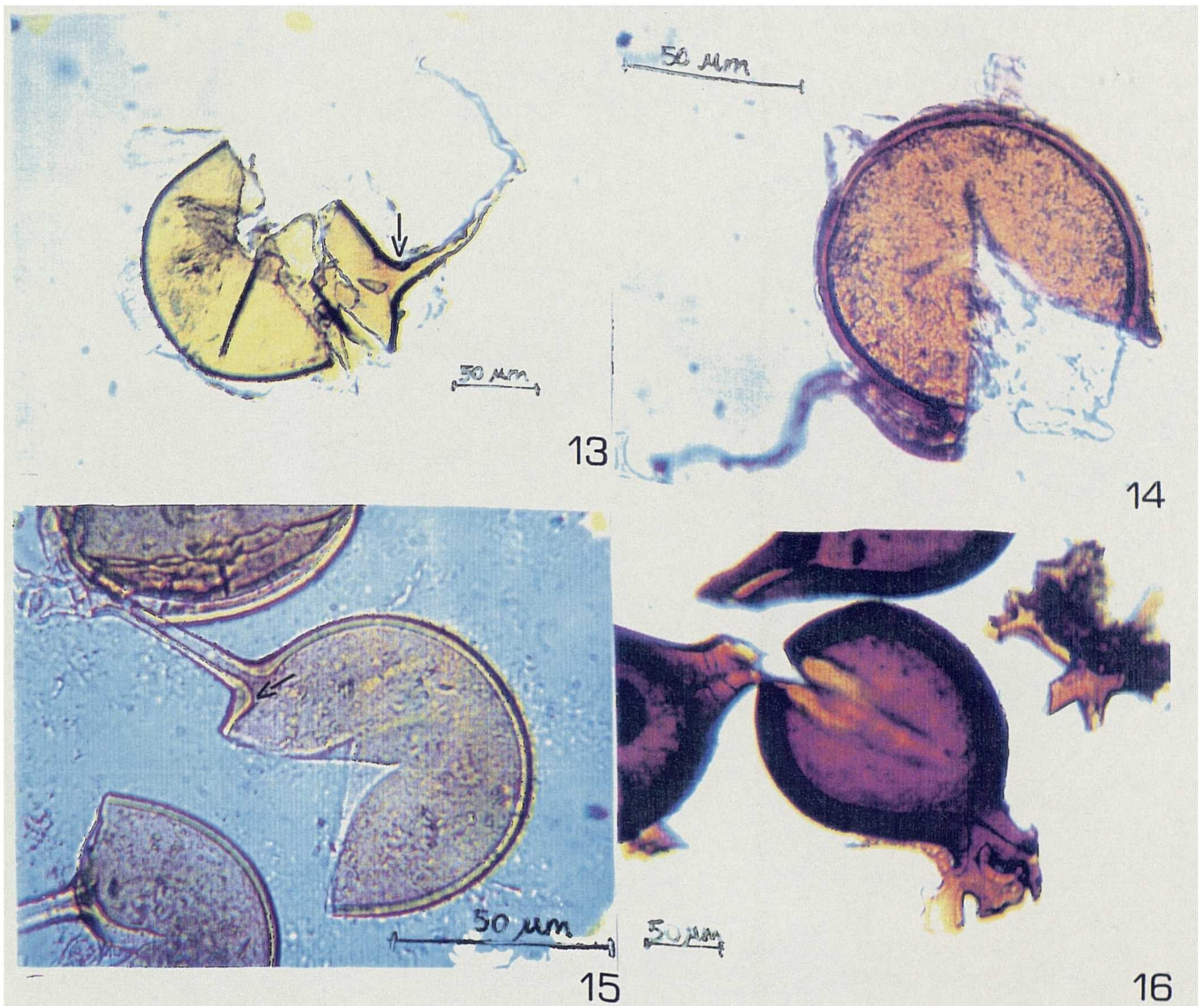
Figs 1-4. Micrographs of spores from SHF. 1: An undescribed species of *Scutellospora* 'rugose' showing the purple reaction of the innermost wall (arrow) to Melzer's reagent. 2: An undescribed species of *Scutellospora* 'smooth' showing reaction of outer wall layers to Melzer's reagent (upper arrow) and the germination shield (lower arrow) 3: A loose cluster of an apparently undescribed *Glomus* sp. of the *maculosum* group. 4: A different *Glomus* sp. in the *maculosum* group. Note the presence of the thin innermost wall (upper arrow) an the remains of the outermost wall (lower arrow).



Figs 5-8. Micrographs of spores from THF. 5: A spore of *Scutellospora tricalypta* showing its ornamented outer wall. 6: An undescribed *Scutellospora* in PVLG similar to one found elsewhere in Brazil and currently under study there (M.J. Miranda & J.L Spain). Note its evident germination shield (arrow). 7: another specimen of the spores shown in Fig 6. again showing an evident germination shield. 8: An unusual type of auxiliary cells, possibly of a *Gigaspora* sp.



Figs 9-12. Micrographs of spores from LERF. 9: Part of a spore-cluster of *Glomus microcarpum*. 10: *Acaulospora foveata*, showing its main characteristics, the thick walls and the excavations of the outermost ornamented wall. 11: *Acaulospora* sp., possibly of *A. largula*. Note the deep purple reaction of the inner wall (arrow) with Melzer's. 12: An undescribed spore which shares some of the characteristic of the genus *Glomus* sp. (a thick laminated wall; upper arrow) and some of the *Acaulospora* sp. (a detachable inner wall; lower arrow).



Figs 13-16. Micrographs of spores from LERF. 13: *Glomus mosseae* with its characteristic funnel-shaped hyphal attachment (arrow). 14: an apparently undescribed spore in PVLG which shares some characteristics of *G. mosseae* and some of the characteristics of *G. constrictum* (see text). 15: Another specimen of *Glomus microcarpum*. Note the thickening of the wall at the spore base. 16. *Sclerocystis rubiformis*, individual spores taken from a sporocarp.

DISCUSSION

Plants show different degrees of colonization by VAM or ECM which may be adaptations to different soil conditions and opportunities of mycorrhizal infection. In some habitats the mycorrhizal association has been found to be exclusively by ECM, and in others exclusively by VAM fungi while in yet others both ECM and VAM associations have been recorded (Sieverding 1991). The causal factors for ECM or VAM or both have been largely overlooked in the literature. It is generally recognized that ectomycorrhizas tend to be more restricted to certain types of habitat than do VA associations. ECM fungi have been shown to be favoured by seasonal dryness, inhibited nitrification, and extremely low mineral retention by soils such as the spodosols of the heath forests under study (Janos 1975). For instance, in Cameroon, Newbery *et al.* (1988) comparing two forests with different ectomycorrhizal status, found that soil phosphorus concentrations were higher only in the dry season in the forest with high ECM associations than in the forest with few ECM associations. They suggested these differences were related to water supply and that the ability of ECM fungi to store water in their Hartig net caused the increase in phosphorus availability. However, Chuyong (1994) showed for the same forests in Cameroon that though most of nutrients were cycling faster in the highly ectomycorrhizal forest no evidence was found for the role of ECM in the nutrient acquisition. It is noteworthy that neither the highly nor the poorly ECM-infected forests were particularly limited by any nutrients.

On the other hand, Lodge (1989) showed that ECM fungi were active in moist and well-drained soils under controlled conditions, but under field conditions where VAM fungi were also present the pattern was reversed. She observed that infection by VAM fungi in the field was the lowest where infection by ECM fungi was high, suggesting a possible antagonism among fungal symbionts (Lodge 1989). Despite the fact that the soils of the forests under study were all well drained, their different vegetation and surface soil organic layers created distinctly different moisture levels in the soil. Hence, the SHF with sparse vegetation showed the lowest weekly moisture content for both organic and mineral layers throughout the study (Luizão 1995), which may have selected one type of mycorrhizal association over the other. The ECM fungus *Cenococcum geophilum*, can actively grow at low water potentials (Mexal & Reid 1973) and occurs in dry soils (Worley & Hacskaylo 1959). It was in SHF, the driest forest, that *C. geophilum* was more frequently observed. Following Lodge's (1989) observations, the dominance of ECM fungi under these conditions may be the possible reason for the low VAM diversity in SHF. However, though the THF and LERF had similar moisture contents, the LERF soils showed the greatest diversity of VAM spores. Again this could be related to the antagonism between ECM and VAM associations. Janos (1987) suggested that many ECM host-species are among the plant families known to produce resins and polyphenols in forests on spodosols, features of the THF but not of the LERF. More studies are

required to understand the relationship between VAM and ECM fungi in these forests.

Additionally, the apparent lower diversity in mycorrhizal spores in the THF compared with the LERF may be a consequence of the lower pH in THF (Table 2.4), since some VAM species are inhibited by acid soils (Mosse 1972). This is supported by the fact that the majority of species are apparently undescribed or rare. The low numbers of spores in the soil do not necessarily indicate that the fungi are present in low populations. The relationships between colonization of roots and propagules (including spores) formation, and between propagule distribution and abundance and subsequent formation of the association for different fungi in field environments, are not well understood (Abbot & Gazey 1994). Consequently, spore numbers cannot represent anything other than the species composition and not even that in cases of species which are rarely fertile.

The identification of fungi in the Glomales is a difficult and specialized task. There are currently six genera in the order (Morton & Benny 1990) with a total of about 130 species (Walker & Trappe 1993). The observations needed for definite identification of a new species can rarely be made on a few spores. Usually, it is necessary to have a series of at least twelve spores from a single-species pot culture or perhaps more from a field soil. Proper characterization of the germ plasm in these plots can only be made if the fungi are obtained in pure pot culture. Making such pot cultures provides a source of material for future testing in plant growth response experiments

and in the examination of the role of the fungi in protection against plant pathogens. The SHF, THF and LERF plots, therefore represent a rich source of fungal biodiversity, including several undescribed species of arbuscular mycorrhizal fungi. Further work would probably be rewarded by discoveries in the areas of mycorrhizal taxonomy, systematics and plant physiology.

The actual importance of mycorrhizas in many diverse aspects of the plant's ability to grow and survive in natural and disturbed tropical environments is little known. *P. schomburgkiana*, a tree species frequently found in the THF and in the closed sites of SHF, is highly ectomycorrhizal and also a producer of toxic substances. These two characteristics are the basis for Singer & Araujo's (1979) hypothesis to explain the formation of a deep layer of raw humus on the heath forest soils. The relative scarcity of saprophytic decomposing fungi is suggested to result from the competition for nutrients between mycorrhizal fungi and saprophytic microorganisms (Gadgil & Gadgil 1971, 1975). Mycorrhizal fungi may, by immobilizing nitrogen and phosphorus, reduce the availability of those nutrients so that they limit the growth of saprophytic microorganisms and thereby retard decomposition (Allen 1991). The accumulation of nutrients in the humus found in these heath forests is believed to be a nutrient-conserving mechanism and allegedly the sole reason for the existence of these forests on such highly leached spodosols (Singer & Araujo 1979; Singer 1984).

Today it is widely accepted that the inoculation of tree seedlings with ECM fungi is a crucial step in reforestation programs in regions where these fungi

are sparse due to land degradation or of inappropriate species (Alexander 1989; Reid 1990). Thus, an improved understanding of the function of the mycorrhizal associations in heath forests could provide an important tool for the management of their soils and may be of help in interpreting species composition and forest dynamics.

Chapter VIII. General Discussion and Conclusions.

Lowland tropical rain forests are rapidly disappearing and vast tracts equivalent to 10.5% of the original undisturbed forests in Brazilian Amazonia were already cleared by 1991 (Fearnside 1993). While large areas of lowland evergreen forest have been brought into commercial timber production, new settlement projects, and highway and dam constructions, heath forest soils are being exploited to provide sand for the increased building activity. A sound knowledge of the carbon and mineral nutrient cycles of heath forest is essential if their viability as natural stands, with the many associated benefits, is to be sustained. It is hoped that the present work has contributed to such knowledge.

Many of the theories that govern our thinking on low nutrient soils of tropical forests are built on the premise that they are very old vegetation in a climax state, and that their survival is mainly based on closed and tight nutrient cycles with a high recycling efficiency in which virtually no nutrient leaching occurs (Klinge 1973, Stark & Spratt 1977, Herrera *et al.* 1978, Jordan & Herrera 1981). The observations which allowed those theories to develop are based on some of the characteristics of the forests found in San Carlos de Rio Negro, Venezuela: nutrients supposedly mainly stored in the plant biomass, small sclerophyllous leaves, low foliar and litterfall nutrient

concentrations, and a mat of mostly mycorrhizal roots supposedly cooperating in nutrient extraction from organic matter and perhaps also in the organic matter decomposition (Klinge 1973, Stark & Spratt 1977, Herrera *et al.* 1978, Jordan & Herrera 1981).

Nutrient limitation

Nutrient poverty has been discussed as a possible cause of the distinctive structure and physiognomy of the heath forests (Whitmore 1990). The maintenance of a root mat has frequently put forward as an adaptive feature of tropical forest on nutrient-poor sites (Klinge 1973, Medina *et al.* 1978). Vitousek & Sanford (1986) compared foliar and fine litterfall nutrients of various rain forests, and showed that nitrogen and phosphorus appeared to cycle less in heath forest than in other lowland forests, even though the total amounts in the soil were not unusually low. Gower (1987) hypothesised that phosphorus availability is the primary factor governing fine root biomass in both heath and lowland evergreen tropical rain forests. Soil calcium concentrations are generally considered adequate for plant growth in tropical forests (Jordan 1985) but forests growing on spodosols may be exceptions (Cuevas & Medina 1988).

In this study, the experimental assay with nutrient additions described in Chapters III and IV demonstrates that calcium, either as a nutrient or as a pH corrective is a major limiting nutrient for microbial activity, including nitrogen transformation rates. The increase in *vitro* microbial activity caused

by the sulphate additions was unexpected but suggests that sulphur is possibly limiting for soil microbes too. In parallel with the results of Saggar *et al.* (1981) who observed higher rates of soil respiration in soils initially amended with cellulose plus sulphate, addition of other nutrients may have created conditions where sulphur was limiting. Unfortunately, no sulphate was applied in the field experiments.

From the low concentrations of nitrate ions and net nitrification rates, nitrification seems to be inhibited in the heath forests. Despite the addition of nutrients including nitrogen, phosphorus, calcium and potassium, net nitrification was negligible in both SHF and THF. As shown by Olson & Reiners (1983) nitrifier populations might be present but somehow unable to respond, perhaps inhibited by polyphenols concentrated near the soil surface. Undoubtedly these inhibitory chemicals are released from leaves and leaf litter in the SHF and THF which are capable of inhibiting the activity of microorganisms.

The relatively high biomass-N compared with the low rates of mineralization and nitrification measured in all the forest types suggests that most of the nitrogen made available is immediately immobilized in the microbial tissues. Taking into account that soil microorganisms are better competitors for nutrients than plant roots and their known preference for ammonium ions, these results are not surprising.

In the assay of nutrient addition on fine root growth (Chapter VI), it was shown that in general, independently of either treatment or substrate,

significantly less fine root growth was found in the ingrowth bags from the SHF sites than in those in the other two forest types. The vermiculite bags placed within the soil had a higher production of fine roots compared with the bags placed on the soil surface but even the latter produced more fine roots ($p < 0.001$) than the bags containing soil. In the LERF, calcium chloride and calcium carbonate additions to vermiculite bags placed on the surface or in the soil were the only treatments which significantly increased fine root growth. The generally lowest fine root production in the SHF particularly when sand was used may be due to the leaching of the added nutrients from the poorly retentive medium. During the experiment 1440 mm of rain fell and, in January, the first month of the experiment, rainfall was more than 100 mm per week (see Chapter II).

The lack of difference between the effect of calcium carbonate and calcium chloride addition in the root mass in the LERF site is likely to be a result of the higher soil pH (in comparison with the THF soils) and better soil buffering in this forest type. However, F. J. Luizão (1995) also working on the same forest soils testing possible nutrient limitation for seedling growth in both field and glasshouse found a significant positive effect of calcium carbonate addition but a significant negative effect of calcium chloride addition. Although Luizão (1995) did not obtain positive results from calcium addition on seedling growth in either field and glasshouse it is worth pointing out that increased fine root production may be an earlier phase of the

fertilization, and strong immobilization of calcium by microorganisms ($CaCl_2$) may result in an initial negative effect on plants.

Role of roots and mycorrhizas

Root mat development has been viewed as an important mechanism that enhances nutrient conservation in Amazonian forest ecosystems on poor soils where the roots absorb nutrients released from decomposing organic matter (Medina *et al.* 1978). However this study showed that neither nitrogen nor phosphorus were released faster in the presence of roots (Chapter V). In fact, nitrogen never showed any significant difference between treatments for all experiments and in all forest types. Unexpectedly, in the THF, the only instance where phosphorus release was different between treatments it showed a slower rate in the presence of roots. On the other hand, faster release, possibly caused by root uptake was observed in the THF and the LERF for other elements including calcium and magnesium (Chapter V).

A feature common to all forest types, for the experiment started in the dry season, was the significant contribution of roots to the accumulation of iron and aluminium. This phenomenon was possibly related to the predominance of fungi (Chapter III) among the microbial population through deposition of their dead residues on the leaves (Lodge *et al.* 1994) and also to the mycorrhizal fungi associated with the roots (Chapter VII). Nevertheless, a possibility of a higher input of contaminant residues from the litterfall

usually observed in the dry season (Luizão & Schubart 1987) cannot be dismissed.

In conclusion, the alleged role of fine roots (and their fungal associates) in the efficient recovery of minerals during decomposition (St John 1985) is apparently only a partial explanation. The results found in this study suggest that fine root development in the leaf-litter bags may contribute to the accumulation of some elements as well as to the release and eventual uptake of others by higher plants.

Fragmentation of organic matter, channelling, and mixing of soil components are key roles by which soil fauna stimulate microbial activity, and enhance the rate of organic matter decomposition. Despite the difficulties in assuring that the absence of roots (rather than the disturbance associated with root-excluding treatments) was the cause of the fewer litter animals in the bags without roots, some animal groups were clearly related to the presence of roots. This should not be a surprise since most of the invertebrates found on the forest floor are usually microbial feeding, particularly on mycorrhizal fungi (Coleman 1985).

The mycorrhizal work was restricted to making a collection of the fungi in the three forest types (Chapter VII). Nevertheless, since only limited mycorrhizal identification has been completed in Amazonia, there exists a vast scope for adding valuable information to the existing species' lists and knowledge of their ecology. Apparent higher VAM fungal diversity was found in the LERF soils compared with the SHF and LERF. It was suggested,

based on Lodge's (1989) observations that in field conditions, antagonism between ECM and VAM is related to soil water availability and that the different vegetation cover and organic layers on the soil surface create different moisture concentrations in each forest soil. Hence, the SHF with sparse vegetation showed the lowest moisture for both organic and mineral layers throughout the study, which may have favoured the ECM type of mycorrhizal association, which is active at low water potentials, over the other. Despite the fact that the THF and LERF have shown similar moisture concentrations, the LERF soils showed the greatest diversity of VAM spores which suggests that ECM fungi may have a higher resistance to resins and polyphenols produced in forests on spodosols (Janos 1987). These forest types are a rich source of fungal biodiversity, including several undescribed species of arbuscular mycorrhizal fungi, and further work to understand the diversity within populations of mycorrhizal fungi and the processes that influence their presence in forest soils is necessary.

Seasonality and drought

The soil microbial biomass is of importance in most ecosystems because it forms the base of the detritus food-web and serves as sink and source for most plant-available nutrients (Jenkinson & Ladd 1981). For Amazonian lowland evergreen forests on oxisols, climate is a known factor controlling microbial population dynamics and activities through seasonal changes in soil moisture (Luizão *et al.* 1992, Grimaldi *et al.* 1992). In this study, however,

no seasonal variation was detected in either the microbial population or its respiratory activity in the SHF, THF, and LERF. It has been pointed out that this could have been either a result of the organic layers at the soil surface which created a buffered environment for microorganisms, protected from desiccation (Van Veen *et al.* 1987) or by the similar amount of rainfall at the two sampling periods. The buffering effect probably does not apply to the open sites of the SHF but where such organic layers do exist, keeping high soil moisture levels, there will be some microbial activity. Such a mechanism is of special importance in well-drained heath forest soils where water supply can be scarce and might prevent microbial biomass build up. The differences in microbial population and activity found between the layers in the LERF showed that saprophytic microorganisms will be inactive (low soil respiration) because of a lack of suitable substrate, and only specialized organisms able to participate in the decomposition of resistant organic matter will be active as seems to be the case in the LERF soils. In fact, by their resistance to low pH, low nitrification ability and apparent tolerance to the allelochemicals (Olson & Reiners 1983) it was concluded that fungi are the dominant group among the microbial population in those soils.

Despite the lack of seasonality in the microbial population and activity, the nitrogen dynamics in all forest soils is highly seasonal. In all forest types, there were more ammonium ions available during the wet season while, the opposite was found for nitrate. It is clear that microbial transformations of nitrogen are more sensitive to the fluctuations in soil moisture than the

microbial population itself. The high nitrogen immobilization in the first phase of the decomposition is usually associated with an increase in microbial biomass carbon. Hence microbial biomass can serve as a potential source of mineralizable nitrogen which can then be used by plants as shown by Bonde *et al.* (1988) who observed that microbial biomass nitrogen accounted for 55-89% of mineralized nitrogen in 40 weeks in a lowland evergreen rain forest on oxisols in Amazonia.

Other possible limiting factors: pH and phenolic toxicity

The expectation that calcium carbonate addition alone with consequent raising of soil pH would enhance microbial activity in soils was not fulfilled. The reason for these results may be related to the fact that only in the SHF calcium carbonate applied to soils had actually neutralised the pH and increased the microbial activity. In both the THF and LERF neither pH raising nor increased microbial activity was observed after calcium carbonate addition, though enhanced nitrogen mineralization was recorded in the LERF. Apparently, there is so much acidity in these soils that the calcium carbonate addition was insufficient to neutralize it.

Nitrification was very slow in all forest soils. Even the addition of urea did not stimulate nitrification. A suggestion, based on the results of Olson & Reiners (1983) is that the low numbers of nitrifiers may inhibited by the relatively insoluble polyphenols concentrated near the soil surface. As expected for heath forests, there are a number of chemicals released from

leaves and leaf litter in the SHF and THF (Anderson & St John 1981) which are capable of inhibiting the activity of microorganisms (Rice & Pancholy 1972).

The numbers of Acari and Collembola varied, the most abundant animals varied with forest type. The density of Acari decreased and Collembola increased in more acidic soils. The relationship between the Collembola communities and the vegetation became stronger as conditions became more extreme and they showed highest densities in the SHF, an observation in agreement with studies elsewhere (Bååth *et al.* 1980, Hågvar 1982).

The rates of leaf-litter decomposition for *C. racemosa* on spodosols found in this study were slower than in the LERF, and much lower than former studies with the same species in forests on oxisols (Luizão & Schubart 1987). Klinge (1973) had suggested that litter on spodosols would decompose faster owing to the activity of the fine roots exploiting the organic matter.

Many factors have been suggested as the cause of heath forests, such as, low soil nutrients, low soil pH, phenolic compounds, high litter C:N ratio, and periodic water shortage or waterlogging. The evidence found in this study revealed a complex situation. Calcium (irrespective of the pH) seemed to limit soil respiration and fine root growth; sulphur seemed to limit soil respiration and nitrogen transformations. However the most important factor, judging from the experiments on soil respiration, nitrogen transformations, and fine root growth, seemed to be the low soil pH of the SHF and THF. The causes of the differences between SHF and THF seemed

to be connected with the low organic matter content of the SHF soils and that is likely to have resulted from human disturbance.

In summary, though some of the theories to explain nutrient dynamics on low-nutrient tropical forests are appealing, as more data accumulates it has become clear these theories are oversimplifications and based on a limited number of forests (Proctor 1992, Whitmore 1989). The present study shows that even for heath forests, generally viewed as among the poorest forest ecosystems, the generalizations of many rain forest functions do not apply. Hence, this study puts emphasis on the need for further research on the dynamics of nutrients in tropical forests.

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APPENDICES

Appendix 1.1: Mean soil respiration ± SD in each of the three plots for each treatment of the laboratory nutrient addition and the mean ± SD for all three plots in the SHF, THF and LERF.

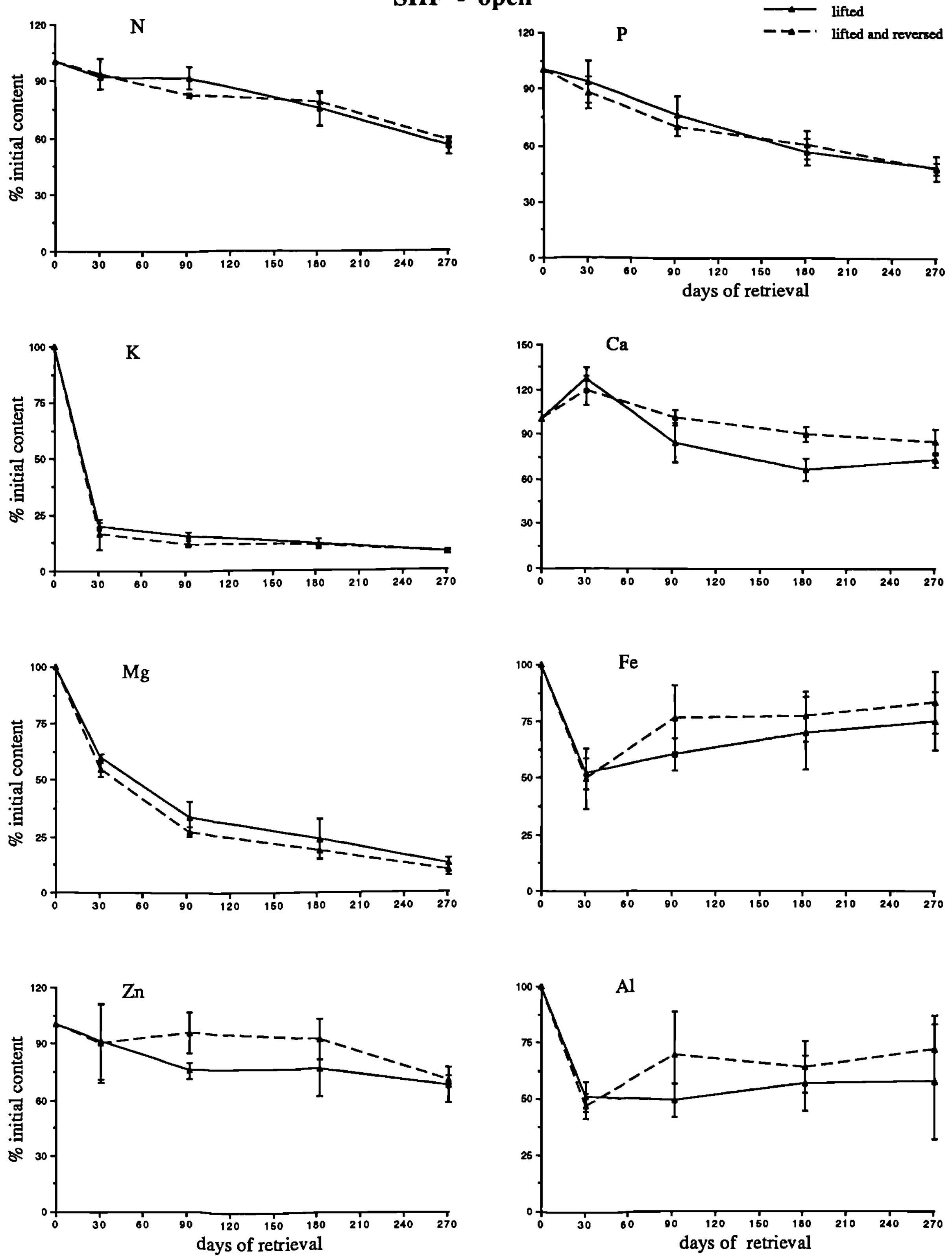
		Treatments											
Forest Types	Plot	Control	N	N + P	N + K	N + $CaCO_3$	N + $CaSO_4$	N + $NaSO_4$	P	K	$CaCO_3$	$CaSO_4$	$NaSO_4$
SHF	1	48.3 ± 15.3	86.1 ± 28.1	132 ± 29.0	100 ± 38.6	58.8 ± 22.0	61.3 ± 4.8	110.0 ± 14.3	70.6 ± 12.1	57.0 ± 15.3	66.8 ± 18.3	69.4 ± 23.0	48.9 ± 17.8
	2	6.1 ± 5.8	24.4 ± 17.6	28.1 ± 21.1	29.3 ± 16.8	33.6 ± 18.0	25.6 ± 8.0	38.5 ± 12.2	34.8 ± 19.0	65.3 ± 25.0	33.6 ± 8.1	25.6 ± 12.0	28.1 ± 10.3
	3	8.02 ± 8.5	27.8 ± 20.1	30.2 ± 18.7	30.2 ± 19.0	24.1 ± 11.8	38.2 ± 17.5	45.6 ± 13.7	29.0 ± 9.5	38.2 ± 17.6	41.9 ± 15.3	52.4 ± 11.9	29.6 ± 8.7
	x ± SD	20.8 ± 20.6	46.1 ± 30.0	63.4 ± 58.4	53.5 ± 35.6	38.3 ± 15.6	41.7 ± 15.7	64.6 ± 33.9	44.8 ± 19.5	53.5 ± 12.0	47.5 ± 15.0	49.1 ± 19.1	35.5 ± 10.1
THF	4	46.1 ± 8.2	66.3 ± 8.1	50.4 ± 3.4	55.5 ± 22.5	59.8 ± 4.5	98.7 ± 8.7	85.0 ± 6.9	63.4 ± 9.1	67.7 ± 4.1	59.79 ± 5.3	75.6 ± 11.7	87.2 ± 9.3
	5	50.3 ± 10.0	48.2 ± 10.4	53.0 ± 3.7	57.8 ± 11.3	50.97 ± 5.3	59.1 ± 5.6	59.8 ± 12.0	75.44 ± 5.3	58.4 ± 3.6	48.25 ± 8.3	90.39 ± 16.2	56.4 ± 11.0
	6	39.8 6.3	38.5 ± 14.0	45.59 ± 5.0	43.7 ± 26.0	48.16 ± 8.7	48.16 ± 17.6	75.8 ± 5.3	41.73 ± 12.3	43.7 ± 7.8	41.1 ± 10.6	66.1 ± 5.8	49.4 ± 6.5
	x ± SD	45.4 ± 12.4	51.0 ± 12.9	49.7 ± 3.26	52.3 ± 35.6	53.0 ± 5.25	68.6 ± 23.0	73.5 ± 11.0	60.2 ± 14.8	56.6 ± 10.5	49.7 ± 8.17	77.4 ± 10.6	64.5 ± 17.4
LERF	7	59.1 ± 12.0	63.9 ± 5.1	70.2 ± 3.8	67.4 ± 9.2	66.7 ± 8.0	69.5 ± 6.7	48.0 ± 12.1	52.1 ± 2.8	61.2 ± 5.2	63.9 ± 9.6	63.3 ± 10.2	87.6 ± 7.3
	8	55.9 ± 8.5	49.1 ± 3.7	55.5 ± 4.3	68.6 ± 11.3	47.1 ± 6.5	58.5 ± 5.5	71.3 ± 5.3	45.1 ± 5.3	54.51 ± 7.2	59.2 ± 6.9	66.6 ± 6.8	63.9 ± 9.1
	9	63.4 ± 16.2	59.9 ± 4.8	72.5 ± 3.9	97.2 ±7.9	58.4 ± 5.1	69.0 ± 4.8	64.8 ± 9.7	51.4 ± 3.9	67.6 ± 4.5	66.9 ± 5.4	81.7 ± 4.5	71.8 ± 6.6
	x ± SD	59.4 ± 18.9	57.6 ± 6.63	66.1 ± 8.0	77.7 ± 14.6	57.4 ± 8.5	65.7 ± 5.4	61.4 ± 10.4	49.5 ± 3.35	61.1 ± 5.7	63.4 ± 3.3	70.5 ± 8.5	74.4 ± 10.4

Appendix 2.1: Mean net mineralization rates ± SD (µg N g^{-1} oven-dry soil 10 d^{-1} in each of the three plots for each treatment of the laboratory nutrient addition and the mean ± SD for all three plots in the SHF, THF and LERF.

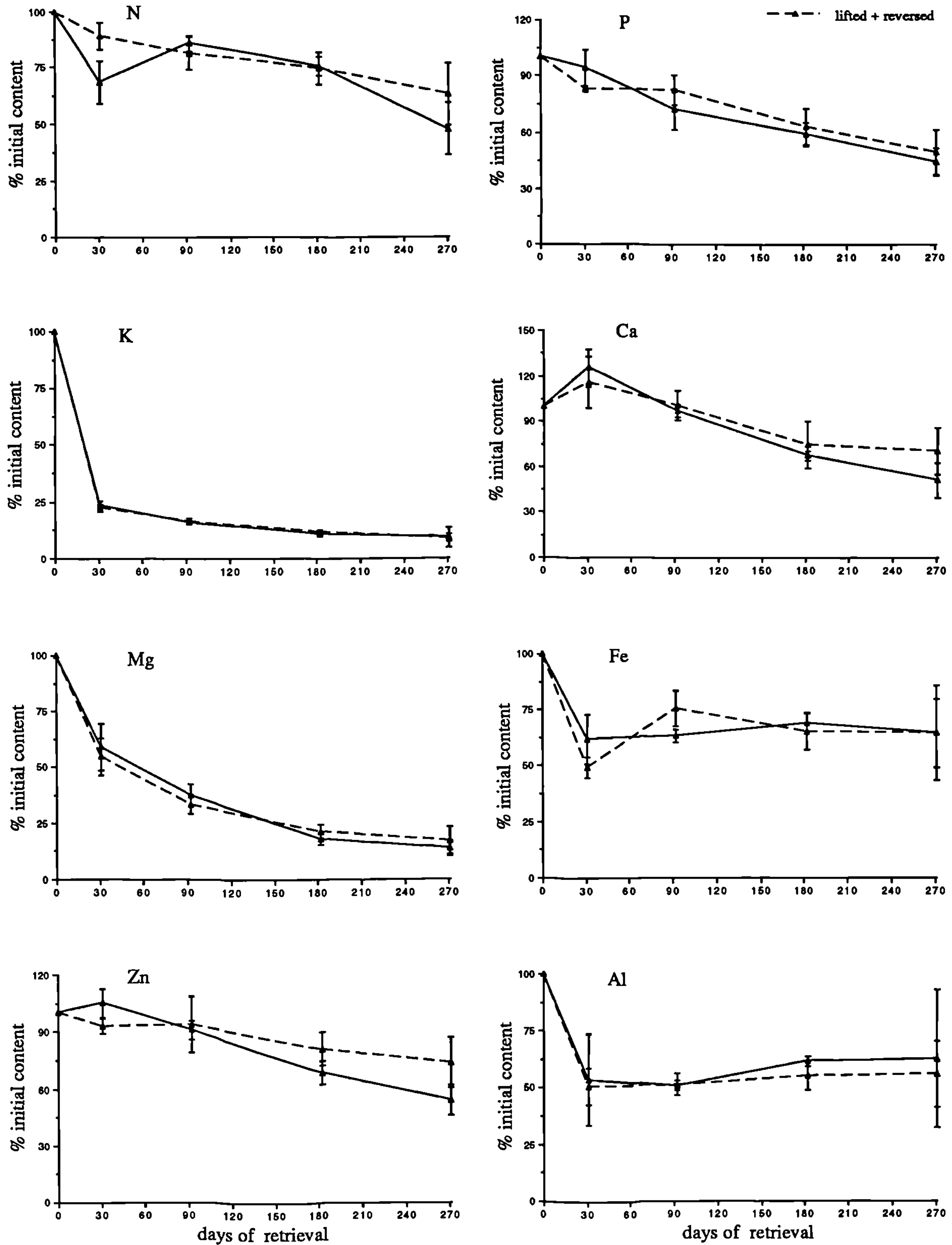
Forest Type	Plot	Treatments: Control	N	N + P	N + K	N + $CaCO_3$	N + $CaSO_4$	N + $NaSO_4$	P	K	$CaCO_3$	$CaSO_4$	$NaSO_4$
SHF	1	6.25 ± 5.0	32.23 ± 12.7	24.57 ± 23.8	-6.92 ± 1.29	35.06 ± 3.6	21.16 ± 8.4	9.74 ± 7.8	-3.15 ± 3.5	8.14 ± 2.3	11.8 ± 10.4	13.13 ± 4.8	11.73 ± 3.5
	2	3.80 ± 3.4	28.3 ± 5.6	22.0 ± 3.4	19.6 ± 12.5	24.62 ± 2.0	22.31 ± 10.9	7.28 ± 2.4	-1.99 ± 1.1	-0.98 ± 1.0	14.94 ± 2.0	-3.02 ± 8.5	2.82 ± 2.7
	3	3.35 ± 1.9	32.33 ± 6.5	23.7 ± 7.9	26.87 ± 8.0	17.47 ± 4.3	27.06 ± 4.5	7.72 ± 3.4	28.58 ± 28.0	-1.45 ± 3.6	8.59 ± 6.4	9.26 ± 2.1	9.88 ± 1.7
	x ± SD	4.47 ± 3.4	30.97 ± 7.9	23.42 ± 12.7	13.18 ± 17.1	25.72 ± 8.2	23.51 ± 7.7	8.25 ± 4.6	7.81 ± 21.0	1.90 ± 5.2	11.77 ± 6.7	6.46 ± 8.8	8.14 ± 4.7
THF	4	7.34 ± 14.5	59.98 ± 22.6	40.55 ± 10.2	51.76 ± 16.8	55.48 ± 37.5	63.74 ± 2.8	58.01 ± 11.2	28.45 ± 23.3	18.83 ± 1.9	58.10 ± 32.6	32.9 ± 9.0	38.94 ± 9.4
	5	12.9 ± 4.0	24.80 ± 18.0	48.03 ± 3.7	40.89 ± 11.2	40.0 ± 2.5	39.45 ± 8.7	20.09 ± 24.5	0.12 ± 8.5	21.71 ± 4.4	9.31 ± 9.4	9.11 ± 0.9	22.82 ± 2.6
	6	0.8 ± 1.8	17.73 ± 8.8	34.16 ± 1.1	38.76 ± 10.9	19.51 ± 15.8	42.02 ± 8.6	7.45 ± 4.1	7.77 ± 11.0	17.23 ± 2.8	16.5 ± 4.9	9.35 ± 2.6	11.8 ± 6.1
	x ± SD	6.99 ± 9.2	44.17 ± 21.6	40.92 ± 8.1	43.80 ± 12.9	38.34 ± 25.7	48.41 ± 13.1	28.52 ± 26.5	12.11 ± 18.6	19.26 ± 3.4	27.98 ± 28.5	17.12 ± 12.7	24.51 ± 13.2
LERF	7	19.11 ± 3.8	43.68 ± 42.8	43.05 ± 5.0	49.55 ± 9.1	43.23 ± 9.7	10.23 ± 5.2	8.03 ± 2.8	8.13 ± 7.3	14.86 ± 3.4	19.72 ± 2.4	39.67 ± 2.5	19.27 ± 0.9
	8	15.84 ± 4.9	54.82 ± 5.8	47.3 ± 5.6	58.5 ± 8.8	43.89 ± 9.7	3.60 ± 9.3	5.08 ± 3.8	7.36 ± 2.4	12.2 ± 9.5	45.89 ± 31.2	37.70 ± 1.4	20.6 ± 3.4
	9	8.59 ± 7.9	65.0 ± 8.5	40.78 ± 8.2	42.2 ± 4.5	47.7 ± 4.1	-7.40 ± 4.1	-5.95 ± 3.1	0.73 ± 0.8	13.44 ± 4.3	35.65 ± 25.0	42.37 ± 8.8	9.35 ± 3.0
	x ± SD	14.51 ± 6.8	54.52 ± 23.9	43.73 ± 6.3	50.12 ± 9.7	44.94 ± 6.9	2.15 ± 9.6	2.39 ± 7.0	5.47 ± 5.3	13.51 ± 5.6	33.75 ± 23.1	39.92 ± 5.0	16.41 ± 5.8

Appendix 2.2 : Net nitrification rates ± SD (μg N g^{-1} oven-dry soil 10 d^{-1}) in each of the three plots for each treatment of the laboratory nutrient addition and the mean ± SD for all three plots in the SHF, THF and LERF.

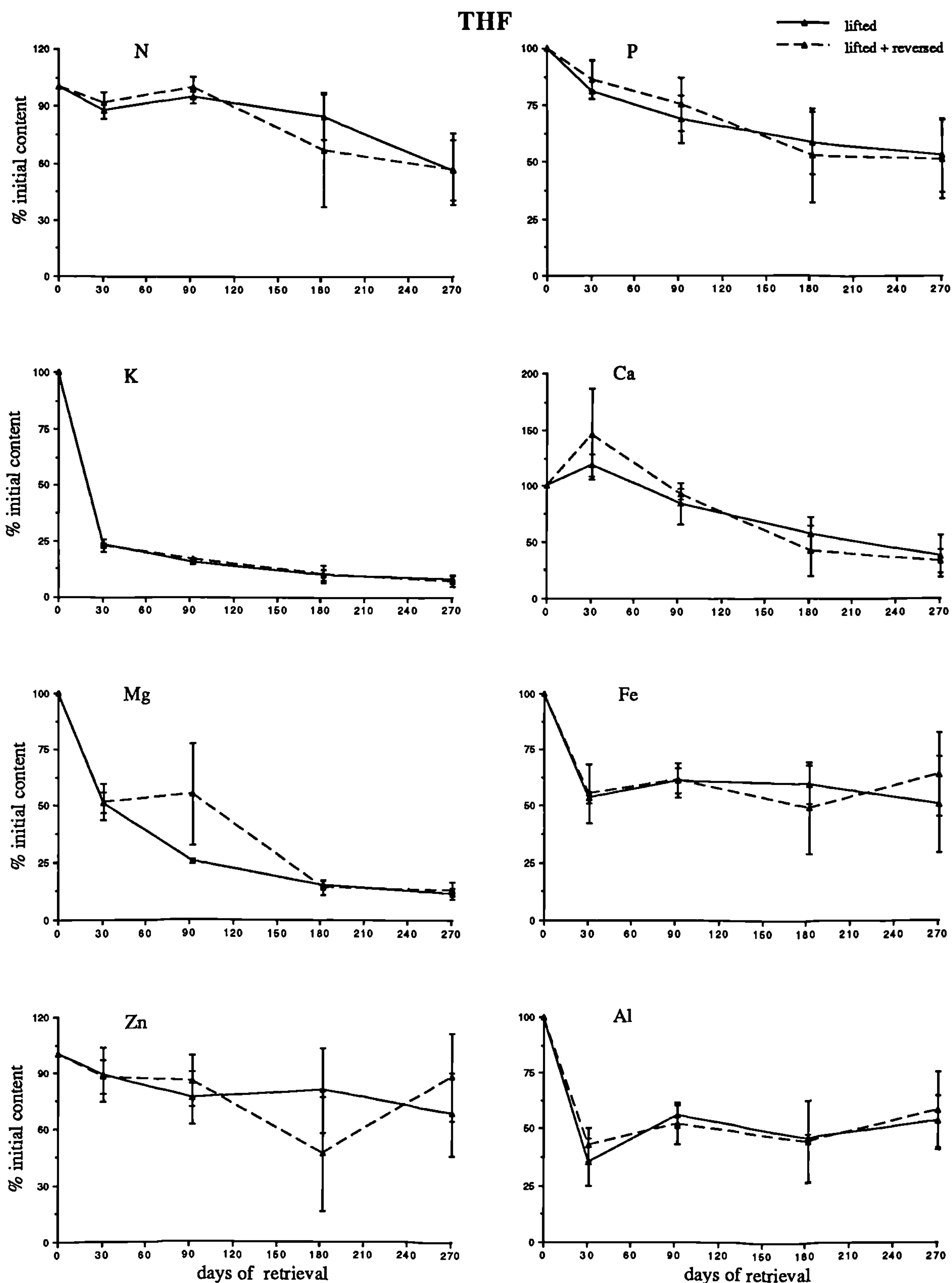
Forest Type	Plot	Control	N	N + P	N + K	N + $CaCO_3$	N + $CaSO_4$	N + $NaSO_4$	P	K	$CaCO_3$	$CaSO_4$	$NaSO_4$
		Treatment											
SHF	1	-0.26 ± 0.04	-0.26 ± 0.04	1.24 ± 0.31	-0.27 ± 0.04	-0.26 ± 0.04	-0.26 ± 0.04	-0.27 ± 0.04	-0.27 ± 0.04	-0.26 ± 0.04	-0.27 ± 0.04	-0.27 ± 0.04	-0.26 ± 0.04
	2	-0.21 ± 0.02	-0.21 ± 0.02	0.03 ± 0.12	-0.21 ± 0.02	-0.06 ± 0.04	-0.21 ± 0.02	-0.21 ± 0.02	-0.21 ± 0.02	-0.21 ± 0.02	-0.21 ± 0.02	-6.48 ± 10.8	-0.21 ± 0.02
	3	-0.33 ± 0.07	-0.33 ± 0.07	0.25 ± 0.33	-0.33 ± 0.07	-0.33 ± 0.07	-0.33 ± 0.07	-0.33 ± 0.07	-0.11 ± 0.07	-0.33 ± 0.07	-0.33 ± 0.07	-0.33 ± 0.07	-0.33 ± 0.07
	x ± SD	-0.27 ± 0.07	-0.27 ± 0.07	0.50 ± 0.61	-0.27 ± 0.07	-0.22 ± 0.13	-0.27 ± 0.07	-0.27 ± 0.07	-0.19 ± 0.08	-0.27 ± 0.07	-0.27 ± 0.07	-2.36 ± 6.24	-0.27 ± 0.07
THF	4	0.12 ± 0.53	-0.56 ± 0.07	2.21 ± 0.31	-0.56 ± 0.07	-0.56 ±0.07	-0.56 ± 0.07	-0.56 ± 0.07	-0.34 ± 0.07	-0.56 ± 0.07	0.28 ± 0.07	-0.56 ± 0.07	-0.56 ± 0.07
	5	2.19 ± 0.31	-0.45 ± 0.08	5.29 ± 0.17	0.02 ± 0.23	-0.29 ± 0.21	-0.67 ± 0.08	0.17 ± 0.08	2.23 ± 0.51	-0.47 ± 0.08	0.47 ± 0.08	0.27 ± 0.10	-0.36 ± 0.16
	6	-0.00 ± 0.16	-0.38 ± 0.15	3.60 ± 0.16	-0.38 ± 0.15	-0.38 ± 0.15	-0.38 ± 0.15	-0.38 ± 0.15	4.98 ± 5.58	-0.383 ± 0.15	-0.38 ± 0.15	1.13 ± 0.08	-0.38 ± 0.15
	x ± SD	0.08 ± 1.16	-0.46 ± 0.12	3.70 ± 1.35	-0.31 ± 0.29	-0.41 ± 0.18	-0.54 ± 2.0	-0.26 ± 0.35	2.29 ± 3.6	-0.47 ± 0.12	0.12 ± 0.40	0.28 ± 0.74	-0.44 ± 0.15
LERF	7	9.02 ± 0.58	12.4 ± 2.91	7.97 ± 2.69	6.44 ± 1.08	5.85 ± 1.18	0.62 ± 1.19	-0.74 ± 1.07	11.2 ± 1.80	1.65 ± 1.76	14.73 ± 1.19	7.31 ± 0.29	5.36 ± 0.78
	8	8.90 ± 0.45	12.7 ± 0.74	11.2 ± 1.70	10.5 ± 2.60	12.13 ± 1.53	0.66 ± 0.47	1.98 ± 2.05	14.0 ± 1.4	4.42 ± 2.16	15.2 ± 3.97	8.04 ± 0.18	6.66 ± 0.74
	9	7.15 ± 2.16	10.4 ± 0.76	11.4 ± 4.54	5.38 ± 0.6	11.2 ± 0.81	-3.03 ± 0.73	-2.7 ± 0.89	12.5 ± 1.85	1.54 ± 2.29	15.3 ± 1.59	6.01 ± 0.56	2.76 ± 1.57
	x ± SD	8.36 ± 1.46	11.8 ± 1.9	10.2 ± 3.2	7.44 ± 2.7	9.75 ± 3.1	-0.59 ± 2.0	-0.50 ± 2.39	12.6 ± 1.9	2.54 ± 2.3	15.1 ± 2.2	7.12 ± 0.9	4.93 ± 1.97



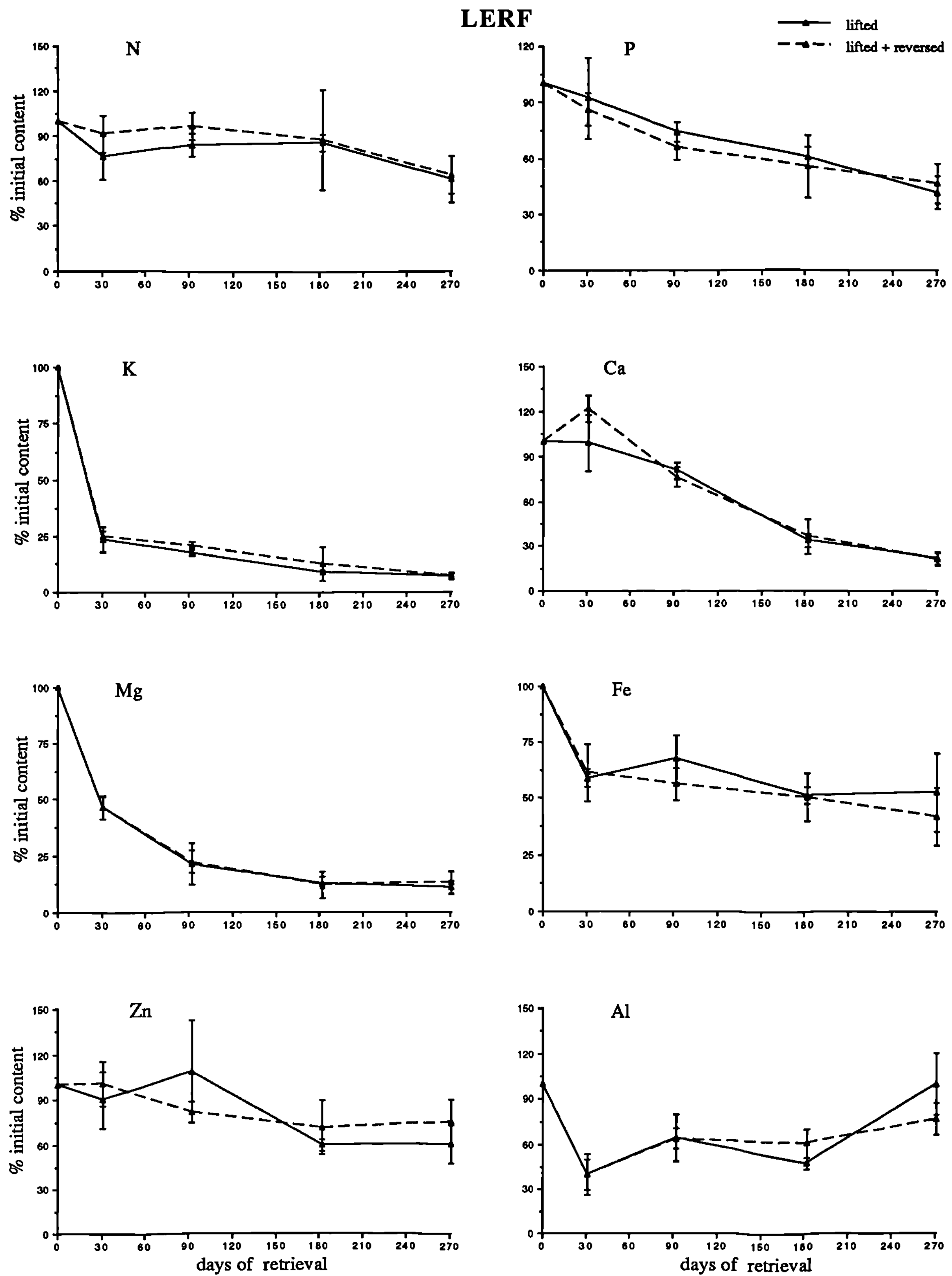
Appendix 3.1: Percentage initial content of mineral elements in the decomposing leaves in the lifted bags and in the lifted and reversed bags in the open sites of SHF during the experiment 3. Values are means ± SD of four bags retrieved in the plot.



Appendix 3.2: Percentage of initial content of mineral elements in the decomposing leaves in the lifted bags and in the lifted and reversed bags in the closed sites of SHF during the experiment 3. Values are means ± SD of four bags retrieved in the plot.



Appendix 3.3: Percentage of initial content of mineral elements in the decomposing leaves in the lifted bags and in the lifted and reversed bags in the THF during the experiment 3. Values are means ± SD of four bags retrieved in the plot.



Appendix 3.4: Percentage initial content of mineral elements in the decomposing leaves in the lifted bags and in the lifted and reversed bags in the LERF during the experiment 3. Values are means ± SD of four bags retrieved in the plot.

Appendix 4.1: Mean number of invertebrate individuals in several taxonomic gropus per litter bag (of *C. racemosa* leaves) in the bags with roots for each retrieval time in the SHF. Values are means ± SD.

	Days of retrieval						
Taxonomic groups	30 d	60 d	120 d	180 d	270 d	360 d	Total
Acarina	5.2±4.9	67±53	50±61	2.9±4.7	65±46	70±68	43±52
Collembola	0.3±0.9	28±29	12±19	0.0±0.0	20±13	12±8.9	12±18
Pseudoscorpiones	0.1±0.3	1.0±1.0	0.2±0.4	0.0±0.0	0.9±1.4	0.3±0.7	0.4±0.9
Formicidae	0.4±0.7	3.0±2.8	2.0±4.5	0.1±0.3	3.5±8.3	6.7±20	2.7±9.1
Dipteran larvae	0.1±0.3	1.3±1.9	0.8±1.1	0.0±0.0	0.1±0.3	0.1±0.3	0.4±1.0
Copepoda	0.0±0.0	2.6±4.9	0.4±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.5±2.2
Psocoptera	1.2±1.2	0.3±0.5	0.4±0.9	0.2±0.5	0.4±0.7	0.1±0.3	0.5±0.8
Diplopoda	0.0±0.0	0.1±0.3	1.6±3.0	0.0±0.0	0.7±1.2	0.4±0.7	0.4±1.1
Aranae	0.0±0.0	0.1±0.3	0.4±0.9	0.0±0.0	0.2±0.6	0.2±0.4	0.1±0.4
Homoptera juveniles	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.7	0.4±0.7	0.1±0.3	0.1±0.4
Thysanoptera	0.0±0.0	2.2±3.1	0.0±0.0	0.1±0.3	0.3±0.5	0.3±1.0	0.5±1.5
Coleoptera adults	0.2±0.6	0.4±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.4	0.2±0.5
Isopoda	0.0±0.0	0.2±0.4	0.4±0.5	0.0±0.0	0.3±.5	0.2±0.4	0.2±0.4
Coleoptera larvae	0.4±1.3	0.4±1.0	0.0±0.0	0.1±0.3	0.2±0.4	0.1±0.3	0.2±0.7
Diplura	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.4	0.04±0.2
Opiliones	0.0±0.00	0.1±0.3	0.2±0.4	0.0±0.0	0.2±0.4	0.1±0.3	0.1±0.3
Protura	0.0±0.0	0.4±1.0	0.0±0.0	0.0±0.0	0.1±0.3	0.3±0.7	0.2±0.5
Pauropoda	0.0±0.0	0.1±.3	0.0±0.0	0.0±0.0	0.3±0.7	0.2±0.7	0.1±0.4
Symphyla	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.6	0.2±0.4	0.08±0.3
Earhworms	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Isoptera	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0	0.02±0.1
Others*	0.4±1.0	0.1±0.3	0.2±0.4	0.0±0.0	0.1±0.3	0.3±1.0	0.2±0.6
TOTAL	8.3±7.9	108±74	69±84	3.9±6.0	92±56	93±74	63±70
No. of groups	2.7±1.4	6.1±1.8	4.4±2.7	1.5±1.3	5.3±2.4	4.8±2.6	4.2±2.5
n	10	9	5	8	10	9	51

* the twenty minor taxonomic groups pooled together were: Chilopoda, Diptera adults, Embioptera, Enchytraeidae, Hemiptera adults, Hemiptera juveniles, Homoptera adults, Lepidoptera juveniles, Microhymenoptera, Nematoda, Odonata, Opilioacaridae, Orthoptera, Ostracoda, Palpigradrae, Peripatus, Scorpionidae, Tricoptera, Thysanura.

Appendix 4.2: Mean number of invertebrate individuals in several taxonomic groups per litter bag (of *C. racemosa* leaves) in the bags without roots for each retrieval time in the SHF. Values are means ± SD.

	Days of retrieval						
Taxonomic groups	30 d	60 d	120 d	180 d	270 d	360 d	Total
Acarina	na	60±44	51±20	4.2±3.9	27±17	14±12	22±0.2
Collembola	na	22±8.0	11±9.0	0.2±0.4	6.7±5.0	2.7±2.6	6.0±8.7
Pseudoscorpiones	na	0.3±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3
Formicidae	na	2.3±2.5	10±17	0.0±0.0	0.0±0.0	0.5±1.0	1.8±6.4
Dipteran larvae	na	1.0±1.7	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.7
Copepoda	na	0.0±0.0	0.7±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3
Psocoptera	na	0.0±0.0	0.3±0.6	0.0±0.0	1.0±1.7	0.2±0.5	0.2±0.7
Diplopoda	na	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.2±0.5	1.0±0.3
Aranae	na	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.0±0.0	0.04±0.2
Homoptera juveniles	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Thysanoptera	na	1.3±2.3	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.9
Coleoptera adults	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Isopoda	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.5	0.05±0.2
Coleoptera larvae	na	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.04±0.2
Diplura	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Opiliones	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Protura	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Pauropoda	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Symphyla	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Earhworms	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Isoptera	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Others*	na	0.0±0.0	0.0±0.0	0.3±0.8	0.0±0.0	0.7±1.0	0.2±0.6
TOTAL	na	87±47	75±17	4.8±3.9	35±18	18±17	32±37
No. of groups	na	3.6±2.1	5.3±1.1	1.2±0.7	2.3±0.6	4.8±2.6	4.2±2.5
n	na	3	3	6	3	4	22

* the twenty minor taxonomic groups pooled together were: Chilopoda, Diptera adults, Embioptera, Enchytraeidae, Hemiptera adults, Hemiptera juveniles, Homoptera adults, Lepidoptera juveniles, Microhymenoptera, Nematoda, Odonata, Opilioacaridae, Orthoptera, Ostracoda, Palpigradrae, Peripatus, Scorpionidae, Tricoptera, Thysanura.

[na] not analysed

Appendix. 4.3: Mean number of invertebrate individuals in several taxonomic groups per litter bag (of *C. racemosa* leaves) in the bags with roots for each retrieval time in the THF. Values are means ± SD.

	Days of retrieval						
Taxonomic groups	30 d	60 d	120 d	180 d	270 d	360 d	Total
Acarina	43±27	81±63	73±47	26±29	92±65	190±145	83±86
Collembola	6.1±11	7.1±3.9	5.5±5.5	0.2±0.7	18±12	36±25	12±17
Pseudoscorpiones	0.2±0.4	1.0±0.8	0.9±1.1	0.4±1.1	0.3±0.5	1.5±2.0	0.7±1.1
Formicidae	12±37	0.7±1.7	2.0±2.6	0.2±0.5	1.2±2.2	5.4±6.8	3.7±16
Dipteran larvae	0.4±0.5	0.4±0.7	0.1±0.3	0.0±0.0	0.0±0.0	0.7±1.2	0.3±0.6
Copepoda	1.7±3.5	2.6±3.5	0.1±0.3	0.0±0.0	0.0±0.0	0.5±0.8	0.8±2.2
Psocoptera	1.4±1.0	0.2±0.4	0.0±0.0	0.0±0.0	0.2±0.4	0.1±0.3	0.3±0.7
Diplopoda	0.0±0.0	2.4±4.5	0.4±0.7	0.0±0.0	1.2±1.3	1.7±1.6	1.0 ±2.2
Aranae	0.4±1.0	0.1±0.3	1.4±1.7	0.1±0.3	0.9±0.9	2.2±2.2	0.8±1.4
Homoptera juveniles	0.2±0.7	0.0±0.0	3.1±8.0	4.1±7.4	3.1±4.7	9.4±9.6	3.2±6.5
Thysanoptera	0.9±0.8	0.3±1.0	0.0±0.0	0.5±0.5	0.2±0.4	0.0±0.0	0.3±1.6
Coleoptera adults	0.0±0.0	0.0±0.0	0.2±0.5	0.0±0.0	0.7±1.0	0.6±0.7	0.2±0.6
Isopoda	0.8±2.0	1.6±3.6	0.4±1.1	0.0±0.0	0.2±0.4	0.5±0.8	0.6±1.8
Coleoptera larvae	0.3±1.0	0.1±0.3	0.0±0.0	0.0±0.0	0.1±0.3	0.4±0.5	0.2±0.5
Diplura	0.0±0.0	0.2±0.7	1.5±1.5	0.0±0.0	0.8±1.0	0.7±0.5	0.5±0.9
Opiliones	0.0±0.00	0.1±0.3	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.04±0.2
Protura	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.5	0.04±0.2
Pauropoda	0.1±0.3	0.0±0.0	0.0±0.0	0.2±0.5	0.6±0.7	1.1±1.7	0.3±0.8
Symphyla	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0	0.2±0.7	1.0±0.9	0.2±0.6
Earhworms	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.5	0.04±0.2
Isoptera	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0	1.6±4.6	0.3±1.8
Others*	0.1±0.3	0.2±0.4	0.9±0.8	0.5±0.8	1.3±1.3	1.2±0.7	0.7±0.9
TOTAL	68±60	98±70	89±60	32±38	121±72	255±181	110±111
No. of groups	4.9±2.4	5.7±2.5	6.5±2.2	3.2±2.5	7.8±2.7	11±3.2	6.6±3.6
n	9	9	8	8	9	8	51

* the twenty minor taxonomic groups pooled together were: Chilopoda, Diptera adults, Embioptera, Enchytraeidae, Hemiptera adults, Hemiptera juveniles, Homoptera adults, Lepidoptera juveniles, Microhymenoptera, Nematoda, Odonata, Opilioacaridae, Orthoptera, Ostracoda, Palpigradrae, Peripatus, Scorpionidae, Tricoptera, Thysanura.

Appendix 4.4: Mean number of invertebrate individuals in several taxonomic groups per litter bag (of *C. racemosa* leaves) in the bags without roots for each retrieval time in the THF. Values are means ± SD.

Taxonomic groups	Days of retrieval						
	30 d	60 d	120 d	180 d	270 d	360 d	Total
Acarina	na	67±39	11±9.0	1.5±1.2	19±18	14±3.1	15±23
Collembola	na	16±6.0	2.9±3.1	0.0±0.0	8.7±7.8	22±8.4	6.2±8.7
Pseudoscorpiones	na	0.0±0.0	0.2±0.4	0.3±0.8	0.0±0.0	0.0±0.0	0.1±0.4
Formicidae	na	0.0±0.0	0.3±0.7	0.0±0.0	0.0±0.0	0.6±0.6	0.2±0.5
Dipteran larvae	na	0.3±0.6	0.1±0.3	0.0±0.0	1.0±0.0	1.3±1.5	0.3±0.7
Copepoda	na	5.7±8.1	0.6±1.0	0.0±0.0	0.0±0.0	0.6±1.5	0.9±2.9
Psocoptera	na	0.0±0.0	0.0±0.0	0.0±0.0	1.3±1.1	0.0±0.0	0.1±0.5
Diplopoda	na	1.3±1.1	0.8±2.0	0.0±0.0	1.3±1.5	0.7±0.6	0.6±1.3
Aranae	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Homoptera juveniles	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Thysanoptera	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Coleoptera adults	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Isopoda	na	0.0±0.0	0.1±0.3	0.0±0.0	0.7±1.1	0.0±0.0	0.1±0.4
Coleoptera larvae	na	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.04±0.2
Diplura	na	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.04±0.2
Opiliones	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Protura	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Pauropoda	na	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.8
Symphyla	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.04±0.2
Earhworms	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Isoptera	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Others*	na	0.0±0.0	0.1±0.3	0.5±0.8	0.3±0.6	0.3±0.6	0.2±0.5
TOTAL	na	91±42	16±11	2.3±1.7	33±21	41±14	24±31
No. of groups	na	4.3±1.1	3.1±1.3	1.3±0.8	4.7±1.1	5.0±2.0	2.9±1.9
n	na	3	9	6	3	3	27

* the twenty minor taxonomic groups pooled together were: Chilopoda, Diptera adults, Embioptera, Enchytraeidae, Hemiptera adults, Hemiptera juveniles, Homoptera adults, Lepidoptera juveniles, Microhymenoptera, Nematoda, Odonata, Opilioacaridae, Orthoptera, Ostracoda, Palpigradrae, Peripatus, Scorpionidae, Tricoptera, Thysanura.

[na] not analysed

Appendix 4.5: Mean number of invertebrate individuals in several taxonomic groups per litter bag (of *C. racemosa* leaves) in the bags with roots for each retrieval time in the LERF. Values are means ± SD.

	Days of retrieval						
Taxonomic groups	30 d	60 d	120 d	180 d	270 d	360 d	Total
Acarina	41±19	125±79	354±757	118±58	166±61	237±105	170±318
Collembola	29±30	13±16	14±14	20±22	30±19	38±20	24±22
Pseudoscorpiones	0.8±0.7	1.1±1.3	1.2±1.4	0.9±1.0	2.5±1.4	1.4±1.1	1.3±1.2
Formicidae	0.6±0.8	3.7±8.4	2.8±0.8	1.2±1.3	22±47	1.4±2.0	4.9±19
Dipteran larvae	1.0±1.4	1.8±1.5	0.6±1.0	0.7±0.7	0.4±0.5	1.6±1.7	1.0±1.3
Copepoda	8.8±7.5	4.9±3.4	0.1±0.3	0.0±0.0	0.1±0.3	0.2±0.4	2.7±5.0
Psocoptera	0.8±1.1	0.0±0.0	0.0±0.0	0.1±0.3	0.1±0.3	0.0±0.0	0.2±0.6
Diplopoda	0.2±0.4	1.0±1.4	1.3±1.2	0.4±0.5	0.6±1.1	0.9±1.2	0.7 ±1.0
Aranae	32±103*	0.7±1.0	1.3±1.7	0.6±0.5	1.9±2.8	0.7±0.9	7.3±47
Homoptera juveniles	0.0±0.0	0.8±2.0	0.2±0.4	3.0±5.3	2.0±2.1	14±25	3.2±11
Thysanoptera	0.4±0.7	0.8±1.1	0.7±0.9	0.1±0.3	0.1±0.3	0.7±1.3	0.5±0.9
Coleoptera adults	0.2±0.4	0.3±0.5	0.8±1.3	0.1±0.3	0.4±0.5	1.2±1.9	0.5±1.0
Isopoda	0.1±0.3	0.3±0.5	1.2±1.8	0.2±0.5	0.9±1.1	0.2±0.4	0.5±1.0
Coleoptera larvae	0.2±0.4	0.1±0.3	0.4±0.7	0.0±0.0	0.7±0.1	0.4±0.5	0.3±0.6
Diplura	0.2±0.4	0.2±0.4	1.3±1.6	0.5±0.9	1.0±1.1	1.2±1.9	0.7±1.2
Opiliones	0.2±0.4	0.0±0.0	0.2±0.4	0.0±0.0	1.3±2.4	0.4±0.5	0.3±1.0
Protura	0.0±0.0	0.1±0.3	0.0±0.0	1.6±2.0	2.2±2.5	1.4±2.1	0.8±1.7
Pauropoda	0.0±0.0	0.0±0.0	0.1±0.3	0.1±0.3	0.9±2.5	0.3±0.5	0.2±1.0
Symphyla	0.0±0.0	0.0±0.0	0.1±0.3	0.1±0.3	0.5±1.1	0.6±0.7	0.2±0.6
Earhworms	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.7	0.07±0.3
Isoptera	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.1±3.2	0.0±0.0	0.2±1.2
Others**	0.6±0.7	0.9±1.3	1.4±1.7	1.4±1.4	2.0±2.1	2.2±1.7	1.4±1.6
TOTAL	116±93	155±97	382±758	150±76	237±120	305±117	221±322
No. of groups	7.3±1.4	8.2±2.4	9.0±3.1	8.4±2.8	11±2.1	12±2.7	9.2±2.9
n	11	9	9	8	8	9	54

* 1 nest in one of the samples

** the twenty minor taxonomic groups pooled together were: Chilopoda, Diptera adults, Embioptera, Enchytraeidae, Hemiptera adults, Hemiptera juveniles, Homoptera adults, Lepidoptera juveniles, Microhymenoptera, Nematoda, Odonata, Opilioacaridae, Orthoptera, Ostracoda, Palpigradae, Peripatus, Scorpionidae, Tricoptera, Thysanura.

Appendix 4.6: Mean number of invertebrate individuals in several taxonomic groups per litter bag (of *C. racemosa* leaves) in the bags without roots for each retrieval time in the LERF. Values are means ± SD.

	Days of retrieval						
Taxonomic groups	30 d	60 d	120 d	180 d	270 d	360 d	Total
Acarina	28±25	61±10	54±27	47±50	46±20	31±16	43±32
Collembola	2.0±1.4	12±13	3.1±2.1	7.0±8.2	7.0±5.0	8.0±4.4	5.4±6.4
Pseudoscorpiones	0.0±0.0	0.0±0.0	0.3±0.5	0.5±0.5	0.7±0.6	0.0±0.0	0.3±0.4
Formicidae	0.0±0.0	0.0±0.0	0.3±0.7	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.4
Dipteran larvae	2.5±2.1	1.3±1.5	1.4±1.2	0.7±1.0	0.0±0.0	0.3±0.6	0.9±1.2
Copepoda	4.0±1.4	22±6.5	1.2±1.6	0.3±0.8	1.0±1.0	4.7±6.4	3.6±7.1
Psocoptera	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.3±1.5	0.3±0.6	0.2±0.6
Diplopoda	0.5±0.7	1.7±1.5	2.6±3.5	2.2±2.7	1.0±0.0	3.3±4.1	1.9±2.7
Aranae	0.5±0.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.07±0.3
Homoptera juveniles	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.03±0.2
Thysanoptera	1.0±1.4	1.0±1.0	0.1±0.3	0.2±0.4	0.0±0.0	0.7±1.1	0.3±0.7
Coleoptera adults	0.0±0.0	0.3±0.6	0.1±0.3	0.3±0.5	0.3±0.6	0.3±0.6	0.2±0.4
Isopoda	0.0±0.0	0.3±0.6	0.4±0.5	0.7±1.0	0.7±1.1	0.7±1.1	0.4±0.7
Coleoptera larvae	0.5±0.7	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.3±0.6	0.1±0.3
Diplura	0.5±0.7	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.07±0.3
Opiliones	0.0±0.0	0.3±0.6	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.07±0.3
Protura	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Pauropoda	0.0±0.0	0.0±0.0	0.1±0.03	0.0±0.0	0.0±0.0	0.0±0.0	0.03±0.2
Symphyla	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Earhworms	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.03±0.2
Isoptera	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Others*	0.0±0.0	0.0±0.0	0.4±0.7	1.2±2.4	0.0±0.0	0.3±0.6	0.4±1.2
TOTAL	40±32	101±14	64±27	60±58	58±25	51±24	57±39
No. of groups	6.5±2.1	6.3±1.5	6.0±1.2	4.3±2.9	5.7±0.6	6.0±2.6	5.1±2.5
n	2	3	9	6	3	3	29

* the twenty minor taxonomic groups pooled together were: Chilopoda, Diptera adults, Embioptera, Enchytraeidae, Hemiptera adults, Hemiptera juveniles, Homoptera adults, Lepidoptera juveniles, Microhymenoptera, Nematoda, Odonata, Opilioacaridae, Orthoptera, Ostracoda, Palpigradrae, Peripatus, Scorpionidae, Tricoptera, Thysanura.