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Soil biochemical and microbial indices in wet tropical forests: Effects of deforestation and cultivation

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Summary – Zusammenfassung

Little information is available about the long-term effects of deforestation and cultivation on biochemical and microbial properties in wet tropical forest soils. In this study, we evaluated the general and specific biochemical properties of soils under evergreen, semi-evergreen, and moist deciduous forests and adjacent plantations of coconut, arecanut, and rubber, established by clear felling portions of these forests. We also examined the effects of change in land use on microbial indices and their interrelationships in soils.

Significant differences between the sites occurred for the biochemical properties reflecting soil microbial activity. Microbial biomass C, biomass N, soil respiration, N mineralization capacity, ergosterol, levels of adenylates (ATP, AMP, ADP), and activities of dehydrogenase and catalase were, in general, significantly higher under the forests than under the plantations. Likewise, the activities of various hydrolytic enzymes such as acid phosphomonoesterase, phosphodiesterase, casein-protease, BAA-protease, ß-glucosidase, CM-cellulase, invertase, urease, and arylsulfatase were significantly higher in the forest soils which suggested that deforestation and cultivation markedly reduced microbial activity, enzyme synthesis and accumulation due to decreased C turnover and nutrient availability. While the ratios of microbial biomass C : N and microbial biomass C : organic C did not vary significantly between the sites, the ratios of ergosterol : biomass C and ATP : biomass C, *q*CO2 and AEC (Adenylate Energy Charge) levels were significantly higher in the forest sites indicating high energy requirements of soil microbes at these sites.

Biochemische und mikrobiologische Indizes in Böden feuchter Tropenwälder: Auswirkungen von Rodung und Inkulturnahme

Über die langfristigen Auswirkungen von Waldrodung und Kultivierung auf die biochemischen und mikrobiellen Eigenschaften von Waldböden der feuchten Tropen ist wenig bekannt. In dieser Arbeit untersuchten wir die allgemeinen und speziellen biochemischen Bodeneigenschaften unter immergrünen, halb-immergrünen und feuchten Laubwäldern und unmittelbar benachbarten Plantagen von Kokosnuss, Arekanuss und Gummi, die auf Rodungsflächen dieser Wälder angelegt sind. Außerdem untersuchten wir die Auswirkungen dieser Landnutzungsänderungen auf mikrobiologische Indizes und entsprechende Wechselbeziehungen in den Böden.

Zwischen den Standorten zeigten sich signifikante Unterschiede hinsichtlich der biochemischen Eigenschaften, welche die mikrobielle Aktivität im Boden widerspiegeln. Die Gehalte an C und N in der mikrobiellen Biomasse, Bodenatmung, N-Mineralisationskapazität, Ergosterol, Adenylatgehalte (ATP, AMP, ADP) sowie die Dehydrogenase- bzw. Kataloseaktivitäten waren im allgemeinen auf den Waldstandorten signifikant höher als in den Plantagen. In gleicher Weise waren die Aktivitäten verschiedener hydrolytischer Enzyme wie saure Phosphomonoesterase, Phosphodiesterase, Casein-Protease, BAA-Protease, ß-Glucosidase, CM-Cellulase, Invertase, Urease und Arylsulfatase in den Waldböden signifikant höher. Dies legt nahe, dass nach Waldrodung und Inkulturnahme wegen geringerer C-Umsätze und Nährstoffverfügbarkeit die mikrobielle Aktivität, Enzymaktivität, Enzymsynthese und -akkumulation deutlich herabgesetzt ist. Während die Verhältnisse C_{mik} : N und C_{mik} : C_{org} zwischen den Standorten kaum variierte, waren die Verhältnisse Ergosterol : C_{mik} und ATP : C_{mik} sowie die Höhe von *q*CO2 and AEC (Adenylate Energy Charge) unter Wald signifikant höher. Dies ist ein Hinweis auf die hohen Energieansprüche der Bodenmikroorganismen an diesen Standorten.

Key words: soil biochemical properties / soil microbial activity / soil enzymes / adenylates / wet tropical forests / deforestation

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1 Introduction

Large areas of forests in the tropics are presently undergoing changes owing to anthropogenic influences like human settlement, change in land use, substantial clearance, and conversion into agricultural land etc. Understanding these long-term effects on forest soils is difficult since parameters like tree growth and soil organic matter are slow in revealing changes (*Staddon* et al., 1998). A more practical approach would be to assess soil quality using microbial indicators, especially those that are related to nutrient transformation and availability (*Pankhurst* et al., 1997). Since soil microorganisms are the driving force for nutrient supply in soils (*Smith* and *Paul*, 1990), any kind of environmental anthropogenic perturbation can lead to distinct effects on the abundance, composition, and activity of microorganisms responsible for nutrient transformations in soils.

Though numerous studies on the soil microbial properties of temperate (*Leirós* et al., 2000; *Smolander* and *Kitunen*, 2002; *Zhong* and *Makeschin*, 2003) and tropical (*Salamanca* et al., 2002; *Menyailo* et al., 2003) forest systems have been made, little is known about the changes induced by clearance and conversion of forests for cultivation. Major detrimental effects of forest clearance for agricultural use are the increase in soil

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degradation, decrease in biodiversity, and loss of ecosystem structure. Besides, it affects the quantity and quality of the potential organic inputs to soil through modification of the microclimatic condition at the ground level. Information on biochemical and microbial indices of such soils subjected to long-term disturbance, especially in the tropics would provide valuable insight into the extent of soil deterioration, help evolve or adopt management strategies that contribute to retention of soil quality, besides setting the limits for its exploitation.

The wet tropical forests of South Andaman are categorized into Evergreen, Semi-Evergreen, and Moist Deciduous. These forests are rich in biodiversity with almost 2500 angiospermic species belonging to 682 genera and 137 families of which 14 % are endemic, 54 % are common with other parts of India, and 32 % are of extra-Indian origin (*Champion* and *Seth*, 1968). Portions of these forests were clear-cut to establish plantations of coconut, arecanut, and rubber during the late 1960s. The major objective of this study was to assess the influence of such changes in land use on various soil biochemical indices in the A horizon of three major tropical forests and three adjacent plantations of coconut, arecanut, and rubber established by clearing a portion of evergreen, semi-evergreen, and moist deciduous forests respectively.

2 Materials and methods

2.1 Site details

The study sites were located in South Andamans, India (10°30′–13°42′N latitude and 92°14′–94°16′E longitude). The climate is wet tropical with a total rainfall of 3100 mm spread over seven months from May to November. The mean annual temperature is 30.1 °C, with a minimum of 18.6 °C and a maximum of 33.0 °C. The relative humidity varies from 63–90 %.

The wet tropical forests of S. Andaman are categorized into Evergreen (EG), Semi-Evergreen (SE), and Moist Deciduous (MD). The site characteristics of the soils under these forests are given in Tab. 1. The soils of all the sites are Inceptisols developed from the same parent material *viz*., sand stones and shales, but are covered at present with different vegetation. The soil at 0–6 cm depth is dark yellowish brown, sandy loam, sub-angular blocky, and non-sticky. At 6–40 cm, it is pale brown, clay loam, sub-angular blocky, and slightly to medium sticky. Portions of the forests were cleared to establish coconut, arecanut, and rubber plantations at varying periods of time. The plantations of coconut (stand age 31–33 yr) were developed by clearing moist deciduous forest, arecanut (stand age 24–25 yr) by clearing semi-evergreen forests and rubber (stand age 20–27 yr) by clearing evergreen forests (Tab. 1). The forests were clear-felled manually and the dried plant biomass burnt. Unburnt wood was removed before planting of the respective trees. The interspaces of coconut and arecanut are either barren or contain weeds like *Mikania cordata*, *Eupatorium odoratum*, and *Euphorbia hirta*, while the interspace of rubber is occupied by thick ground vegetation consisting of mainly *Pueraria phaseoloides, Dioscorea oppositifolia, D. alata, Asparagus racemosus*, and *Momordica dioica*. Fertilization, through either chemical or organic sources, in these plantations has not been done for the past five years and when done was confined only to the basins of the respective trees. However, soil samples from these plantations were always drawn from a distance of 5 m from the edge of the bases of the respective trees.

2.2 Soil sampling

For the study, four locations in the forest type and the adjacent plantations were selected and soil cores (0–30 cm) were taken from 15 randomly selected spots distributed over an area of 10 ha from each location. The four locations were treated as replications of each forests and plantation types. The total number of samples collected for each vegetation type was therefore 60, i.e. 15 spots × 4 replications. The soil samples were cleared of any organic debris and transferred for storage in sealed plastic bags. All samples were collected after removal of the litter layer (1.0–1.2 cm and < 0.5 cm in

Table 1: Site characteristics of soils under forests and plantations.
Tabelle 1: Standort- und Bodeneigenschaften unter Wald und Plantage.

Forest type	Predominant tree species				Adjacent plantation
	I storey	II storey	III storey	Ground storey	
Evergreen	*Dipterocarpus alatus* *Dipterocarpus gracilis* *Artocarpus chaplasha* *Calophyllum soulatri*	*Amoora wallichi* *Pterocymbium tinctorium* *Pterocarpus dalbergioides* *Sageraea elliptica* *Artocarpus gomeziana*	*Pometia pinnata* *Mesua ferrea* *Terminalia manii* *Myristica andamanica*	*Sterculia villosa* *Artocarpus lakoocha* *Baccaurea sapida* *Agalia andamanica*	Rubber (20–27)[a]
Semi-Evergreen	*Dipterocarpus pilosus* *Artocarpus chaplasha* *Pterogyta alata* *Planchonia andamanica*	*Pterocarpus dalbergioides* *Amoora wallichi* *Lannea coromandelica* *Podocarpus nerifolia*	*Lagersatroemia hypoleuca* *Dillenia pentagyna* *Lannea coromandelica* *Albizzia lebbek*	*Moosa andamanica* *Baccaurea sapida* *Myristica spp* *Saprosma ternatyum*	Arecanut (24–25)[a]
Moist Deciduous	*Canarium euphyllum* *Tetrameles nudiflora* *Salmalaia insignis* *Ganophyllum falcatum*	*Diospyros marmorata* *Sageraea elliptica* *Amoora wallichi* *Terminalia bialata*	*Nauclea gageana* *Adenanthera pavonica* *Pterocymbium tinctorium* *Anthocephalus cadamba*	*Podocarpus neriifolia* *Artocarpus gomeziana* *Ventilago madraspatana* *Buttneria andamanensis*	Coconut (31–33)[a]

[a] Stand age

Table 2: Relevant properties of soils under forests and plantations (Values are means of 60 determinations; standard errors are given in parentheses; values in one row followed by the same letter are not significantly different from each other at $P < 0.05$).
Tabelle 2: Wichtige Bodeneigenschaften unter Wald und Plantage (Die Zahlen sind Mittelwerte von 60 Bestimmungen; Standardfehler in Klammern; Werte in einer Reihe gefolgt von demselben Buchstaben unterscheiden sich nicht signifikant voneinander, $P < 0.05$).

	Forests[a]			Plantations[b]		
	EG	SE	MD	C	A	R
pH (1:2.5 $CaCl_2$)	5.12 (0.22)	5.30 (0.24)	5.24 (0.17)	5.15 (0.21)	5.21 (0.19)	5.02 (0.24)
Clay (%)	28 (4)	29 (4)	26 (6)	26 (5)	29 (7)	31 (5)
CEC ($\mu mol_c\ g^{-1}$)	365 (17)	377 (20)	376 (21)	364 (17)	365 (12)	361 (22)
Total N ($g\ kg^{-1}$)	1.41 (0.30) b	1.54 (0.29) b	2.13 (0.26) a	0.83 (0.16) d	0.86 (0.14) d	1.05 (0.12) c
Organic C ($g\ kg^{-1}$)	14.4 (2.3) b	16.4 (1.9) b	22.4 (2.6) a	6.9 (1.2) d	7.6 (1.3) d	10.8 (1.3) c
Bray P ($g\ kg^{-1}$)	0.008 a	0.008 a	0.009 a	0.003 b	0.003 b	0.004 b
K ($g\ kg^{-1}$)	0.19 a	0.20 a	0.23 a	0.06 b	0.07 b	0.07 b
Ca ($g\ kg^{-1}$)	0.038	0.037	0.036	0.031	0.036	0.034
Mg ($g\ kg^{-1}$)	0.041	0.043	0.043	0.038	0.049	0.039
Al_2O_3 (%)	0.73	0.76	0.71	0.71	0.73	0.81
Fe_2O_3 (%)	0.75	0.79	0.78	0.84	0.80	0.81

[a] Forests: EG-Evergreen, SE-Semi-evergreen, MD-Moist deciduous; [b] Plantations: C-Coconut, A-Arecanut, R-Rubber

the forest and plantation sites, respectively) and after the end of monsoon (in December–January 2001). The soils were then sieved (< 2 mm), analyzed for their moisture content on the day of collection, and stored at 4 °C for not more than one week before analyses. Sub samples for the determination of organic C and total N were sieved to pass a 0.5-mm mesh.

2.3 Soil physical and chemical properties

The relevant properties of the soil samples studied are given in Tab. 2. The soil pH was determined in 1:2.5 soil : water suspension. Total N was determined by the regular Kjeldahl method of *Bremner* and *Mulvaney* (1982), organic C by the modified Mebius method (*Nelson* and *Sommers*, 1982), clay content by the pipette method (*Gee* and *Bauder*, 1986), and cation exchange capacity by the method of *Gillman* (1979). Available K was determined by the ammonium acetate method (*Thomas*, 1982), Bray P using the dilute acid-fluoride extractant (*Olsen* and *Sommers*, 1982), and soil Al and Fe were estimated using the spectrophotometric methods described by *Barnhisel* and *Bertsch* (1982) and *Olson* and *Ellis* (1982), respectively.

2.4 General biochemical properties

Basal respiration was measured in soil samples (adjusted to 55 % water-holding capacity) by pre-incubating for 3 days at 20 °C in the dark. The CO_2 production was finally measured for another three days by trapping in 0.05 M NaOH and titration of the excess NaOH with 0.05 M HCl. The metabolic quotient (qCO_2) was calculated as follows:

$$\frac{\mu g CO_2 - C \text{ evolved in 3 days } (g \text{ soil})^{-1}}{\left[\left(\mu g \text{ biomass C } (g \text{ soil})^{-1}\right) / 3 \text{ days}\right] \times 1000}$$

$$= \mu g\ CO_2 - C\ (mg \text{ biomass C})^{-1} day^{-1}$$

The results are expressed in mg CO_2-C (g biomass C)$^{-1}$ day^{-1}.

The microbial biomass C and N were estimated by fumigation-extraction (*Vance* et al., 1987; *Brookes* et al., 1985). Briefly, two portions of moist soil (equivalent to 25 g oven-dry soil) were taken from 100 g moist soil used for measuring basal respiration. One portion was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$ and the other portion was kept as such. Both the portions were then extracted with 100 ml 0.5 M K_2SO_4 for 30 min by oscillating shaking at 200 rpm and filtered (Schleicher and Schuell 595). Organic C was determined in the extracts and microbial biomass C was calculated as follows: microbial biomass C = E_C/k_{EC}, where E_C is the difference between organic C extracted from fumigated soils and organic C extracted from non-fumigated soils and k_{EC} = 0.45 (*Joergensen*, 1995). The extracts were also analyzed for their total N content, and microbial biomass N was calculated as follows: Microbial biomass N = E_N/k_{EN}, where E_N is the difference between total N extracted from fumigated soils and total N extracted from non-fumigated soils and k_{EN} = 0.54 (*Brookes* et al., 1985; *Joergensen* and *Mueller*, 1996).

Ergosterol was determined in 2 g moist soil taken from the 100-g soil sample used for measuring basal respiration. This sample was extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 revolutions min^{-1} (*Djajakirana* et al., 1996). Ergosterol contents in the extracts were then quantitatively determined by reversed-phase HPLC analysis as described by *Joergensen* and *Castillo* (2001). Determination of adenine nucleotides and calculation of AEC (adenylate energy charge) were done according to the procedure of *Dyckmans* and *Raubuch* (1997). The adenylates were determined in a 4 g moist soil taken from the 100-g soil sample used for measuring basal respiration. Dimethylsulphoxide (DMSO), Na_3PO_4 (10mM) buffer + EDTA (20 mM) at pH 12 and NRB (Nucleotide Releasing Buffer for microbial ATP, Cel-

sis) were used as extractants. After derivatization with chloracetaldehyde, the adenine nucleotides were determined by HPLC as described by *Joergensen* and *Castillo* (2001). The energetic status of soil microorganisms was evaluated by determining the adenylate energy charge (AEC) (*Brookes* et al., 1983), which is defined as follows: AEC = (ATP + 0.5 × ADP)/(ATP + ADP + AMP).

Nitrogen mineralization capacity was determined by extracting 10 g soil with 50 ml of 2 M KCl for 30 minutes before and after incubation for 10 days at 30 °C with optimal moisture content (i.e. water retained at 33 kPa). The NH_4^+-N and total inorganic N were determined in the extracts by steam distillation (*Keeney* and *Nelson*, 1982). The difference between the values obtained before and after incubation indicates nitrogen mineralization capacity (*Leirós* et al., 2000). Arginine ammonification rate was determined by the addition of 0.5 ml of 2 g l^{-1} arginine solution to 2 g soil, incubating for 3 h at 30 °C at optimal moisture content, extracting with 8 ml of 2 M KCl (*Alef* and *Kleiner*, 1986) and determination of ammonium in the extracts. The arginine ammonification rate was calculated as the difference between the values of the arginine-treated samples and the untreated samples.

Dehydrogenase activity was estimated by the addition of 3 % aqueous solution of 2,3–5 Triphenyltetrazolium chloride (TTC) to 6 g soil, incubation at 37 °C for 24 h and extraction of triphenyl formazan (TPF) with methanol (*Casida* et al., 1964). Catalase activity (EC 1.11.1.6) was determined by suspending 0.5 g soil sample in a mixture of 40 ml distilled water and 5 ml 0.3 % H_2O_2, stirring for 10 min at room temperature, addition of 5 ml of 3 M H_2SO_4, and estimation of the residual H_2O_2 by titration with 0.1 M $KMnO_4$ (*Johnson* and *Temple*, 1964).

2.5 Specific biochemical parameters

Urease (EC 3.5.1.5) was assayed by incubating soil with an aqueous urea solution (for 2 h at 37 °C), extracting NH_4 with 1 M KCl and 10 mM HCl, and analyzing NH_4^+ colorimetrically by a modified indophenol reaction (*Kandeler* and *Gerber*, 1988). Proteases (EC 3.4.4) hydrolyzing benzoyl argininamide (BAA-protease) and casein (casein-protease) were determined as described by *Gil-Sotres* et al. (1992). BAA-protease activity was determined using a-benzoyl-N-argininamide (BAA) as substrate, and the NH_4^+ released was estimated by steam distillation. Casein-hydrolyzing activity was determined with casein as the substrate, and the amino acids released were measured by the Folin colorimetric method.

Acid phosphomonoesterase (EC 3.1.3.2) activity was determined by incubating soils at 37 °C for 1 h in modified universal buffer (pH 6.5) with *p*-nitrophenyl phosphate as the substrate (*Tabatabai* and *Bremner*, 1969). Activity of ß-glucosidase (EC 3.2.1.21) was determined as above, but by using *p*-nitrophenyl-ß-D glucopyranoside as the substrate (*Eivazi* and *Tabatabai*, 1988). Phosphodiesterase (EC 3.1.4.1) activity was assayed by incubating soils at pH 8.0 (THAM buffer) and 37 °C for 1 h with bis-*p*-nitrophenyl phosphate as the substrate (*Browman* and *Tabatabai*, 1978). Arylsulfatase (EC 3.1.6.1) activity was determined by incubating soils at pH 5.8 (acetate buffer) and 37 °C for 1 h with p-nitrophenyl sulfate as substrate (*Tabatabai* and *Bremner*, 1970). The amount of *p*-nitrophenol released in all these cases was estimated spectrophotometrically.

Invertase (EC 3.2.1.26) activity was determined with saccharose as substrate followed by incubation for 3 h at 50 °C (acetate buffer) and determining reducing sugars as described by *Schinner* and *von Mersi* (1990). Carboxymethylcellulase activity was determined as above, but by using carboxymethylcellulose as substrate and incubating for 24 h (*Schinner* and *von Mersi*, 1990).

2.6 Statistics

Analyses were performed on all soil samples, and the values (mean ± S.E.) reported are means of 60 determinations (15 × 4 replications) expressed on an oven-dry soil basis (24 h at 105 °C). The significance of treatment effects was determined by one-way analysis of variance. Where the F values were significant, post hoc comparisons were made using the least significant difference test at the 0.05 probability level. The interdependence of the various soil variables and biochemical parameters were determined by principal component analysis (PCA) using the Varimax rotation to achieve either small or large component loading and an Eigen value of 0.1 as the lower limit. All statistical analyses were performed using Statistica 5.1 (*Statsoft*, 1997).

3 Results and discussion

Soil pH values were acidic and ranged between 5.12–5.30 (Tab. 2). The cation exchange capacity (CEC) also showed minimum variation between the sites, mean CEC being 373 and 363 $\mu mol_c\ g^{-1}$ under the forests and plantations, respectively. Likewise, the clay content did not differ significantly between the sites and was 28 % in the forests and 29 % in the plantation soils. In contrast, soil organic C and total N levels were significantly higher under the forests (mean 17.7 and 1.69 mg g^{-1}, respectively) than the corresponding levels in the plantation soils (mean 8.4 and 0.91 mg g^{-1}, respectively). Similar differences between the sites also occurred for the levels of Bray P and K. At the forest sites, the levels of Bray P (mean 0.82 mg (100 g)$^{-1}$) and K (mean 20.7 mg (100 g)$^{-1}$) significantly exceeded the corresponding levels in the plantation sites (0.33 and 6.8 mg (100 g)$^{-1}$, respectively). The levels of Ca, Mg, Al_2O_3, and Fe_2O_3, however, did not vary significantly between the sites.

3.1 General biochemical parameters

The mean soil microbial biomass C under the forests was 511 ± 98 mg C kg^{-1} (Tab. 3). The biomass C levels in the plantations (mean 188 ± 71 mg C kg^{-1}) were significantly lower than the corresponding levels in the forests indicating a marked decline due to deforestation and cultivation. This can be attributed to reduced input of plant residues due to absence of fresh overstory litter in the plantation sites. Since microbial biomass in forest soils is largely governed by litter fall (*Trofymow*, 1998), greater microbial biomass C in the

Table 3: Values of the general biochemical parameters in soils under forests and plantations (Values are means of 60 determinations; standard errors are given in parentheses; values in one row followed by the same letter are not significantly different from each other at $P < 0.05$).
Tabelle 3: Werte der allgemeinen biochemischen Kenngrößen unter Wald und Plantage (die Zahlen sind Mittelwerte von 60 Bestimmungen; Standardfehler in Klammern; Werte in einer Reihe gefolgt von demselben Buchstaben unterscheiden sich nicht signifikant voneinander, $P < 0.05$).

	Forests[a]			Plantations[b]		
	EG	SE	MD	C	A	R
Microbial biomass C (μg C g^{-1})	422 (26) c	494 (18) b	617 (23) a	145 (8) e	148 (11) e	270 (14) d
Microbial biomass N (μg N g^{-1})	34.8 (4.2) b	45.9 (4.3) a	49.4 (5.1) a	15.2 (3.3) d	16.3 (3.6) d	21.2 (3.1) c
Soil respiration (μg CO_2-C g^{-1} day^{-1})	11.9 (2.1) c	14.4 (1.8) b	26.6 (2.2) a	2.2 (0.6) e	2.3 (0.4) e	5.2 (0.7) d
Ergosterol (μg g^{-1})	2.61 (0.33) bc	3.20 (0.29) ab	4.10 (0.35) a	0.43 (0.06) e	0.44 (0.04) e	0.80 (0.09) d
ATP (nmol g^{-1})	2.71 (0.43) a	2.86 (0.54) a	2.81(0.57) a	0.64 (0.09) c	0.64 (0.10) c	1.21 (0.14) b
AMP (nmol g^{-1})	0.28 (0.06) a	0.28 (0.04) a	0.29 (0.06) a	0.13 (0.04) c	0.15 (0.04) c	0.22 (0.06) b
ADP (nmol g^{-1})	0.42 (0.11) b	0.56 (0.13) a	0.51 (0.09) a	0.21 (0.05) d	0.22 (0.06) d	0.34 (0.06) c
Dehydrogenase (nmol TPF g^{-1} h^{-1})	252 (11) b	262 (17) b	389 (21) a	170 (15) d	169 (18) d	221 (19) c
Catalase (mmol H_2O_2 consumed g^{-1} h^{-1})	1.67 (0.29) b	2.31 (0.26) a	2.62 (0.31) a	0.56 (0.09) d	0.60 (0.11) d	1.31 (0.24) c
NH_4-N mineralized (mg N kg^{-1} (10 d)$^{-1}$)	114 (18) b	125 (21) b	142 (19) a	37 (7) d	36 (7) d	69 (11) c
Tot. inorganic N mineralized (mg N kg^{-1} (10 d)$^{-1}$)	127 (19) b	135 (18) b	152 (22) a	44 (8)d	45 (6) d	83 (14) c
Arginine ammonification (μg NH_4-N g^{-1} h^{-1})	2.1 (0.5) b	2.5 (0.4) b	3.5 (0.5) a	1.1 (0.3) d	1.1 (0.3) d	1.6 (0.4) bc

[a] Forests: EG-Evergreen, SE-Semi-evergreen, MD-Moist deciduous; [b] Plantations: C-Coconut, A-Arecanut, R-Rubber

undisturbed sites reflects greater accumulation of plant residues and organic C, which are substrates for soil microbes (*García-Gil* et al., 2000).

Soils under forests exhibited significantly greater levels of biomass N (mean 43.4 ± 7.6 mg N kg^{-1}) than the corresponding levels in the plantations (mean 17.6 ± 3.2 mg N kg^{-1}; Tab. 3). Higher biomass N under the forest is apparently due to greater microbial activity in response to enhanced organic matter content in these soils. In the present study, soil respiration measured as CO_2 evolution was on an average 17.6 ± 7.9 μg CO_2-C g^{-1} day^{-1} under the forests. The CO_2 evolution in the plantation soils was significantly lower (mean 3.2 ± 1.7 μg CO_2-C g^{-1} day^{-1}; Tab. 3), the lowest values corresponded to those sites with low microbial biomass C. Therefore, significantly higher CO_2 evolution in the forest soils indicated higher microbial activity (*Chander* et al., 1998) in response to higher levels of readily degradable C content. Markedly lower values in the plantation soils were due to a severe depletion of readily decomposable substrates because it has been suggested that substrate availability was the principal determinant to soil respiration (*Wang* et al., 2003). The data on ATP, AMP, and ADP also indicated greater microbial activity under the forests (mean 2.79, 0.28, 0.50 nmol g^{-1}, respectively) compared to the corresponding levels in the plantations (0.83, 0.17, 0.26 nmol g^{-1}, respectively). The inorganic N was predominantly NH_4^+-N, and the soils in the forests registered significantly higher total inorganic N and NH_4^+-N (mean 138 ± 13 and 127 ± 14 mg N kg^{-1} (10 d)$^{-1}$, respectively) compared to the plantations (mean 57 ± 22 and 47 ± 19 mg N kg^{-1} (10 d)$^{-1}$, respectively; Tab. 3). The arginine ammonification rate was also greater under the forests (range 2.7 ± 0.7 μg NH_4-N g^{-1} h^{-1}) compared to the plantations (1.3 ± 0.3 μg NH_4-N g^{-1} h^{-1}; Tab. 3) and reflects the high ammonification capacity of soils under the former.

The data also revealed significantly higher soil dehydrogenase and catalase activities in the forests (Tab. 3). The mean dehydrogenase activity under the forests (301 ± 76 nmol TPF g^{-1} h^{-1}) was markedly higher compared to the mean activity in the plantations (187 ± 30 nmol TPF g^{-1} h^{-1}). Similarly, higher input of organic residues must have stimulated the synthesis of catalase resulting in a significantly higher activity under the forests (mean 2.20 ± 0.48 mmol H_2O_2 consumed g^{-1} h^{-1}) compared to the plantations (mean 0.82 ± 0.42 mmol H_2O_2 consumed g^{-1} h^{-1}; Tab. 5). Higher levels of basal respiration, metabolic quotient, ATP, N mineralization, and dehydrogenase and catalase activities in the forest sites reflect the response of more input of substrate C and organic matter since these are microbial processes that are influenced both by the quantity and quality of soil organic matter (*Scott* and *Binkley*, 1997; *Leirós* et al., 2000).

3.2 Specific biochemical parameters

The results on specific biochemical parameters (Tab. 4) were identical to those observed in case of the general biochemical parameters. All the enzymes exhibited significantly higher activity in the forest sites. At these sites, the mean activities of phosphomonoesterase and phosphodiesterase was 14.2 ± 1.3 and 3.8 ± 0.5 μmol *p*-nitrophenol g^{-1} h^{-1}, respectively, markedly higher than the corresponding activities in the plantation sites (5.9 ± 2.2 and 1.7 ± 0.6 μmol *p*-nitrophenol g^{-1} h^{-1}, respectively; Tab. 4). The forest sites also registered significantly higher β-glucosidase and arylsulfatase activities (mean 4.7 ± 0.7 and 0.61 ± 0.11 μmol p-nitrophenol g^{-1} h^{-1}, respectively) than the corresponding mean levels in the plantation sites (2.3 ± 0.8 and 0.40 ± 0.08 μmol p-nitrophenol $g^{-1}h^{-1}$, respectively). Also, invertase and CM-cellulase activities in the plantation sites were significantly lower compared

Table 4: Values of the specific biochemical parameters in soils under forests and plantations (Values are means of 60 determinations; standard errors are given in parentheses; values in one row followed by the same letter are not significantly different from each other at $P < 0.05$).
Tabelle 4: Werte der spezifischen biochemischen Kenngrößen unter Wald und Plantage (Die Zahlen sind Mittelwerte von 60 Bestimmungen; Standardfehler in Klammern; Werte in einer Reihe gefolgt von demselben Buchstaben unterscheiden sich nicht signifikant voneinander, $P < 0.05$).

	Forests[a]			Plantations[b]		
	EG	SE	MD	C	A	R
Phoshomonoesterase (μmol *p*-nitrophenol g^{-1} h^{-1})	13.1 (2.2) b	14.0 (1.7) ab	15.6 (2.0) a	4.8 (0.8) d	4.4 (0.8) d	8.4 (1.1) c
Phosphodiesterase (μmol *p*-nitrophenol g^{-1} h^{-1})	3.4 (0.8) ab	3.7 (0.9) a	4.3 (0.9) a	1.4 (0.3) c	1.4 (0.3) c	2.4 (0.5) bc
Arylsulfatase (μmol *p*-nitrophenol g^{-1} h^{-1})	0.54 (0.11) bc	0.56 (0.10) b	0.74 (0.13) a	0.36 (0.04) d	0.35 (0.06) d	0.49 (0.08) c
ß-Glucosidase (μmol *p*-nitrophenol g^{-1} h^{-1})	4.4 (0.9) b	4.3 (1.0) b	5.5 (0.8) a	1.7 (0.4) d	2.1 (0.5) d	3.2 (0.6) c
Casein-protease (μmol tyrosine g^{-1} h^{-1})	2.5 (0.6) bc	2. 8 (0.6) b	3.6 (0.9) a	1.4 (0.4) e	1.6 (0.4) e	2.1 (0.6) cd
BAA-protease (μmol NH_3-N g^{-1} h^{-1})	7.2 (1.5) b	7.4 (1.4) b	8.4 (1.7) a	4.2 (0.9) d	4.1 (1.0) d	5.3 (1.2) c
Urease (μmol NH_3-N g^{-1} h^{-1})	7.8 (1.1) b	8.1 (1.6) b	9.7 (1.7) a	4.8 (0.9) d	5.1 (1.0) d	6.3 (1.0) c
CM cellulase (μmol glucose g^{-1} h^{-1})	0.61 (0.09) b	0.63 (0.10) b	0.83 (0.08) a	0.44 (0.04) d	0.42 (0.06) d	0.55 (0.06) bc
Invertase (μmol glucose g^{-1} h^{-1})	9.5 (1.5) b	10.5 (1.9) b	12.4 (1.6) a	3.7 (0.8) d	4.1 (0.9) d	6.3 (0.8) c

[a] Forests: EG-Evergreen, SE-Semi-evergreen, MD-Moist deciduous; [b] Plantations: C-Coconut, A-Arecanut, R-Rubber

to the forest sites. While the mean invertase activity in these sites was 4.7 ± 1.4 μmol glucose g^{-1} h^{-1}, mean CM-cellulase activity at these sites was 0.47 ± 0.07 μmol glucose g^{-1} h^{-1}, both significantly lower than the corresponding activities in the forest sites (10.8 ± 1.5 and 0.69 ± 0.12 μmol glucose g^{-1} h^{-1}, respectively). Similar reductions in the activities of casein-protease, BAA-protease, and urease were observed in the plantation sites. Average activities of urease (5.4 ± 0.8 μmol NH_3-N g^{-1} h^{-1}), casein-protease (1.7 ± 0.4 μmol tyrosine g^{-1} h^{-1}), and BAA-protease (4.5 ± 0.7 μmol NH_3-N g^{-1} h^{-1}) were significantly lower than the respective activities in the forest sites (8.5 ± 1.0 μmol NH_3-N g^{-1} h^{-1}; 3.0 ± 0.6 μmol tyrosine g^{-1} h^{-1}, and 7.7 ± 0.6 μmol NH_3-N g^{-1} h^{-1}, respectively). Accumulation and/or stabilization of organic matter (*Stemmer* et al., 1999), abundance of carbohydrates (*García-Gil* et al., 2000), and plant roots (*Schneider* et al., 2001) coupled with greater microbial activity (*Parham* et al., 2002) might explain the greater levels of enzyme activities in the forest sites.

The general and specific biochemical parameters, however, varied with vegetation type within the sites. Since the stands have established on the same geological formation and on soils that had been similar, the variations in soil microbial and enzyme activities can be attributed to differences in the amount and quality of the present and past substrate (*Haron* et al., 1998), total dissolved organic C and N (*Smolander* and *Kitunen*, 2002), species composition of the forest canopy (*Menyailo* et al., 2003) etc.

3.3 Ratios of various microbial indices

The ratio of microbial biomass C : organic C reflects the availability of substrate to the soil microflora and in reverse, it also reflects the fraction of recalcitrant organic matter in soil (*Joergensen* and *Scheu*, 1999). Though the mean level of biomass C : organic C under the forests (2.8 %) was slightly higher than the mean level under the plantations (2.2 %), the variation was, however, non-significant (Tab. 5). The mean biomass C : N ratio was 11.8 in the forest sites and 10.4 in the plantation sites indicating an increase due to disturbance. However, the biomass C : N ratio is not regarded as a sensitive indicator of shifts in the microbial community structure (*Joergensen* and *Scheu*, 1999). Further, the biomass C : N

Table 5: Ratios of the different soil microbial indices in the forests and plantations (Values in one row followed by the same letter are not significantly different from each other at $P < 0.05$).
Tabelle 5: Verhältnisse verschiedener bodenmikrobiologischer Kenngrößen unter Wald und Plantage (Werte in einer Reihe gefolgt von demselben Buchstaben unterscheiden sich nicht signifikant voneinander, $P < 0.05$).

	EG[a]	SE	MD	C	A	R
Microbial biomass C : organic C (%)	2.9	3.0	2.7	2.1	1.9	2.5
Microbial biomass N : total N (%)	2.5	3.0	2.3	1.8	1.9	2.0
Microbial biomass C:N	12.1	10.8	12.5	9.5	9.1	12.7
Ergosterol : microbial biomass C (%)	0.62 a	0.65 a	0.66 a	0.30 b	0.30 b	0.29 b
ATP : microbial biomass C (μmol g^{-1})	6.42 a	5.79 b	4.55 c	4.41 d	4.32 e	4.48 cd
AEC	0.86 a	0.85 a	0.85 a	0.76 b	0.74 b	0.78 b
qCO_2 (mg CO_2-C (g biomass C)$^{-1}$ (day)$^{-1}$)	28.2 b	29.1 b	43.1 a	15.1 c	15.5 c	19.2 c

[a] Site description given in Tab. 2

ratios at both sites consistently exceeded the corresponding soil organic C : N ratios (Tab. 5). This can be ascribed to low N availability and relatively higher organic matter availability to soil microbes (*Salamanca* et al., 2002) or increased fungal : microbial biomass ratio (*Joergensen* et al., 1995).

It has been suggested that large biomass C : N ratios are caused by increased fungal : microbial biomass ratios (*Joergensen* et al., 1995). We tested this by measuring the ergosterol levels at both the sites. Ergosterol is an important indicator of fungal biomass in soil (*Montgomery* et al., 2000) and the levels in the forest sites (range 2.61 to 4.10 μg g^{-1}; mean 3.30 ± 0.75) were considerably higher than the levels observed in the plantation sites (range 0.43 to 0.80 μg g^{-1}; mean 0.56 ± 0.21; Tab. 3). Similarly, the ergosterol : biomass C ratios in the forest sites (range 0.62–0.66 %) were significantly higher than the levels observed in the plantation sites (range 0.29–0.30 %; Tab. 5). If ergosterol content (Tab. 3) is converted into fungal biomass C by multiplication by a factor of 90 (*Djajakirana* et al., 1996), fungi represent 58 % of the total biomass C in the forest sites and only 27 % of the total biomass C in the plantation sites. This indicated that fungi contribute only a relatively small percentage to the microbial biomass in the plantations sites. However, it is also possible that the fungal biomass in our plantation sites have low ergosterol content as observed by *Salamanca* et al. (2002) in secondary tropical forest soils.

The metabolic quotient (qCO_2), often used as an indicator of maturity of a soil ecosystem (*Anderson* and *Domsch*, 1985), was markedly higher under the forests (mean 33.5 ± 8.3 mg CO_2-C (g biomass C)$^{-1}$ day^{-1}) compared to the plantations (16.6 ± 2.3 mg CO_2C (g biomass C)$^{-1}$ day^{-1}; Tab. 5). Though the metabolic quotient values are larger in microbial communities of young soils (*Insam* and *Domsch*, 1988), it is more likely that the availability of soil organic matter plays the dominant role (*Joergensen* and *Castillo*, 2001). Hence, relatively large qCO_2 in the forest sites is due to greater levels of readily degradable C content and indicates short turnover times of the microbial biomass and relatively large rates of substrate use to fulfill the energetic demand of the soil microflora (*Joergensen* and *Scheu*, 1999).

The ATP : biomass C ratios observed in the forest (range 4.55–6.42 μmol g^{-1}; mean 5.59 ± 0.95) were significantly higher than those observed in the plantation sites (range 4.32–4.48 μmol g^{-1}; mean 4.41 ± 0.08; Tab. 5). The ATP : biomass C ratios at all the sites were, however, lower than the average ratio of 11.7 μmol g^{-1} reported by *Contin* et al. (2001) by employing the enzymatic luciferin/luciferase system. Nevertheless, lower ATP : biomass C ratios of between 3.1 and 5.2 μmol g^{-1} in acid soils (pH 5.9–6.5) of secondary tropical forests of Philippines have been reported (*Salamanca* et al., 2002). They have excluded the possibility of incomplete extraction efficiency of the added ATP to be the major cause for this difference because of the high extraction efficiency (between 90 and 95 %) of the alkaline DMSO extractant (*Dyckmans* and *Raubuch*, 1997). The AEC (Adenylate Energy Charge) levels in the forest sites also significantly exceeded the levels in the plantation sites and were consistently greater than 8.0, indicating rapid microbial proliferation in these sites. High AEC levels coupled with high qCO_2 and larger ATP : biomass C ratios in the forest sites also suggest that the soil microbes at these sites have higher energy requirements compared to the plantation sites. This should mean a decrease in soil organic matter due to increased microbial consumption and turnover in the forests soils. But the soil organic matter contents were still higher under the forests compared to the plantation soils (Tab. 2). This apparent discrepancy might be due to greater input of C to the soils through regular litter fall under the forests.

3.4 Interrelationships of biochemical parameters and various soil properties

In order to describe overall patterns of interrelationships among individual soil properties and to extract common factors responsible for total variation, we performed a joint principal components analysis (PCA) which explained 80 % of the total variation of all soil chemical and biochemical properties examined with four principal components (Tab. 6). The

Table 6: Principal components loadings after Varimax rotation: The soil parameters are grouped according to the maximum fittings to the principal components (PC) (Correlation coefficients > 0.60; $n = 360$).
Tabelle 6: Ladungen der Hauptkomponenten (PC) nach Varimax-Rotation: Die Bodenkenngrößen sind angeordnet nach ihrem maximalen Fitting in den vier Hauptkomponenten (Korrelationskoeffizienten > 0.60; $n = 360$).

	PC1	PC 2	PC 3	PC 4
Microbial biomass C	0.71	0.28	–0.33	–0.11
Microbial biomass N	0.78	–0.21	0.10	–0.24
Basal respiration	0.71	–0.26	0.13	0.14
qCO_2	–0.19	–0.06	0.67	0.12
Dehydrogenase	0.73	0.25	0.12	0.09
Catalase	0.65	0.11	0.10	–0.18
N mineralized	0.11	–0.59	0.66	–0.14
Inorganic N mineralized	–0.15	–0.11	0.72	0.07
Ergosterol	0.63	0.05	–0.16	–0.04
ATP	0.04	0.19	0.65	–0.11
Phosphomonoesterase	0.06	0.71	–0.14	0.07
Phosphodiesterase	0.09	–0.15	0.46	0.08
CM-cellulase	0.03	0.65	–0.14	0.12
β-g1ucosidase	0.65	0.13	–0.22	0.02
Invertase	0.66	0.20	0.11	–0.08
Casein-protease	0.63	–0.20	0.08	–0.20
BAA-protease	–0.15	–0.11	–0.48	0.78
Urease	–0.19	0.05	0.08	0.71
Arylsulfatase	0.08	0.11	–0.03	0.46
Organic C	0.38	0.68	0.04	0.02
Total N	0.21	0.72	0.00	0.19
Bray P	0.72	–0.22	0.13	0.06
K	0.69	0.29	–0.43	0.11
Explained variance	**32.4**	**23.3**	**14.4**	**10.3**

first principal component (PC1), which explained 32.4 % of the total variance, was loaded by microbial biomass C, microbial biomass N, basal respiration, ergosterol, dehydrogenase, catalase, ß-glucosidase, invertase, casein-protease, Bray P, and K, indicating the size and activity of the microbial community and the logical dependence of soil microbes on nutrient content. The high loading of ergosterol indicated a decomposition pathway dominated by fungi, and the high loadings of invertase and casein-protease suggested that the activities of both these enzymes depend on the microbial community than on their extracellular accumulation in these soils. This is in concurrence with the findings of *Trasar-Cepeda* et al. (2000) in temperate forest soils. The second principal component (PC2) explained 23.3 % of the total variance, and was loaded by phosphomonoesterase, CM-cellulase, organic C, and total N, characterizing accumulation of hydrolytic enzymes and organic matter in soils. The third principal component (PC3) explained 14.4 % of the total variance and was defined by N mineralization indices, ATP, and *q*CO2 reflecting mineralization of organic matter and specific metabolic activity of soil microorganisms. The fourth factor (PC4) with 10.3 % of the total variance consisted mainly of BAA-protease and urease confirming the independence of the degradation of low molecular weight N compounds.

4 Conclusion

On a long-term basis, deforestation and establishment of plantations significantly decreased soil microbial activity as evidenced from the markedly lower levels of microbial biomass C, microbial biomass N, and basal respiration. Consequently, the activities of various soil enzymes involved in the cycles of C, N, P, and S were also at significantly lower levels at the plantation sites due to decreased C turnover and nutrient availability. While the ergosterol : biomass C ratio indicated the dominance of fungi at the forest sites, the larger ATP : biomass C ratio and the higher levels of *q*CO2 and AEC (Adenylate Energy Charge) at the forest sites indicated greater microbial proliferation and activity compared to the plantation sites. Overall, the study suggested that deforestation and cultivation markedly reduced microbial and enzyme activities due to significant depletion of organic matter and nutrients at the plantation sites. Contrarily, the more direct short-term supply of nutrients from decomposing leaf litter and the indirect supply of nutrients from the mineralization of organic matter led to significantly higher microbial and enzyme activities at the forest sites.

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