DIVERSITY AND COMMUNITY STRUCTURE OF TESTATE AMOEBAE (PROTISTA) IN TROPICAL MONTANE RAIN FORESTS OF SOUTHERN ECUADOR: ALTITUDINAL GRADIENT, ABOVEGROUND HABITATS AND NUTRIENT LIMITATION

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Für meine Familie
„Egal wie weit der Weg ist, man muß den ersten Schritt tun.“

Mao Zedong
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SUMMARY

The tropical Andes in southern Ecuador constitute a hotspot of plant (especially trees and bryophytes) and animal (especially birds, bats, arctiid and geometrid moths) diversity. However, data on small animals such as testate amoebae as an important component of the soil and aboveground community are lacking. Variations in density, diversity and community structure of testate amoebae along altitudinal transects in tropical regions are largely unknown. Testate amoebae colonize almost any habitat but are most abundant and diverse in soils with high humidity, high organic content and slow rates of decomposition. They preferentially feed on certain bacteria thereby affecting the taxonomic composition and metabolic activity of microbial communities. By altering microbial activity testate amoebae affect nutrient cycling in particular in ecosystems where earthworm populations are depleted.

The present thesis investigates the density and diversity of testate amoebae in litter, soil and aboveground habitats along an elevational gradient of tropical mountain rain forests in southern Ecuador, evaluates correlations with biotic and abiotic factors, and proves the role of nutrient limitation.

The first part of the thesis evaluates if the diversity and density of testate amoebae in the studied tropical mountain rain forests (1) changes with altitude, (2) is as high as in respective forests of the temperate and boreal zone, and (3) is typical for tropical southern hemisphere locations. Species composition of testate amoebae is investigated at three altitudes (1000, 2000 and 3000 m) and two horizons (L and F/H/Ah). A total of 135 species and intraspecific taxa of testate amoebae were found. Rarefaction plots suggest that only few more species are to be expected. The results suggest that species richness of testate amoebae does not decrease continuously with elevation; rather, it peaks at intermediate levels. Further, the data suggest that diversity, but not density of testate amoebae in tropical forests exceeds that in temperate forests. The great majority of testate amoebae species of the studied tropical mountain rain forests are geographically widespread, including temperate regions; however nine of the species (6.7%) are considered tropical, some of these species likely represent Gondwana relicts.

The second part of the thesis analyses altitudinal changes in microbial biomass and community composition and seeks relationships between microorganisms and testate amoebae. Microbial biomass, fungal biomass and microbial community structure at three altitudes (1000, 2000 and 3000 m) and in two soil layers [L/F layer (Layer I) and underlying H/Ah layer (Layer II)] was investigated. Basal respiration, microbial biomass and concentration of ergosterol generally declined from Layer I to Layer II and peaked at 2000 m. Compared to temperate forest ecosystems microbial biomass and ergosterol concentrations were generally low. Presumably, low microbial biomass in soils of tropical forest ecosystems is due to high temperatures associated with high respiration but also low litter quality, with the latter declining with altitude. These conclusions are supported by the fact that at higher altitudes the microbial community changed from a bacterial-dominated to a fungal-dominated system. Parallel to microbial biomass and ergosterol concentrations the density of testate amoebae peaked at 2000 m. However, compared to microbial parameters changes in testate amoebae communities between the two layers were less pronounced. The data suggest that density and community structure of testate amoebae are driven by the
availability of food resources (bacteria and fungi) which at high altitude decrease with increasing moisture and decreasing pH.

The third part of this thesis investigates if testate amoebae in soils of tropical montane rain forests are bottom-up regulated. To prove this hypothesis, carbon (C) and nutrients (N, P) were added to the soil and the response of microorganisms and testate amoebae to the additional resource supply was investigated. We assumed that microorganisms will benefit from the additional resources and that this will propagate to the next trophic level, i.e. protists represented by testate amoebae, since microorganisms are the major food resources for testate amoebae. The results indicate that saprotrophic fungi in tropical montane rain forests are mainly limited by carbon whereas gram positive and gram negative bacteria benefit from increased availability of P. Testate amoebae suffered from increased dominance of saprotrophic fungi in glucose treatments but benefited from increased supply of N presumably by increased availability of high quality detritus, certain bacteria and increased performance of endosymbiotic algae. The results show that testate amoebae of tropical montane rain forests are controlled by bottom-up forces relying on specific food resources rather than the amount of bacterial biomass with saprotrophic fungi functioning as major antagonists. Microbial food webs in soil therefore may be much more complex than previously assumed with trophic links being rather specific and antagonistic interactions potentially overriding trophic interactions.

The fourth study of this thesis evaluates if testate amoebae from aboveground epiphytic communities respond to altitudinal gradients (macroscale) and to the height the epiphyte is located on the tree (microscale). Changes in diversity and density of testate amoebae in epiphytic microhabitats along tree height from 0 to 1 to 2 m and regional macrohabitats (forests at 1000, 2000 and 3000 m) were investigated. A total of 115 taxa of testate amoebae were found. The results suggest that species diversity and density peak at intermediate elevation. Furthermore, density in aboveground habitats was higher than that in litter and soil (weight based data). Density and diversity of testate amoebae significantly changed along both the micro- and macroscale, however, variations in density were more pronounced at the macroscale whereas variations in diversity were more pronounced at the microscale, suggesting that microhabitat characteristics are most decisive in selecting for testate amoebae species.

Overall, the results document that with a total of 166 species determined in this study testate amoebae in Ecuador are highly divers compared with other tropical and temperate forests. Density and diversity of testate amoebae are controlled by bottom-up forces with their density depending on the availability of high quality detritus resources, certain bacterial groups, the performance of endosymbiotic algae and antagonistic interactions with saprotrophic fungi. The availability of food resources decreases with increasing elevation, with increasing moisture and with decreasing pH, resulting in maximum density and diversity at intermediate elevation.
ZUSAMMENFASSUNG


Im ersten Teil der Arbeit wurde untersucht, ob sich die Dichte und Diversität von Thekamöben (1) entlang des Höhnengradienten ändert, (2) der Dichte und Diversität in Wäldern der gemäßigten und borealen Klimazone ähnlich sind, und (3) typisch für die Standorte der tropischen Südhemisphäre sind. Die Diversität wurde in drei Höhenlagen (1000, 2000 und 3000 m) und zwei Bodenschichten (Schicht I: L/F und Schicht II: H/Ah) untersucht. Insgesamt wurden 135 Arten und intraspezifische Taxa von Thekamöben gefunden. „Rarefaction plots“ deuten daraufhin, dass nur wenig mehr Arten zu erwarten sind. Die Ergebnisse weisen zudem darauf hin, dass der Artenreichtum von Thekamöben nicht kontinuierlich mit der Höhe abnimmt, sondern dass ihr Verbreitungsschwerpunkt auf intermediärer Höhe liegt (2000 m). Dabei ist die Diversität, nicht aber die Dichte von Thekamöben in tropischen Bergregenwäldern, größer als in Wäldern der gemäßigten Breiten. Die Mehrzahl der nachgewiesenen Arten ist geographisch weit verbreitet, nur neun Arten (6.7%) besitzen eine rein tropische Verbreitung, wovon einige Arten wahrscheinlich Gondwana-Relikte repräsentieren.

Im zweiten Teil der Arbeit wurde die mikrobielle Biomasse und Diversität, und die Interaktionen von Mikroorganismen und Thekamöben entlang eines Höhengradienten (1000, 2000 and 3000 m) in zwei Bodenschichten (Schicht I: L/F und Schicht II: H/Ah) untersucht. Die mikrobielle Biomasse und Diversität nahm mit der Bodentiefe ab und erreichte ihr Maximum auf der mittleren Höhenstufe bei 2000 m. Sie war im Vergleich zu Untersuchungen in gemäßigten Wäldern sehr gering, was vermutlich auf eine hohe Respiration aufgrund hoher Temperaturen, sowie auf die insgesamt geringe, mit zunehmender Höhe weiter abnehmende Streuqualität zurückzuführen ist. Der Wechsel von einer Bakterien- zu einer Pilzdominierten mikrobiellen Gemeinschaft mit zunehmender Höhe unterstützt diese Hypothese. Wie die mikrobielle Gemeinschaft, hatten auch die Thekamöben ihren Verbreitungsschwerpunkt auf der mittleren Höhenstufe bei 2000 m, jedoch veränderte sich ihre Dichte und Diversität mit der Bodentiefe nur wenig. Die Ergebnisse deuten darauf hin, dass die Dichte und...
Diversität von Thekamöben durch die Verfügbarkeit von Nahrungsressourcen (vor allem Bakterien und Pilzen) gesteuert wird, die aufgrund ungünstigerer Umweltbedingungen, z.B. niedrigem pH, mit zunehmender Höhe abnimmt.

Im dritten Teil der Arbeit wurde die Hypothese untersucht, dass die Dichte und Diversität von Thekamöben in tropischen Bergregenwäldern durch die Verfügbarkeit ihrer Nahrungsressource (bottom-up) reguliert ist. Es wurden Testflächen mit Kohlenstoff (C) und Nährstoffen (N, P) gedüngt und die Veränderung der Dichte und Diversität von Mikroorganismen und Thekamöben untersucht. Wir vermuteten, dass die mikrobielle Gemeinschaft aufgrund der zusätzlichen Nahrungsressource zunimmt, und dass sich dieser Effekt in der nächsten tropischen Ebene, den Thekamöben, widerspiegelt. Während saprotrophe Pilze primär durch die Verfügbarkeit von Kohlenstoff limitiert waren, waren gram-positive und gram-negative Bakterien in erster Linie P-limitiert. Zugabe von Glukose führte zur Dominanz saprotropher Pilze, wohingegen die Dichte und Diversität der Thekamöben abnahm. Stickstoffzugabe führte dagegen zu einem Anstieg die Dichte und Diversität der Thekamöben, was vermutlich auf eine erhöhte Menge von leicht verfügbarem organischer Substanz und erhöhte Biomasse bestimmter Bakterien und endosymbiontischen Algen zurückzuführen ist. Die Ergebnisse deuten darauf hin, dass Thekamöben in tropischen Bergregenwäldern „bottom-up“ reguliert sind, die Verfügbarkeit einer spezifischen mikrobiellen Nahrungsquelle bedeutender ist als die mikrobielle Biomasse an sich, und dass saprotrophe Pilze als Antagonisten von Thekamöben fungieren. Das mikrobielle Nahrungsnetz in Böden tropischer Bergregenwälder scheint komplexer zu sein als bisher vermutet und primär durch spezifische und antagonistische Interaktionen gesteuert zu sein.

Im vierten Teil der Arbeit wurde die Thekamöben-Gemeinschaft auf Epiphyten unterschiedlicher Stammhöhe (Mikroskala) entlang des Höhengradienten (Makroskala) untersucht. Dazu wurde die Veränderung der Dichte und Diversität von Thekamöben in epiphytischen Mikrohabitaten auf Bäumen in einer Höhe von 0-2 m entlang des Höhengradienten (1000, 2000 und 3000 m) erfasst. Insgesamt wurden 115 Arten von Thekamöben nachgewiesen. Die Dichte und Diversität erreichte ihren Höhepunkt in der intermediären Höhenstufe bei 2000 m und war höher als in der Streu und im Boden. Die Thekamöben-Gemeinschaft unterschied sich sowohl auf der Ebene der Mikro- als auch der Makroskala signifikant, wobei die Dichte primär auf der Ebene der Makroskala, die Diversität auf der Ebene der Mikroskala beeinflusst wurde. Mikrohabitatstrukturen scheinen insbesondere die Zusammensetzung der Thekamöben-Gemeinschaft zu steuern.

Chapter 1

General Introduction
CHAPTER 1. GENERAL INTRODUCTION

1.1. Tropical montane rain forests

Tropical montane rain forests are among the most species-rich ecosystems of the world (Myers et al., 2000) and represent 10% of the rain forests worldwide. Using plant species richness data of 1800 „operational geographical units“ Kier et al. (2005) recognized 867 terrestrial ecoregions worldwide. Richness of plants and animals was at a maximum in the Borneo lowlands ecoregion (10,000 species), followed by nine ecoregions in Central and Southern America (Fig 1.1). Very high species numbers of a variety of animal and plant taxa are also found in the tropical montane rain forest in the Ecuadorian Andes. In these biodiversity „hotspots“ the number of species of animal and plant taxa may exceed that of temperate forests by orders of magnitude, e.g. the number of tree species per hectare in tropical montane rain forests may be as high as 100, whereas in temperate forests only a handful of taxa are present per hectare (Whitmore, 1998; Oosterhoom and Kappelle, 2000; Kessler et al., 2005). These forests have very high levels of endemism of both fauna and flora, however, they are under severe pressure from the rapidly increasing population in the Interandean valleys due to agricultural encroachment, grazing, hunting and cutting for fuelwood. Therefore, the enormous species richness in Ecuador is severely threatened by an alarming annual loss of 4% of total forest cover – the second highest rate in South America (Whimore and Sayer, 1992; Miller, 1998); primary montane rain forests show an even higher deforestation rate than the lowland forest (Doumenge et al., 1995).

Fig. 1.1. Ecoregions in the Neotropics (from Kier et al., 2005, based on Olson et al., 2001).
Study area

The majority of montane rain forests in Ecuador is located along the Andean Cordillera which runs the length of the country. The study area of this project is located in southern Ecuador on the northern fringes of the Podocarpus National Park on the eastern slopes of the Andes, south-east of the province capital Loja, within the Reserva Biologica San Francisco (RBSF). The park covers 146,300 ha and has a very irregular topography covering altitudes from 950 to 3700 m. The forests are undisturbed by man but frequent land slides result in a mosaic of mature and regeneration forests (Madsen and Øllgaard, 1994). The area Bombuscaro in the eastern gate to the park represents the lowest part of the National Park ca. 1000 m a.s.l; Cajanuma in the north-western gate to the park represents the elfin forest with an altitude ca. 3000 m a.s.l. (Leuschner et al., 2007); RBSF is located close to the Podocarpus National Park, in the province Zamora-Chinchipe and represents upper montane forest at ca. 2000 m a.s.l. (Fig. 1.2). With altitude and decreasing air temperature in tropical mountains tree height (Whitmore, 1998), aboveground biomass (Röderstein et al., 2005), leaf litter production and soil pH also decrease, whereas the thickness of organic layers, litter C-to-N ratio, annual rainfall and soil moisture increase (Leuschner et al., 2007; Moser et al., 2007).

In general, the climate is semihumid with 8 to 10 humid months per year. Accordingly, annual rainfall is high with ca. 2200, 3500 and 4500 mm y\(^{-1}\) at 1000, 2000 and 3000 m, respectively. Precipitation is particularly high during the wet season from April to July and less pronounced from September to December. The mean annual air temperature is decreasing with altitude from 14.9, to 12.3 to 8.9°C at 1000, 2000 and 3000 m, respectively. The coldest month on average is August, the warmest November (Röderstein et al., 2005). The bedrock consists mainly of weakly metamorphosed Palaeozoic schists and
sandstones with some quartz veins (Wilcke et al., 2001). The soil types are mainly Aquic and Oxaquic Dystropepts (Schrumpf et al., 2001). The organic layers has a mass of 30-713 t ha\(^{-1}\) (Wilcke et al., 2002) and a thickness of ca. 6, 11 and 28 cm at 1000, 2000 and 3000 m, respectively.

The studied forests are characterized by exceptionally high plant and animal diversity but only few groups were investigated. Inventories have been carried out on birds, bats, and parts of the arthropod clades Lepidoptera, Orthoptera, and Arachnida (Beck et al., 2008). Major gaps include the highly diverse insect orders Coleoptera, Hymenoptera and Diptera. The RBSF harbours more then 500 species of bryophytes, probably the highest number ever recorded from such a small area in the tropics (Gradstein et al., 2007; Kürschner and Parolly, 2007). Within the spermatophytes, the orchids are the most speciose family at the RBSF with a total of ca. 340 species. This is the highest number recorded for a neotropical forest site. Brehm et al. (2005) observed 1266 morphospecies of Geometridae – a higher number than observed anywhere else in the world.

Many plants families such as Araceae, Arecaceae, and Fabaceae show a high richness in Amazon lowland and Andean foothill forests that quickly drops as elevation increases (Jørgensen and León-Yánez, 1999), however, elevational animal richness patterns are less known. Some groups are exceptionally rich at the RBSF (2000 m) site while others are not; probably they peak at low elevations (Fig. 1.3).

![Fig. 1.3. Hypothetical curves of relative species richness of three animal taxa along an altitudinal gradient (after Brehm et al., 2008).](image-url)
1.2. Testate amoebae

Function

Testate amoebae are widespread and an ecologically important group of unicellular organisms, contained wholly or partially within a protective covering. They are ideally suited to the vagaries of the soil environment as their ability to encyst enables them to survive at transiently inhospitable conditions (Clark, 2003). Testate amoebae are of ancient origin, dating from at least the Mesozoic (250-65 Mya), with possible ancestors as old as the Neoproterozoic (Cryogenian period, 850-630 Mya) (Smith et al., 2008). They colonize almost any habitat but are most abundant and diverse in soils with high humidity, organic content and slow rates of decomposition (Geltzer et al., 1985), and they are a dominant group of organisms among the soil microbial community. Many critical transformations of the major biogeochemical cycles in the biosphere occur in soils and are facilitated by soil organisms (Coûteaux and Darbyshire, 1998), e.g. microorganisms and single celled eukaryotes play key roles in the carbon and nitrogen cycles by regulating both the decomposition of organic matter and specific metabolic pathways. Schönborn (1992) concluded that the contribution of testate amoebae to nutrient cycling is generally important, but in particular in ecosystems where earthworm populations are depleted. The role of testate amoebae in organic matter cycling may be summarized as follows:

1) predation upon and modification of microbial populations;
2) acceleration of the turnover of soil microflora biomass;
3) acceleration of the turnover of soil organic matter;
4) possible degradation of plant residues, humic substances and other materials;
5) prey for other organisms (Lousier and Parkinson, 1984).

Diversity

Global species richness of protists is still unknown. Corliss (1991) estimated the number of known non-fossil protist species in the world as 40,000, Hawksworth (1992) suggested that a total world number of 100,000 could well prove to be too low.

Cailleux (1978) analysing the publications of Decloitre (1952-1975) with testate amoebae species lists noted the doubling in numbers of described species from 800 to 1600 during 13 years, with 1031 species in Europe, 684 in Africa, 428 in Australia and Melanesia, 424 in North and Central America, 424 in South America, 229 in Asia, 220 in the Arctic, and 89 in Antarctica. Smith et al. (2008) suggested that with increasing attention of testate amoebae in America, Asia and Australasia taxon-richness is likely to double from ca. 2000 to 4000. Decloitre (1985) estimated that 1800 species, sub-species and varieties of testate amoebae had been recorded globally. Foissner (1996) has reported about 200 species of testate amoebae which are obligate soil-dwellers; the description of these species is not finished yet. Testate amoebae research has contributed significantly to the debate about the relevance to protists of Beijerinck’s dictum (1913) „Everything is everywhere, the environment selects“ (Smith et al., 2008).
Apologists of this paradigm have presented evidence for the cosmopolitan dispersion of all microorganisms up to 1 mm in size (Finlay, 2002; Finlay and Fenchel, 2004). Foissner (2006) suggested that this assumption is flawed as more than half of the protist world is still undescribed, especially the rare species (Foissner et al., 2002). Furthermore, Foissner (2006) suggested that historical events (e.g. the split-up of Pangaea), limited cyst viability and, especially, time as major factors for dispersal and provinciality of micro-organisms. They are neither cosmopolitan nor ubiquitous (Meisterfeld, 2000a,b). Several species, mainly from the taxa Nebelidae, Distomatopyxidae and Lamptopyxidae, have a restricted geographical distribution, which is certainly not a result of uneven sampling effort (because of morphology) (Smith and Wilkinson, 1986; Foissner, 2006). Arcellinidae are common in all freshwater habitats and mosses (Meisterfeld, 2000a,b). More molecular data and more regional information are necessary to answer this question.

**Morphology**

The main elements of living amoebae are the cell itself and the test (shell) in which it lives (Fig. 1.4). Parts of the cytoplasm form the pseudopodia used for locomotion, attachment and feeding. Two types of pseudopodia are used characterizing higher taxonomic levels such as Lobosea (rounded lobose) and Filosea (pointed or filiform pseudopodia). The test is the outer part of the organism. There are two basic types of test construction; autogenous (idiosomic), arranged irregularly or in imbricated ranks across its surface and bound in organic cement, or it may be calcareous and smooth (Clark, 2003), and xenogenous (agglutinated or xenosomic) the composition may be of proteinaceous material, binding to it exosomes; these are mineral grains, diatom frustules and/or small remains of the other organisms. Bonnet (1964, 1975) and Coûteaux (1976) divided the test of amoebae into 16 morphological types (e.g. Fig. 1.5), depending on location of pseudostome and form of the test. Coloration of the test display wide interspecific variation e.g. neutral, grey-blue, purple, grey-green, yellow even red. Tests between species can range in length or diameter from 7 and 500 μm.

Reproduction

Testate amoebae mostly reproduce by asexual binary fission (Fig. 1.6). However, there have been two studies that suggested that sexual reproduction occurs occasionally (Valkanov, 1962 for *Valkanovia*; Schönborn and Peschke, 1988 for *Assulina muscorum*). Mignot and Raikov (1992) observed meiosis from electron microscopy studies of *Arcella vulgaris*. Hedley and Ogden (1973) and Seravin and Gudkov (1984) observed multiple pseudoconjugation in *Euglypha rotunda* (Fig 1.7).

Rates of reproduction depend on species and are highly variable from 2 to 100 generations per year and depend on humidity and temperature and on the availability of food and materials for constructing tests (Ogden, 1981; Lousier, 1984a, b; Lousier and Parkinson, 1984; Clark, 2003).
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Fig. 1.5. Environmental scanning electron microscope (ESEM) pictures of some test types of testate amoebae.

Fig. 1.6. Division and daughter shell morphogenesis of *Euglypha* (from Ogden, 1991). A, B – protrusion of cytoplasmic bud, idiosome migration and first stages of daughter shell formation; C, D – increase of cytoplasm volume of the daughter cell by means of vacuolarisation and completion of daughter shell formation; E – nucleus division and migration of one of the nuclei to the daughter cell; F – separation of the daughter cell.
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Fig. 1.7. Multiple pseudoconjugation in *Euglypha rotunda* (after Seravin and Gudkov, 1984).

**Classification**

Testate amoebae are a morphologically diverse group (Wylezich et al., 2002). Moreover, it has been shown that population growth as well as shell morphology of testate amoebae is highly variable depending on the species and environmental conditions. Furthermore, this variability is reproducible and even reversible (Wanner, et al., 1997). On the one hand, this points to a new and fascinating tool in bioindication, but on the other hand to serious taxonomical problems, because classification of closely related testate amoebae is primarily based on highly variable shell characteristics. Testate amoebae are phylogenetically divided into those with lobose and those with filose pseudopodia (Cavalier-Smith, 2004). Testate lobose amoebae are Amoebozoa (Nikolaev et al., 2005) and include the families Difflugiidae, Centropyxidae, Arcellidae and Hyalospheniidae, with the larger species (>100 μm). Testate filose amoebae are closely related to the Foraminifera and Cercozoa (Longet et al., 2004) and include the families Euglyphidae and Trinematidae. These contain small species (<100 μm).

There are no modern monographs with new classification of the testate amoebae available, only Meisterfeld (2000a,b) summarized some new information about some species from the order Arcellinida (Amoebozoa) and amoebae with filopodia. Penard (1902), Cash et al. (1915), Grospietsch (1972), Bonnet and Thomas (1960a,b,c) also contain species descriptions. Ogden and Hedley (1980) illustrated 95 mainly aquatic and moss species by SEM micrographs. Geltzer et al. (1985, 1995) illustrated about 110 soil species from the temperate zone and Clark (2003) characterized 90 species. Masei and Zhiganov (2006) illustrated about 714 fresh water species.

For some families the following papers helped finding original descriptions:

- *Arcella* (Deflandre, 1928; Decloitre, 1976);
- *Centropyxis* (Deflandre, 1929; Decloitre, 1978, 1979);
- *Cryptodifflugia* (Grospietsch, 1964; Schönborn, 1965a);
Cyclopyxis (Deflandre, 1929; Decloitre, 1977a);
Diffugia (Stepanek, 1952; Gauthier-Lievre and Thomas, 1958; Chardez, 1961, 1967; Ogden, 1979, 1980, 1980a, 1983, 1984; Ogden and Fairman, 1979);
Euglypha (Thomas, 1958a; Decloitre, 1962; Coûteaux et al., 1979);
Hyalosphenia (Grospietsch, 1965);
Nebela (Deflandre, 1936; Jung, 1942; Gauthier-Lievre and Thomas, 1954; Decloitre, 1977b);
Plagiopyxis (Thomas, 1958b);
Quadrulella (Chardez, 1967);
Trinema (Chardez, 1960).

Trophic biology and environment

There are limits to the size of food which testate amoebae can consume as most taxa are limited to prey
that is smaller in diameter than their pseudostome (Charman et al., 2000; Clark, 2003). Large testate
amoebae ingest protists, including small testate and naked amoebae (Bonnet, 1964; Laminger, 1978), and
small metazoan such as rotifers and nematods (Yeates and Foissner, 1995); smaller species feed mainly
on bacteria, yeasts, microfungi and unicellular algae (Coûteaux and Devaux, 1983; Foissner, 1987). Both
large and small species also feed on detritus and humus particles (Schönborn, 1965b, 1982). Certain
testate amoebae species (Amphitrema flavum, Placocista spinosa) contain algal symbionts (zoochlorella)
(Schönborn, 1965b). Some species of the genera Assulina, Corythion and Schönbornia have been
observed „over-feeding“ under favourable condition. The amoebae gather humus and other material
around the pseudostome into „food bundles“ which appear to be stored for consumption when conditions
deteriorate. However, Lüftenegger et al. (1988), suggested that these bundles of materials are used to plug
the pseudostome during cell encystment.

The amount of food available considerably affects the testate amoebae community. The availability of
food may be related to soil moisture and chemistry. The most important factor for growth and
reproduction of testate amoebae is water (Clark, 2003). The water film on particles is the living space for
testate amoebae (Charman et al., 2000). High or low moisture is also reflected by morphological features
of testate amoebae, with species changing from acrostom to plagiostom at dryer conditions (see Fig. 1.5).
Morphological features, such as plagiostomy, may be used for evaluating soil moisture conditions. Soil
pH is reported to be the second most important factor affecting the distribution and their species
composition of testate amoebae, and may be used as a diagnostic tool for evaluating soil acidity (Geltzer
carbon concentration may also be important (Tolonen, 1986). At higher temperatures generation times are
decreased (Schönborn, 1962). Light and oxygen affect the vertical living space of testate amoebae.
Species such as Hyalosphenia papilio, Placocista spinosa, Amphitrema flavum and A. wrightianum may
be limited by the availability of light (Meisterfeld, 1977) because of symbiotic algae. Solar ultraviolet-B
radiation appears to increase the abundance or biomass of testate amoebae (Searles et al., 2001). Likewise
as levels of free oxygen decrease with depth, there are fewer living testate amoebae (Corbet, 1973).
1.3. Objectives

The study was carried out as part of the integrated DFG projects „Functionality in a Tropical Mountain Rainforest: Diversity, Dynamic Processes and Utilization Potentials under Ecosystem Perspectives“, and „Biodiversity and Sustainable Management of a Megadiverse Mountain Ecosystem in South Ecuador“. The project offered the unique opportunity to investigate the diversity and density of testate amoebae in the southern hemisphere, relationships between biodiversity and ecosystem processes, i.e. interactions between altitudinal changes in community structure of testate amoebae and microbial composition, interaction between microorganisms and testate amoebae, resource control and micro- and macroscale effects on testate amoebae.

In **CHAPTER 2**, changes in diversity and community structure of testate amoebae along an altitudinal gradient (1000, 2000 and 3000 m a.s.l.) in two horizons (L and F/H/Ah) in the Andes of Ecuador were investigated. Further, the study provides first faunistic data on testate amoebae of southern Ecuador. Little is known on changes in diversity and density of testate amoebae along altitudinal gradients of mountains in tropical regions of the southern hemisphere, and only few publications exist about relationships between testate amoebae and altitude. Results of these studies suggest that the diversity of testate amoebae generally declines with elevation (Bonnet, 1978; Todorov, 1998). Acidic soils of the temperate and boreal zone typically harbour few decomposer macrofauna species and are dominated by meso- and microfauna with testate amoebae reaching high density and diversity (Geltzer et al., 1985; Schaefer, 1991). This, however, needs further testing, in particular in tropical regions. There are contrasting views on the global distribution and diversity of protist (Finlay, 2002; Foissner, 2006, 2008; Cotterill et al., 2008); the great majority of testate amoebae species are geographically widespread but at least some are regionally restricted or endemic, however, this needs further investigation in particular in the southern hemisphere, virtually no information is present for South America.

Based on the above considerations we investigated the following hypotheses:

1. The diversity and density of testate amoebae declines with altitude in tropical montane forests.
2. The diversity and density of testate amoebae in tropical acidic forest soils is similar to respective forests of the temperate and boreal zone.
3. The diversity of testate amoebae of the studied region is typical for tropical southern hemisphere locations.

In **CHAPTER 3**, the altitudinal changes in microbial biomass and community composition were analysed to evaluate relationships between microorganisms and testate amoebae in the studied tropical montane forests in southern Ecuador, at three altitudes (1000, 2000 and 3000 m a.s.l.) and two soil layers (L and F/H/Ah). With altitude and decreasing air temperature leaf litter production and soil pH also decrease, whereas the thickness of organic layers, litter C-to-N ratio, annual rainfall and soil moisture increase (Leuschner et al., 2007; Moser et al., 2007). Further, in high organic matter soils (Frostegård and Bååth,
1996) and in soils with higher acidity (Bååth and Anderson, 2003) fungi gain in dominance, as bacteria more heavily rely on more easily available carbon resources (Anderson and Domsch, 1980; Scheu and Parkinson, 1995). However, until today virtually all studies on bacterial - fungal ratios were performed in the temperate zone and therefore validity of the results needs proof from tropical regions. Laboratory experiments showed that testate amoebae primarily feed on bacteria (Bonnet, 1964; Stout and Heal, 1967), but also on fungal spores and yeasts (Coûteaux and Devaux, 1983; Ogden and Pitta, 1990), algae and other protists (Laminger, 1978, 1980), some of them even feed on small metazoans (Yeates and Foissner, 1995) and humus particles (Schönborn, 1965, 1982). According to our previous investigations (Chapter 1) community composition of testate amoebae changed markedly along the altitudinal gradient studied. However, it is unclear if these changes are associated with changes in microbial community structure.

Therefore, we investigated the following hypotheses:

(1) With declining pH the dominance of fungi increases with altitude.
(2) Bacterial dominance generally decreases from Layer I to Layer II irrespective of altitude.
(3) Parallel to testate amoebae microbial biomass peaks at intermediate altitude.

In CHAPTER 4, the role of carbon, nitrogen and phosphorus as limiting factors for microorganisms and microbial grazers (testate amoebae) in tropical montane rain forest were investigated. It is generally assumed that microorganisms and saprophagous animals are mainly controlled by the availability of resources, i.e. by bottom-up forces (Hairston et al., 1960; Hunt et al., 1987; Hairston, 1989). However, studies on the role of bottom-up forces in decomposer communities are scarce, and no information is available on tropical montane rain forests at all. Therefore, we added carbon, nitrogen and phosphorus separately and in combination to experimental plots at one of our study sites (ECSF). We investigated the response of testate amoebae to the addition of these resources. Further, for relating the observed changes to microbial food resources we also investigated microbial biomass, fungal biomass and microbial community composition using phospholipid fatty analysis (PLFA; Frostegård et al., 1993). Soil microorganisms are generally assumed to be primarily limited by carbon (Wardle, 1992; Demoling et al., 2007). Since testate amoebae are feeding on microorganisms (Bonnet, 1964; Stout and Heal, 1967), we expected the response of testate amoebae to be closely linked to that of microorganisms. Previous studies (Vitousek, 1984; Vitousek and Sanford, 1986) suggested that phosphorus limits plant growth in tropical rain forests more often than nitrogen, and that lowland tropical forests are relatively rich in nitrogen (Martinelli et al., 1999).

In detail, the following hypotheses were investigated:

(1) Microorganisms are primarily limited by carbon and thereby also bacterial and fungal feeding testate amoebae.
(2) Phosphorus functions as secondary limiting element for microorganisms and testate amoebae.
(3) Nitrogen is generally of little importance.
In **CHAPTER 5**, changes in diversity and density of testate amoebae in epiphytes of trees in tropical montane rain forests in the Andes of southern Ecuador were investigated. Local – microscale [tree height of 0 (base of tree trunk), 1 and 2 m; TH I, TH II and TH III, respectively] and regional – macroscale, (forests at 1000, 2000 and 3000 m) changes were investigated. A recent study in tropical montane rain forests (Brehm et al., 2008; Beck et al., 2008) suggests that the diversity and density of plant and animals (testate amoebae, geometrid moths, birds) peaks at intermediate altitude probably because of favourable abiotic conditions. Therefore, we hypothesised that total diversity and density of testate amoebae in epiphytes also peak at intermediate altitude irrespective of tree microhabitat (macroscale hypothesis, H 1).

Generally, testate amoebae in epiphytes on trees received little attention and this applies in particular to epiphytes of montane rain forests, e.g. no information is available on changes in diversity and density of testate amoebae in epiphytes with tree height. Bonnet (1973) investigated epiphytes growing on trees and soil and found that epiphytes on trees are colonized by ubiquitous species. Therefore, we hypothesised that diversity and density of testate amoebae varies little with tree height thereby contributing little to testate amoeba diversity (microscale hypothesis, H 2). Temperature and pH contribute to turnover of testate amoebae shells, i.e. low pH supports the conservation of empty shells (Schönborn, 1973; Geltzer et al., 1985) and soil pH decreases with increasing altitude in the studied montane rain forests (Moser et al., 2007). Further, low temperature slows down the turnover of testate amoebae (Meisterfeld and Heisterbaum, 1986) and temperature decreases with increasing altitude (Mosser et al., 2007; Röderstein et al., 2005). In addition, the turnover of testate amoebae increases with increasing stress and stress conditions, such as variations in humidity, are likely to be more pronounced higher up in trees (Bonnet, 1973; Meisterfeld, 1978; Bohlman et al., 1995). Therefore, we hypothesised that the percentages of live forms decreases and empty shells increases with altitude and height of epiphyte location on trees (H 3).

In **CHAPTER 6** the results are discussed in a comprehensive way and an outlook for future research is given.
Testate amoebae (protista) of an elevational gradient in the tropical mountain rain forest of Ecuador
CHAPTER 2. TESTATE AMOEBAE (PROTISTA) OF AN ELEVATIONAL GRADIENT IN THE TROPICAL MOUNTAIN RAIN FOREST OF ECUADOR

2.1. Abstract

We investigated the species composition of testate amoebae at three altitudes (1000, 2000 and 3000 m) and two horizons (L and F/H/Ah) of a tropical mountain rain forest in southern Ecuador. A total of 135 species and intraspecific taxa of testate amoebae were found (36 samples). Rarefaction plots suggest that only few more species are to be expected. Species number in the L horizon was at a maximum at 2000 m (109 species) and similar at 1000 and 3000 m (75 and 71 species, respectively). Species numbers in the F/H/Ah horizon were 29, 67 and 48 at 1000, 2000, 3000 m, respectively. The density of testate amoebae in the L horizon increased significantly in the order 1000 < 3000 < 2000 m with 3147 ± 129, 9198 ± 144 and 12,497 ± 1317 ind. g\(^{-1}\) dry matter and in the F/H/Ah horizon with 176 ± 25, 3118 ± 97 and 4986 ± 102 ind. g\(^{-1}\) dry matter, respectively. The significant Horizon x Elevation interaction reflects the exceptionally low abundance of testate amoebae in the Ah horizon at 1000 m. The results suggest that species richness of testate amoebae does not decrease continuously with elevation; rather, it peaks at intermediate elevation. Further, the data suggest that diversity, but not density, of testate amoebae in tropical forests exceeds that in temperate forests. Morphological features of testate amoebae reflected semiaquatic habitat conditions. The great majority of testate amoebae species of the studied tropical mountain rain forests are geographically widespread, including temperate regions; however 9 of the species (i.e. 6.7%) are considered tropical, some of these species likely represent Gondwana relicts.

2.2. Introduction

Testate amoebae are widespread and an ecologically important group of soil unicellular organisms. They colonize almost any habitat but are most abundant and diverse in fresh water and soils with high organic content and slow rates of decomposition (wetlands, mosses, peats, and montane and forest soils) (Geltzer et al., 1985). Despite the ubiquity and often worldwide distribution of testate amoebae species, information on differences between testate amoebae communities of the southern and northern hemisphere and the number of species with local distribution are limited (Bobrov, 2001). Information on changes in testate amoebae diversity with latitude is scarce; some information is given in Bonnet (1983), Foissner (2006), Smith (1992, 1996) and Smith and Wilkinson (1986). Little is known on changes in diversity and density of testate amoebae along altitudinal gradients of mountains in tropical regions of the southern hemisphere. Generally, only few publications exist about relationship between testate amoebae and altitude. Todorov (1998) investigated changes in the structure of testate amoebae communities along a gradient from 400 to 2500 m above sea level and observed a reduction at higher altitudes (Pirin Mountain, Bulgaria). Mitchell et al. (2004) analysed moss samples from altitudes between 1000 and 2200 m a.s.l. (in the south-eastern Alps of Italy); they also found a decline in the testate amoebae diversity with altitude, but there was no significant correlation between altitude and species richness (except in few
species). Results of these studies suggest that the diversity of testate amoebae generally declines with elevation; this, however, needs further testing, in particular in tropical regions.

This study investigates changes in diversity and community structure of testate amoebae along an altitudinal gradient from 1000 to 3000 m a.s.l. in the Andes of Ecuador, i.e. a tropical southern hemisphere locality. The study evaluates (1) if the diversity and density of testate amoebae indeed declines with altitude in tropical mountain forests, (2) if the diversity and density of testate amoebae in tropical acidic forest soils is as high as in respective forests of the temperate and boreal zone, and (3) if the diversity of testate amoebae of the studied region is typical for tropical southern hemisphere locations. Further, the study provides faunistic data on testate amoebae of southern Ecuador.

2.3. Material and Methods

Study sites and sampling

The study area is located in southern Ecuador on the northern fringes of the Prodocarpus National Park on the eastern slopes of the Andes, south-east of the province capital Loja. Along an elevation transect three study sites at 1000, 2000, 3000 m a.s.l. were investigated. The maximum distance between the sites was 30 km. The study site at 1000 m a.s.l. was located in Bombuscaro near the province capital Zamora (S 04°06'54", W 78°58'02"), the study site at 2000 m a.s.l. was located in the Reserva Biologica San Francisco in the valley of the Rio San Francisco (S 3°58'18", W 79°4'45"). The study site at 3000 m a.s.l. was located in the Cajanuma area at the north-west gate of the Podocarpus National Park south of Loja (S 04°06'711", W 79°10'581). Samples were taken in October / November 2006.

The soil types of the study sites are alumic acrisols at 1000 m, gley cambisols at 2000 m, and podzols at 3000 m (Soethe et al., 2006). Soil pH (CaCl$_2$) decreases with elevation from 3.9 to 3.5 to 2.9 at 1000, 2000 and 3000 m, respectively. The climate is semihumid with 8 to 10 humid months per year. Accordingly, annual rainfall is high with ca. 2200, 3500 and 4500 mm y$^{-1}$ at 1000, 2000 and 3000 m, respectively. The mean annual air temperature is decreasing with altitude from 14.9, to 12.3 to 8.9°C at 1000, 2000 and 3000 m, respectively. The coldest month on average is August, the warmest November (Röderstein et al., 2005).

The studied forests are characterized by exceptionally high plant diversity with a total of 1117 vascular plants and more than 280 tree species, with about 100 tree species per 0.6 ha. Lauraceae, Melastomataceae and Rubiaceae are most species rich and most frequent at all study sites (for details see Homeier et al., 2002; Homeier, 2004; Röderstein et al., 2005; Richter and Moreira-Muñoz, 2005). The organic layers had a mass of 30-713 t ha$^{-1}$ (Wilcke et al., 2002) and a thickness of ca. 6, 11 and 28 cm at 1000, 2000 and 3000 m, respectively.

At each elevational site, a sampling area of ca. 100-200 m$^2$ was selected and 6 sampling points were randomly chosen. Litter and soil samples were taken with a corer (5 cm diameter) up to a depth of 30 cm, depending on the depth of the soil profile, to collect material from the L (Horizon I) and F/H/Ah layer (Horizon II). The samples were air-dried for transport and investigated in the laboratory.
CHAPTER TWO

Extraction and analysis of testate amoebae

To detach the amoebal tests from the air dry litter and soil, the samples were rewetted for 24 h with sterile tap water (250 ml per 5 g litter or soil). The next day, the samples were filtered through a 500 µm sieve to separate coarse organic particles. Testate amoebae were subsequently collected from the filtrate on a 250 µm mesh, and small forms were recovered by a final filter step using a 25 µm sieve. Microscopic slides were prepared and tests were identified and counted at 200x and 400x magnification with an upright Leitz Ortholux II and a Nikon Inverted Microscope DIAPHOT-TMD. Additionally, we used an environmental scanning electron microscope (ESEM®, Philips Electron Optics, Eindhoven, The Netherlands) to verify species identification. The following taxonomic references were used for identification: Bonnet (1964, 1965, 1974, 1975, 1980a), Chardez (1967, 1969), Coûteaux et al. (1979), Decloitre (1962, 1978, 1981), Grospietsch (1965), Ogden and Hedley (1980), Schönborn et al. (1987), Geltzer et al. (1995). For each sample a minimum of 150 individuals were inspected. The number of tests was expressed per gram of air dry litter or soil material.

Classification

The classification of species is based on morphological characters (morphospecies) according to recent publications (Meisterfeld 2002a, b; Cavalier-Smith 2002; Cavalier-Smith et al. 2004). Testate amoebae are considered paraphyletic but morphologically and ecologically they form a well defined group consisting of two monophyletic clades (Rhizaria and Amoebozoa; Adl et al., 2005). No comprehensive phylogeny of testate amoebae is available until to date and therefore we adopt a species level approach in this study.

Statistical analysis

Differences in species number and abundance among the three study sites were analysed by repeated-measures ANOVA with Elevation as fixed factor and Horizon as repeated factor using SAS 8e (SAS Institute Inc., Cary, USA). To investigate the effect of the number of samples taken on richness estimates using Michaelis-Menten, ICE and Jackknife, K. Colwell's program EstimateS (Version 8) was used (Colwell, 1994-2004. EstimateS: statistical estimation of species richness and shared species from samples. URL http://viceroy.eeb.uconn.edu/estimates). Sample-based rarefaction curves were produced for each data set. For all calculations involving multiple random orderings of the samples 50 randomizations were used. The ICE calculations were carried out using the EstimateS default «cut-point» of 10 (Lee and Chao, 1994). Michaelis-Menten estimates were calculated using the smoothed species accumulation curve (MMeans) (Longino et al., 2002).

Principal component analysis (PCA) was used to analyse the response at the species and plot level. In these analyses the altitudinal gradient was coded by dummy variables and included using the passive analysis procedure in CANOCO (Ter Braak, 1988; Jongman et al., 1995). Prior to the analyses data were log-transformed.
2.4. Results

A total of 135 species and intraspecific taxa (53 of Rhizaria and 81 of Amoebozoa), of 38 genera and 15 families of testate amoebae were identified and counted (see Appendix p.22-24, for full scientific names of species). The 20 most abundant genera made up 95% of the total testate amoebae. The genera with ≥ 1% of total individuals comprised *Trinema* 21.6% (12 spp.), *Hyalosphenia* 17.6% (3 spp.), *Nebela* 12.6% (14 spp.), *Euglypha* 9.4% (21 spp.), *Cyclopyxis* 6.6% (8 spp.), *Centropyxis* 3.8% (12 spp.), *Heleopera* 3.0% (5 spp.), *Plagiopyxis* 2.6% (6 spp.), *Phryganella* 2.3% (4 spp.), *Sphenoderia* 2.3% (4 spp.), *Argynnia* 2.0% (3 spp.), *Placocista* 1.5% (1 sp.), *Tracheleuglypha* 1.5% (2 spp.), *Trigonopyxis* 1.3% (1 sp.), *Quadrulella* 1.2% (4 spp.), *Assulina* 1.1% (3 spp.), *Apodera* 1.0% (1 sp.), *Certesella* 1.0% (1 sp.), *Pseudodifflugia* 1.0% (2 spp.) and *Corythion* 1.0% (4 spp).

Species numbers were at a maximum in Horizon I with 75, 109, 71 species at 1000, 2000 and 3000 m, respectively. Respective species numbers in Horizon II were 29, 67 and 48. In general, the dominant taxa in Horizon I also dominated in Horizon II. However, strong differences in species composition occurred among rare species.

**Density**

Density of testate amoebae significantly increased in the order 1000 < 3000 < 2000 in Horizon I with 3147 ± 129, 9198 ± 144 and 12,497 ± 1317 ind. g⁻¹ dry matter and in Horizon II with 176 ± 25, 3118 ± 97 and 4986 ± 102 ind. g⁻¹ dry matter, respectively (F²,15 = 523.6, P = 0.0001 and F²,15 = 878.5, P = 0.0001 for Elevation and Horizon, respectively). The significant Horizon x Elevation interaction (F²,15 = 50.2, P = 0.0001) reflects the exceptionally low abundance of testate amoebae in Horizon II at 1000 m.

**Dominance and diversity**

At 1000 m the average species richness (ASR) in Horizon I was 24.3 (min = 19, max = 32, SD 5.6). The following six species with a relative density of > 5% (considered dominant) accounted for 42% of the total abundance: *Trinema enchelys* (11%), *T. lineare* (7%), *Cyclopyxis eurystoma* v. *parvula* (7%), *Cy. lithostoma* (6%), *Euglypha* sp. (6%) and *Heleopera petricola* (5%). In Horizon II ASR declined to 9.5 (min = 4, max = 15, SD 4.1). Most dominant were *T. lineare* (35%), *T. enchelys* (11%), *E. laevis* (6%), *E. simplex* (6%), *Cy. lithostoma* (6%) and *E. sp. 1* (5%), which together made up 69% of the total testate amoebae.

At 2000 m ASR in Horizon I was highest (65, min = 43, max = 87, SD 7.9). Here only four species were dominant, making up 38% of total testate amoebae: *T. enchelys* (15%), *T. lineare* (11%), *Hyalosphenia subflava* (7%), *Centropyxis constricta* (5%). Also, in Horizon II ASR was high (30.0, 23 = min, 34 = max, SD 3.9). The following three dominant species accounted for 43% of the total abundance: *H. subflava* (32%), *T. enchelys* (6%), and *Cy. eurystoma* v. *parvula* (5%).

At 3000 m ASR in Horizon I was 23.0 (min = 17, max = 35, SD 5.6). Here five species were dominant and accounted for 50% of total testate amoebae: *H. subflava* (22%), *T. enchelys* (13%) and *Nebela tincta*
(9%). In Horizon II ASR dropped to 19.6 (min = 14, max = 28, SD 6.1). Three taxa were dominant and accounted for 55% of total testate amoebae: *H. subflava* (39%), *T. enchelys* (10%) and *Placocista spinosa* (6%).

The increase in species number from 1000 to 3000 to 2000 m was highly significant ($F_{2,15} = 24.21$, $P = 0.0001$). Also, the decline in species number from Horizon I to Horizon II was highly significant ($F_{2,15} = 63.14$, $P = 0.0001$) and, as in abundance, the decline was most pronounced at 1000 m (significant Horizon x Elevation interaction, $F_{2,15} = 8.76$, $P = 0.003$).

Remarkably, similar species dominated at all altitudes. *T. enchelys* was dominant at each site and *H. subflava* was dominant at each site except at 1000 m. The coefficient of faunal similarity (see Krebs, 1999, Chap. 11) of Horizon I (Horizon II) between 1000 and 2000 m was 56% (27%) respectively, between 1000 and 3000 m it was 41% (25%), and between 2000 and 3000 m it was 53% (51%). The coefficients of faunal similarity between Horizon I and Horizon II for 1000, 2000 and 3000 m were 35%, 55%, 50%, respectively.

The ICE estimate of the sample based rarefaction curves stabilized at ca. 150 species (Fig. 2.1). The Michaelis-Menten estimate closely converged to the observed richness. The species accumulation curve was slightly sigmoid, ending at 149 species. The unique curve was flat and declined at higher sampling intensity. The duplicates curve was also flat. Neither estimator stabilized with sample size. However, the estimates were close to the observed richness (135) with 149, 151 and 164 for Michaelis-Menten, ICE and Jackknife richness, respectively.

**Morphological types**

Of the 16 morphological types of testate amoebae described by Bonnet (1964, 1975) and Coûteaux (1976) we found ten (Fig. 2.2). At each site ACC, PLV, ACS, AXI dominated with ACC being most abundant (for explanation of abbreviations see Fig. 2.2). ARC and some ACC types with spines and thorns are typical indicators of for non-limited water conditions. CRS and CRV types of shells predominately occurred in Horizon II at 1000 m. AMP, PRO, ARC and CRV were rare.
Figure 2.1. Sample-based rarefaction curves and corresponding estimators. ICE, incidence-based coverage estimator; MMMean, asymptote of Michaelis–Menten curve estimated from the sample-based rarefaction curve; Jack 2 Mean, second-order Jackknife richness estimator. Sobs, observed species richness; Uniques, number of species each present in only one sample; Duplicates, number of species each present in exactly two samples.

Figure 2.2. Dominance of different morphological types of testate amoebae shells. ACC: compressed acrostomy; PLV: plagiostomy with visor; ACS: simple acrostomy; AXI: axial; CRS: simple cryptostomy; PLS: simple plagiostomy; CRV: cryptostomy with visor; ARC: arcella; AMP: amphystomy; PRO: propylostomy.
Community structure

PCA clearly separated the three altitudes with the two horizons of each altitude clustering close together (Fig. 2.3). The community at 1000 m was characterised by species of the genus Euglypha (E. sp. 1, E. polylepis, E. tuberculata, E. acanthophora, E. laevis, E. filifera), Lamtopyxis travei, Cy. lithostoma, Arcella vulgaris, A. arenaria, Heleopera petricola v. amethystea; these species also occurred at 2000 and 3000 m but in lower numbers. In contrast, T. complanatum v. aerophilae, Nebela lageniformis v. minor, Cy. machadoi, Corythion asperulum, Planhoogenraadia acuta were found only at 1000 m. At 3000 m the density of species of the genera Nebela (N. bohemica, N. barbata, N. tubulata v. spatha, N. tinctor, N. wailesi and N. lageniformis) and the species Porosia bigibbosa and Argyonia vitrea reached maximum density. P. spinosa, E. rigosa and Quadrulella symmetrica v. longicollis preferentially colonized the site at 3000 m. At 2000 m Cy. eurystoma v. parvula, Ar. caudata, Q. quadrigera, Ce. constricta, Plagiopyxis callida, Phryganella acropodia, Trigonopyxis arcula, Cornuapexyxis lunaristoma, L. cassagnau, new species (Sp. nov. 1) were most abundant or were only found at this site.

Figure 2.3. PCA-log plot of testate amoebae in Horizons I and II at 1000, 2000 and 3000 m. Eigenvalues of axes 1 and 2 were 0.34 and 0.28, respectively. Full species names are given in the Appendix 2.1 (p. 22-24).
2.5. Discussion

In acidic forest soils which are typical for many tropical regions, litter accumulates on the forest floor forming ectorganic matter (humus forms mor, moder). Mor and moder soils typically harbour few decomposer macrofauna species, such as earthworms, millipedes and isopods. On the contrary, decomposer meso- and microfauna flourish (Schaefer, 1991). The protozoan community of mor and moder soils is dominated by testate amoebae, which contrasts to mull soils where naked amoeba and flagellates are most abundant (Schaefer and Schauermann, 1990). Typically in mor and moder soils testate amoebae are 10-20 times more abundant than in mull soils (Geltzer et al., 1985). The density and diversity of testate amoebae in these soils therefore reflect biotic and abiotic conditions and form a major component of the bacterial energy channel.

Bonnet and Thomas (1960), Bonnet (1964), Schönborn (1973) defined complexes of species typical for mor and moder soils including *Plagiopyxis declivis*, *Trigonopyxis arcula*, *Assulina* spp. and *Corythion* spp. This “acidophil” community is worldwide distributed and was also present at our study sites; however, other species were more dominant. At almost all sites *Hyalosphenia subflava* dominated followed by *N. wailesi*, *N. penardiana*, *Cy. ambigua*, *Heleopera* spp., *Pl. declivis*, *As. muscorum*, *Q. quadrigera*, *Q. symmetrica*, *Corythion* spp. and *Schoenbornia humicola*.

A total of 135 species and intraspecific taxa of testate amoebae were found with the density in the litter layer varying between 176-12,497 ind. g\(^{-1}\) dry weight. A similar number of species (104) but higher density (up to 367,000 ind. g\(^{-1}\) dry weight) has been found in a rain forest of Puerto Rico (Bamforth, 2007). In a temperate forest in Germany Wanner (1989) reported 62 species and densities of 7000-23,000 ind. g\(^{-1}\) dry weight. These data suggest that diversity but not density of testate amoebae in tropical mountain forests exceeds that in temperate forests. However, there is a large overlap in species between temperate and tropical forests of similar soil pH and humus form.

Tropical mountain rain forest soils are continuously moist, and this is also true for the studied mountain rain forests in Ecuador as indicated e.g., by the presence of numerous Rotifers such as *Habrotrocha*. High moisture at the study sites is also reflected by morphological features of testate amoebae; species with cryptostomy were rare whereas the diversity and frequency of acrostome species exceeded that typically occurring in non- permanently moist soils (Chardez, 1968). Presence and absence of species of the genera *Nebela*, *Euglypha* spp. (*E. acanthophora*, *E. cristata*, *E. compressa*, *E. filifera*, *E. strigosa*) and flattened *Arcella*-like species e.g., *Ce. aculeate* (Chardez, 1968; Bamforth, 2007), suggest that the soil fauna of the studied rain forest is semiaquatic and similar to that of bryophyte communities. Several species are even typical for submerged sites including *P. spinosa*, *Archerella flavum*, *H. sylvatica*, *H. papilio*, *N. militaris*, *N. tincta*, *As. seminulum*, *He. rosea*, *As. muscorum*, *A. catinus*, *E. strigosa* and *Tr. arcula* (Geltzer and Korganova, 1980).

We investigated three altitudes ranging from 1000 to 3000 m a.s.l. Even though restricted, the results suggest that species richness of testate amoebae does not decrease continuously with elevation; rather, it likely peaks at intermediate elevation (2000 m) and a similar pattern applies to the density of testate
amoebae. This was true despite some tropical species of lower altitudes did not occur at 2000 m. The density of species followed the same pattern. A similar pattern was found for moth species richness by Brehm et al. (2006). The pattern, however, contradicts the study of Bonnet (1978) reporting a decrease in species richness and an increase in cosmopolitan testate amoebae species with altitude.

The low diversity and density of testate amoebae at 1000 m likely is related to the low amount of litter in the litter layer at this site suggesting that litter decomposition at 1000 m is higher than at 2000 and 3000 m (Röderstein et al., 2005; Wilcke et al., 2007). Presence of a thick L/F layer combined with moderate climatic conditions presumably is responsible for the maximum diversity and density of testate amoebae at 2000 m.

Most of the species found were cosmopolitan, however, the distribution of nine species (i.e. 6.7%) appears to be restricted to the tropics: *Co. lunaristoma*, *Cy. lithostoma*, *Cy. machadoi*, *Cy. stephanostoma*, *L. travei*, *L. cassagnai*, *Ar. caudata*, *Pa. acuta*, *Q. quadrigera*. According to Bonnet (1980b, 1987), some of these species evolved on Gondwana and therefore likely represent Gondwana relicts.

At 1000 m, one of the tropical species, *Cy. lithostoma*, dominated the testate amoebae community. At the other altitudes, tropical species were not very abundant, rather, cosmopolitan species dominated. However, the number of tropical species was at a maximum at 2000 m, with some of them only occurring at this altitude (*Co. lunaristoma*, *Cy. machadoi*). Other tropical species only occurred at 1000 m (*L. travei*, *Pa. acuta*) and 3000 m (*Cy. stephanostoma*). *Ap. vas* and *Cr. martiali* which are considered Australian species with circumpolar distribution were found in almost all samples but most frequent at 3000 m. Local environmental factors are important in determining the distribution of species (Smith and Wilkinson, 1986), and changes in environmental factors with altitude likely are responsible for the difference in dominance of testate amoebae species of tropical Gondwana distribution along the studied elevational gradient.

There are contrasting views on the global distribution and diversity of protists (Finlay, 2002; Foissner, 2006). In part our data support the view that 'everything is everywhere’ since the great majority of the testate amoebae morphospecies recorded in our study indeed also occurs in very different geographical regions, such as the temperate and boreal zone of the Northern hemisphere. One species claimed to be endemic to narrow geographical regions, *Playfairina valkanovi* (Foissner, 2006), was also recorded at our study site, suggesting that it is widespread in the Southern hemisphere. Our data suggest that primarily local habitats rather than historical conditions select for testate amoebae species, although small but distinct differences occur between the Southern and Northern hemisphere. In general, however, the community composition of the moist and acidic tropical forest investigated closely resembles habitats with similar abiotic environment at very different geographical regions, such as moist and acidic forests of the Northern hemisphere (Korganova, 1997; Rakhleeva, 2002).
Appendix 2.1. Species list of testate amoebae at three sites (1000, 2000 and 3000 m) and two horizons (H I, L layer; H II, F/H/Ah layer) in the studied tropical mountain rain forest of Ecuador (Nomenclature according to Meisterfeld, 2000a,b).

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SD = Standard Deviation
Microorganisms as driving factors for the community structure of testate amoebae along an altitudinal transect in tropical mountain rain forests
CHAPTER THREE

CHAPTER 3. MICROORGANISMS AS DRIVING FACTORS FOR THE COMMUNITY STRUCTURE OF TESTATE AMOEBAE ALONG AN ALTITUDINAL TRANSECT IN TROPICAL MOUNTAIN RAIN FORESTS

3.1. Abstract

We investigated microbial biomass, fungal biomass and microbial community structure at three altitudes (1000, 2000 and 3000 m) and in two soil layers [L/F layer (Layer I) and underlying H/Ah layer (Layer II)] of tropical mountain rain forests in southern Ecuador. Basal respiration, microbial biomass and concentration of ergosterol generally declined from Layer I to Layer II and peaked at 2000 m. Compared to temperate forest ecosystems microbial biomass and ergosterol concentrations were generally low. Patterns in phospholipid fatty acids indicated that the composition of microbial communities markedly changed from Layer I to Layer II. These differences between layers decreased with increasing altitude. The concentration of the arbuscular mycorrhizal fungal marker PLFA 16:1ω5c decreased with altitude in Layer I but increased in Layer II. The fungal-to-bacterial ratio increased with altitude and was higher in Layer I than in Layer II. Presumably, low microbial biomass in soils of tropical forest ecosystems is due to high temperature associated with high respiration but also low litter quality, with the latter declining with altitude. These conclusions are supported by the fact that at higher altitude the microbial community changed from a bacterial-dominated to a fungal-dominated system. CCA showed that microbial biomass correlated closely with density of a number of putatively bacterial feeding testate amoeba species including Corythion dubium Taranek, 1871, Euglypha cristata Leidy, 1879, Trigonopyxis arcula Penard, 1912, Tracheleuglypha dentata Deflandre, 1928, Trinema lineare Penard, 1890. Ergosterol concentrations, but not the PLFA 18:2ω6c, strongly correlated with the putatively fungal feeding species Phryganella acropodia (Hertwig, Lesser, 1874) Hopkinson, 1909. Generally, parallel to microbial biomass and ergosterol concentrations the density of testate amoebae peaked at 2000 m. However, compared to microbial parameters changes in testate amoebae communities between two layers were less pronounced. The data suggest that density and community structure of testate amoebae are driven by the availability of food resources (bacteria and fungi) which at high altitude decrease with increasing moisture and decreasing pH.

3.2. Introduction

Mountain rainforests of southern Ecuador at the eastern slopes of the Andes are among the most species-rich ecosystems on Earth (Küper et al., 2004; Hilt and Fiedler, 2005). With altitude tropical lowland forest is replaced by Lower Montane Forest with trees reaching up to 40 m, Upper Montane Forest where trees grow up to 20 m, and, close to the timberline, by Elfin forest with trees of a maximum height of 7 m (Leuschner et al., 2007). Parallel to the decrease in size, trees become twisted and gnarled and leaves become thick and hard (Foster, 2001). However, until now these forests have been poorly investigated
and studies on the effect of altitude on ecosystem processes are rare. With altitude and decreasing air temperature in tropical mountains tree height (Whitmore, 1998), aboveground biomass (Röderstein et al., 2005), leaf litter production and soil pH also decrease, whereas the thickness of organic layers, litter C-to-N ratio, annual rainfall and soil moisture increase (Leuschner et al., 2007; Moser et al., 2007). Changes in aboveground animal communities including arthropods and birds have been investigated along altitudinal gradients of tropical mountains (Hasegawa et al., 2006). Beside that, soil microorganisms as an important component of the soil community have rarely been investigated. Variations in biomass and community structure along altitudinal transects are largely unknown.

Soil microorganisms (bacteria and fungi) represent essential components of the soil system (Hackl et al., 2005). They are key organisms for the decomposition of organic matter, central to the cycling of carbon and nitrogen and a sensitive indicator of changes in total organic matter (Beare et al., 1992; Luizao et al., 1992; Beck et al., 1997). Saprophytic fungi are the most important decomposers of lignin and lignin-like compounds (Ingold and Hudson, 1993), whereas mycorrhizal fungi provide plants with mineral nutrients. Compared to the mineral soil little attention has been paid to organic layers of forests (Maraun and Scheu, 1996) which is surprising because microorganisms primarily are concentrated in the upper soil layer where organic material enters the decomposer community and is decomposed by a variety of functional groups of microorganisms (Hackl et al., 2005).

Soil microorganisms are imbedded in a complex food web and form the basis of the bacterial and fungal energy channel (Bonkowski, 2004; Scheu et al., 2005). Presumably, the most important bacterial consumers in soil are protists followed by bacterivorous nematodes (Zwart and Brussaard, 1991). In acidic forest soils, such as prevalent in the investigated tropical mountain forests, protists are dominated by testate amoebae (Geltzer and Korganova, 1976; Geltzer and Korganova, 1980; Krashevska et al., 2007). Testate amoebae primarily feed on bacteria (Bonnet, 1964; Stout and Heal, 1967), but also on fungal hyphae, spores and yeasts (Coûteaux and Devaux, 1983; Ogden and Pitta, 1990), algae and other protists (Bonnet, 1964; Laminger, 1978; Laminger, 1980); some even feed on small metazoans (Yeates and Foissner, 1995), others on humus particles (Schönborn, 1965, 1982). Testate amoebae prefer certain prey-species and reject others (Laminger and Bucher, 1984). Selective grazing may be an important factor determining the taxonomic composition and metabolic activity of microbial communities (Sherr et al., 1992; Bonkowski, 2004).

Our previous work showed that species richness of testate amoebae does not decrease continuously with altitude in tropical mountain rain forests; rather, it peaks at intermediate altitude (Krashevska et al., 2007). The aim of the present study was to analyse altitudinal changes in microbial biomass and community composition to evaluate relationships between microorganisms and testate amoebae in the studied tropical mountain rain forests in southern Ecuador. We hypothesized that (1) with declining pH the dominance of fungi increases with altitude, (2) bacterial dominance generally decreases from Layer I to Layer II irrespective of altitude and (3) parallel to testate amoebae microbial biomass peaks at intermediate altitude. Generally, we expected that the community structure of testate amoebae is closely associated with that of microorganisms.
3.3. Material and Methods

Study sites and field sampling

The study sites are located in southern Ecuador on the northern fringes of the Podocarpus National Park on the eastern slopes of the Andes. Three study sites along an altitudinal transect were selected at 1000, 2000, 3000 m a.s.l. Information on soil, climate and vegetation of the study area has been summarized in Krashevska et al. (2007); more details are given in Beck et al. (2008).

Samples were taken in October/November 2006 (at 3000 m samples were taken on 30 October and 1 November 2006). At each altitude a sampling area of 100-200 m² was selected and 6 sampling sites were randomly chosen. Samples were taken with a corer (5 cm diameter) up to a depth of 48 cm, depending on the depth of the soil profile, to collect material from the L/F layer (Layer I) and H/Ah layer (Layer II), resulting in 36 samples in total. Layer I consisted of little decomposed organic material including fresh leaves, seeds, flowers, and twigs, fine roots, plus some larger woody material. At 1000 m the thickness of this layer was ca. 6 cm, at 2000 and 3000 m it was 11 and 28 cm, respectively. Layer II consisted of strongly decomposed plant material in part mixed with mineral soil and was densely rooted. At 1000 m thickness of this layer was 5 cm and consisted mainly of Ah material, at 2000 and 3000 m it was 10 and 20 cm, respectively, and consisted of H material.

Before start of the analysis the samples were stored at 5°C for a maximum of 6 days which is known to affect microbial biomass and PLFA patterns only little (Zelles et al., 1991; Bååth et al., 2004).

Microbial basal respiration and microbial biomass

Soil respiration and microbial biomass were determined from O₂ consumption measured by an automated microcompensation apparatus (Scheu, 1992). Microbial basal respiration (µl O₂ g⁻¹ soil dry wt h⁻¹) was calculated as mean O₂ consumption during hours 10-20 after attachment to the respirometer. Microbial biomass was assessed by measuring the maximum initial respiratory response (MIRR; mg O₂ g⁻¹ h⁻¹) after glucose addition (SIR method; Anderson and Domsch, 1978; Beck et al., 1997). Glucose (80 and 40 mg g⁻¹ dry wt for Layer I and Layer II, respectively) was added as an aqueous solution adjusting the soil water content to 80-90% of the water holding capacity of the litter and soil materials (cf. Joergensen and Scheu, 1999). The mean of the three lowest measurements during the first 10 h after glucose addition was taken as MIRR.

Ergosterol content

To determine the ergosterol content of the samples 1 g of material of Layer I and II was extracted with 100 ml ethanol in bay-coloured glass flagons for 30 min by shaking (250 rev. min⁻¹ on a rocker). Then samples were transferred into 50 ml screw-cup centrifuge tubes and centrifuged at 3700 g for 40 min. Immediately after centrifugation 20 ml of the supernatant was evaporated in a vacuum rotary evaporator at 40°C and 10 hPa. The residue of ca. 1 ml was diluted with 3 ml ethanol and evaporated again. The
dried extract was collected in 0.5 ml methanol and subsequently filtered through a cellulose acetate membrane (0.45 µm). Due to the fact that ergosterol is photosensitive all steps were carried out in darkness. The samples were stored at -18°C until analysis. Quantitative determination of ergosterol was performed by reversed-phase High Performance Liquid Chromatography analysis using the following set up: main column 10 cm, pre-column 0.5 cm (Spherisorb ODS II, 5 µm diameter), mobile phase 100% methanol, flow rate 1.0 ml h⁻¹ and detection at 282 nm with a Diodenarry Detector (Dionex 170 S). Temperature was maintained constant at 26°C. For calibration a standard solution of ergosterol (Sigma-Aldrich, San Louis, USA) was used (see Djajakirana et al., 1996).

**PLFA extraction and identification**

Lipid extraction was performed on 1-2 g litter or soil according to Frostegård et al. (1993). Lipid fractionation was performed with silica acid columns (0.5 g silicic acid, 3 ml; Varian Medical Systems, Palo Alto, California). Methanolation of phospholipid fatty acids (PLFAs) was conducted with 0.2 M methanolic KOH. The resulting fatty acid methyl esters (FAMEs) were kept at –20°C until analysis with isoocctane as solvent. FAMEs were identified by chromatographic retention time comparison with a standard mixture composed of 37 different FAMEs that ranged from C11 to C24 (Sigma-Aldrich, St Louis, USA). Analysis was performed by gas chromatography (GC) using an Auto System XL (Perkin Elmer Corporation, Norwalk, USA) equipped with a HP-5 capillary column (50 m x 0.2 mm i.d., film thickness 0.33 µm). The temperature program started with 70°C (hold time 2 min) and increased with 30°C per min to 160°C, and then with 3°C per min to 280°C and held for 15 min. The injection temperature was 260°C and helium was used as carrier gas.

For each sample the abundance of individual phospholipid fatty acid methyl-esters was expressed per unit dry weight. The nomenclature for PLFAs followed that of Frostegård et al. (1993). PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω7, 17:0, i17:0, cy17:0, 18:1ω7 and cy19:0 represented bacteria (Frostegård and Bååth, 1996); 16:1ω5c was used as an indicator for arbuscular mycorrhizal fungi (AMF) (Haack et al., 1994). In addition to ergosterol, the concentration of the fungal specific fatty acid 18:2ω6c was used as a fungal marker (Federle, 1986). The ratio of 18:2ω6c to bacterial PLFAs (see above) was used to represent the ratio of fungal-to-bacterial biomass (Frostegård and Bååth, 1996).

**Statistical analysis**

Differences in microbial basal respiration, microbial biomass, PLFAs and ergosterol content between the three study sites were analysed by split plot ANOVA (GLM, type I sum of squares) using SAS 9.13 (SAS Institute Inc., Cary, USA). The treatment analyzed at the plot scale (Altitude) was tested against the variance between plots to avoid pseudoreplication whereas treatments analyzed on the subplot scale (Layer and Layer x Altitude) were tested against the variance between subplots. PLFA patterns were analysed by discriminant function analysis (DFA) using the three elevation sites as independent variables. DFA was carried out with STATISTICA 7.0 for Windows (StatSoft, Tulsa, USA, 2001). Squared
Mahalanobis distances between group centroids (layers) and the reliability of sample classification were determined. Two significant canonical roots were derived and graphically presented in 2-dimensional space.

Testate amoebae data taken from Krashevska et al. (2007) were subjected to canonical correspondence analysis (CCA) using CANOCO (Ter Braak, 1988). CCA was used to sort the sites (dummy variables) with two layers according to their environmental conditions and to relate testate amoebae species abundance patterns to environmental information and to test the data set for trends of ecological preferences of particular species. Only species that were present in more than three of the six replicates per altitude were used for CCA. Environmental variables included microbial biomass, ergosterol, fungal PLFA marker (18:2ω6c), AMF marker (16:1ω5c), Gram-negative bacteria markers (16:1ω7, cy17:0, cy19:0) and Gram-positive bacteria markers (i15:0, a15:0, i16:0, i17:0; Zelles, 1997; Zelles, 1999), animal markers (20:2, 20:4ω6; Chen et al., 2001; Salomonová et al., 2003; Chamberlain et al., 2005), and algae marker (20:5ω3; Dunstan et al., 1994; Boschker et al., 2005). Monte-Carlo tests (999 permutations) were performed to evaluate significance of individual axes (Ter Braak, 1996). Prior to the analyses data were log-transformed.

3.4. Results

Microbial basal respiration and microbial biomass

Basal respiration (BR) was at a maximum at 2000 m, lower at 3000 m and lowest at 1000 m ($F_{2,15} = 14.12, P = 0.0004$; Fig. 3.1a). Generally, BR declined from Layer I to Layer II ($F_{2,15} = 71.37, P < 0.0001$). However, changes in BR between the layers differed between altitudes (Altitude x Layer interaction, $F_{2,15} = 11.63, P = 0.0009$). At 1000 and 2000 m BR in Layer I exceeded that in Layer II by factors of 5.3 and 4.8, respectively, but at 3000 m by a factor of 2.4 only.

Similar to BR soil microbial biomass (MB) was at a maximum at 2000 m, lower at 3000 m and lowest at 1000 m ($F_{2,15} = 22.26, P < 0.0001$; Fig. 3.1b). Also similar to BR, MB generally declined from Layer I to Layer II ($F_{2,15} = 63.25, P < 0.0001$), but changes between the layers differed between altitudes (Altitude x Layer interaction, $F_{2,15} = 18.19, P < 0.0001$). At 1000 m MB in Layer I exceeded that in Layer II by a factor of 6.3, respective factors at 2000 and 3000 m were 3.3 and 1.0 only.

Ergosterol

The concentration of ergosterol ranged between 0.4 and 67.0 µg g$^{-1}$ dry mass (Fig. 3.2.). As BR and MB, ergosterol concentrations declined in the order 2000 m > 3000 m > 1000 m ($F_{2,15} = 46.51, P < 0.0001$). Also similar to BR and MB, changes in ergosterol concentrations from Layer I to Layer II differed between altitudes (Altitude x Layer interaction, $F_{2,15} = 20.63, P < 0.0001$). Concentrations in Layer I exceeded those in Layer II by factors of 37.5, 5.5 and 1.4 at 1000, 2000 and 3000 m, respectively.
Fig. 3.1. Basal respiration (a) and microbial biomass (b) at three altitudes and two soil layers of the studied tropical mountain rain forests. Means with standard errors. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Fig. 3.2. Ergosterol content at three altitudes and two soil layers of the studied tropical mountain rain forests. Means with standard errors. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).
CHAPTER THREE

PLFA

In total 26 PLFAs with a chain length up to C_{24} were detected and identified. The total amount of PLFAs ranged between 121.1 and 1449.1 nmol g^{-1} dry mass with 16:0 being most abundant and 20:5ω3 being least abundant (Fig. 3.3). Contrary to BR, MB and ergosterol, concentrations of phospholipids were at a maximum at 3000 m, lower at 2000 m and lowest at 1000 m ($F_{2,15} = 108.65, P < 0.0001$). At 1000 m and 2000 m the concentration of PLFAs in Layer I exceeded those in Layer II by factors of 9.57 and 6.00, respectively, whereas at 3000 m the concentration in Layer II exceeded those in Layer I by a factor of 2.89 only (significant Altitude x Layer interaction, $F_{2,15} = 92.70, P < 0.0001$).

At 1000, 2000 and 3000 m bacterial PLFAs (i15:0, a15:0, 15:0, i16:0, 16:1ω7, 17:0, i17:0, cy17:0, 18:1ω7 and cy19:0) contributed 39, 41 and 33 % to total PLFAs in Layer I, and 41, 44 and 39 % in Layer II, respectively. The concentration of the AMF decreased with altitude from 22.9 to 21.3 to 19.6 nmol g^{-1} dry mass in Layer I and increased with altitude in Layer II from 5.8 to 7.0 to 49.6 nmol g^{-1} dry mass at 1000, 2000 and 3000 m, respectively (significant Altitude x Layer interaction, $F_{2,15} = 33.96, P < 0.0001$).

In contrast to ergosterol, the fungal marker 18:2ω6c increased with altitude ($F_{2,15} = 10.24, P = 0.002$) from 22.9 to 123.9 to 151.5 nmol g^{-1} dry mass in Layer I and from 8.6 to 17.3 to 160.7 nmol g^{-1} dry mass in Layer II at 1000, 2000 and 3000 m, respectively (significant Layer effect, $F_{1,15} = 5.23, P = 0.04$).

The fungal-to-bacterial PLFA ratio also varied with altitude and layer. In Layer I it was similar at 1000 and 2000 m (0.37 and 0.41, respectively) but considerably higher at 3000 m (0.74). In contrast, in Layer II it was similar at 2000 and 3000 m (0.34 and 0.33, respectively) and considerably lower at 1000 m (0.14) (significant Altitude x Layer interaction, $F_{2,15} = 23.06, P < 0.001$).

Discriminant function analysis (DFA) of microbial PLFAs (14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1ω6c, 16:1ω5c, 17:0, i17:0, cy17:0, 18:1ω7, 18:2ω6c, 18:1ω9c, cy19:0) separated the microbial communities along the altitudinal transect (axis 1), but also separated the communities of the two layers (axis 2) (Wilks' Lambda 0.4641, $F_{4,64} = 7.48, P < 0.001$). Only at 3000 m PLFA patterns were similar in...
both layers; further, the PLFA pattern of Layer I at 3000 m was similar to that of Layer II at 2000 m (Table 3.1; Fig. 3.4).

**Fig. 3.4.** Discriminant function analysis of the fatty acids along the altitudinal transect (axis 1) and between soil layers (axis 2). The scatterplot shows ellipses with $\alpha = 0.05$. Arrows show the tendency of similarity of microbial composition along the altitudinal transect.

**Table 3.1.**

Squared Mahalanobis distances between group centroids and reliability of discrimination based on data on PLFA dominance structure$^a$.

<table>
<thead>
<tr>
<th></th>
<th>1000 m Layer I</th>
<th>1000 m Layer II</th>
<th>2000 m Layer I</th>
<th>2000 m Layer II</th>
<th>3000 m Layer I</th>
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</thead>
<tbody>
<tr>
<td>1000 m Layer II</td>
<td>34.3$^*$</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000 m Layer I</td>
<td>45.9$^{**}$</td>
<td>87.5$^{***}$</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000 m Layer II</td>
<td>111.3$^{***}$</td>
<td>109.6$^{***}$</td>
<td>43.0$^*$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3000 m Layer I</td>
<td>102.4$^{***}$</td>
<td>122.2$^{***}$</td>
<td>34.1$^*$</td>
<td>26.1$^*$</td>
<td></td>
</tr>
<tr>
<td>3000 m Layer II</td>
<td>144.2$^{***}$</td>
<td>160.5$^{***}$</td>
<td>57.1$^*$</td>
<td>34.1$^*$</td>
<td>8.2$^*$</td>
</tr>
</tbody>
</table>

ns = not significant; *P < 0.01; **P < 0.005; ***P < 0.0001

$^a$ See text for details
CHAPTER THREE

Relationships between microorganisms and testate amoebae

In the forward selection procedure of the CCA, three of the eight quantitative variables were significant when tested as a first variable in the model (P < 0.05; Fig. 3.5). The three significant variables explained 56% of the variation in the species data with the trace being significant (F = 1.30, P = 0.01). Axes 1 and 2 explained 22% and 11% of the total variation of the data, respectively.

Including the explanatory variables one after the other using forward selection, gram positive bacteria accounted for most of the variation of the data (15% of total; F = 3.04, P = 0.002). The second environmental variable with significant explanatory value was ergosterol (additional 8% of total; F = 1.60, P = 0.03) and the third variable was MB (additional 7% of total; F = 1.50, P = 0.05).

CCA ordination clearly separated the three altitudes with the two layers of each altitude clustering close together. At 3000 m Layer I and Layer II grouped together at the one end of axis 1 and correlated with gram positive bacteria, at the opposite side Layer I and Layer II of the 2000 m site grouped together and correlated with MB. The first axis mainly represented the difference between these two altitudes. At 1000 m Layer I and Layer II grouped together at the end of axis 2 and correlated negatively with ergosterol.

Parallel to MB and ergosterol most species grouped at 2000 m. Corythion dubium Taranek, 1871, Cyclopyxis eurystoma Deflandre, 1929, Trigonopyxis arcula Penard, 1912, Euglypha cristata Leidy, 1879, Euglypha cristata decora Jung, 1942, Trinema lineare Penard, 1890, Argynnia caudata Leidy, 1879, Plagiopyxis callida Penard, 1910, Heleopera sylvatica Penard, 1890 and Tracheleuglypha dentata Deflandre, 1928 correlated closely with MB, and Phryganella acropodia (Hertwig, Lesser, 1874) Hopkinson, 1909, Euglypha compressa Carter, 1864, Awerintzewia cyclostoma (Penard, 1902) Schouteden, 1906, Difflugiella oviformis Bonnet, Thomas, 1955, Sphenoderia lenta Schlumberger, 1845 with ergosterol. Only four species were associated with the 1000 m site (Euglypha laevis (Ehrenberg, 1832) Perty, 1849, Argynnia dentistoma Penard, 1890, Trinema complanatum v. elongata Decloitre, 1973 and Heleopera petricola Leidy, 1879). Difflugia lucida Penard, 1890, Placocista spinosa (Carter, 1865) Leidy, 1879 and Heleopera sphagni Leidy, 1874 were associated with the 3000 m site and correlated with gram positive bacteria and algae.
Fig. 3.5. CCA biplot of testate amoebae and environmental variables. Variables that do not significantly contribute to the model are projected passively in the ordination (thin arrows; L I, Layer I; L II, Layer II; Gr-, Gram-negative bacteria; Gr+, Gram-positive bacteria; full species names are given in Appendix 2.1, p. 22-24).

3.5. Discussion

Changes with altitude

As hypothesized, most of the microbial parameters measured including BR, MB and ergosterol peaked at 2000 m, and therefore followed a pattern similar to that of testate amoebae. Significant correlation between MB, as measured by substrate-induced respiration, and density of testate amoebae suggests that testate amoebae at the study sites are limited by food resources. As indicated by correlations between MB and a number of species of testate amoebae (in CCA) this is likely to be true for many species assumed to predominantly feed on bacteria including *Corythion dubium*, *Euglypha cristata*, *Trigonopyxis arcula*, *Tracheleuglypha dentata*, *Trinema lineare* (Bonnet, 1964; Coûteaux, 1976; Coûteaux and Ogden, 1988; Laminger, 1980; Cowling, 1986; Gilbert et al., 2000). Interestingly, high concentrations of ergosterol at 2000 m correlated with species of testate amoebae, such as *Phryganella acropodia*, considered to feed on fungi (Coûteaux and Devaux, 1983). However, concentrations of ergosterol did not significantly correlate with the fungal biomarker fatty acid 18:2ω6c but rather with total microbial biomass. Low correlation...
between the two fungal biomarkers may be due to the fact that different fungal taxa contain different amounts of 18:2ω6c (Stahl and Klug, 1996) or do not synthesise 18:2ω6c at all (Malosso et al., 2004).

Some species of the genera *Nebela*, *Hyalosphenia*, *Apodera*, *Argynnia* and *Heleopera*, but in particular *Trinema* and *Euglypha* are considered to feed on animal prey (i.e. nematodes, protists; Laminger and Bucher, 1984; Yeates and Foissner, 1995; Gilbert et al., 2000). Close association between *Argynния caudata* and *Euglypha cristata*, *Quadrulella symmetrica* (Wallich, 1863) Schulze, 1875 and *Nebela tubulosa* Penard, 1902 (Chardez, 1985) in the ordination diagram therefore likely reflects trophic links. *Placocista spinosa*, *Difflugia lucida* and *Heleopera sphagni* significantly correlated with gram positive bacteria and with algae. *Placocista spinosa* and *Heleopera sphagni* consume chloroplasts of algae for acquiring symbiotic partners (Schönborn, 1965), and this may explain the presence of these species in the vicinity of algae, rather than close to gram positive bacteria (see CCA).

Investigating the same altitudinal transect in Ecuador as in the present study Iost et al. (2008) reported microbial biomass to decline with altitude, however, high values also occurred at intermediate altitude (1890 m). The somewhat different results might be due to different methods used to measure microbial biomass (substrate-induced respiration, SIR, as compared to chloroform fumigation extraction, CFE) and by using different sampling sites and sampling time. Both methods do have disadvantages but the SIR method might be advantageous at our study site since the CFE method is unreliable in wet soils with high organic matter content (Coûteaux and Henkinet, 1990).

Results of the present study documented that low amounts of litter at 1000 m are associated with low diversity and density of testate amoebae, probably due to limitation by food resources (Geltzer and Korganova, 1976). At 3000 m food resources were less limiting potentially due to reduced density of competitors at low pH (see Geltzer and Korganova, 1980). At 2000 m the presence of thick layers of organic matter, greater microbial biomass and moderate climatic condition, likely contributed to high density of testate amoebae.

Changes with soil depth

Basal respiration, microbial biomass and ergosterol concentrations generally declined from Layer I to Layer II. Basal respiration of Layer I material exceeded that of Layer II by a factor of 2.8. For microbial biomass and ergosterol the respective factors were 2.5 and 2.9, respectively. Compared to organic layers of temperate forest ecosystems basal respiration, microbial biomass (measured by the same method) and ergosterol concentrations were low (Maraun and Scheu, 1996; Joergensen and Scheu, 1999; Frostegård and Bååth, 1996). Basal respiration, microbial biomass and ergosterol concentrations in L/F-layer material in the beech forests studied by Maraun and Scheu (1996), Joergensen and Scheu (1999) and Frostegård and Bååth (1996) exceeded those of the litter layer at the 2000 m site, where these factors were at a maximum, at least by a factor of 3. Low microbial biomass and ergosterol concentrations at the studied tropical mountain forest ecosystems resemble those reported for other tropical forests (Luizao et al., 1992; Behera and Sahani, 2003; Barbhuiya et al., 2004; Imberger and Chiu, 2002). Presumably, low microbial biomass contents in soils of tropical forest ecosystems are due to high temperature associated
with high respiration but also low litter quality, with the latter declining with altitude. The decrease in basal respiration, microbial biomass and ergosterol concentrations from Layer I to Layer II presumably is caused by a decrease in the amount of available carbon resources with soil depth (Anderson and Domsch, 1980; Scheu and Parkinson, 1995; Maraun and Scheu, 1996) and may also be due to decreasing pH (Anderson and Domsch, 1993; Bååth and Anderson, 2003).

Parallel to ergosterol concentrations, the fungal-to-bacterial ratio was significantly higher in Layer I than in Layer II. Similar to the suggestion by Frostegård and Bååth (1996) the fungal-to-bacterial ratio was at a maximum in high organic matter soils (Layer I) whereas the opposite was true for low organic matter soils (Layer II). In contrast to saprophytic fungi (as measured by ergosterol concentrations), the concentration of AMF decreased with altitude in Layer I and increased in Layer II which presumably was caused by increased root biomass (Leuschner et al., 2007). Obtaining carbon from their associated plants, AMF do not compete with saprophytic fungi for carbon but presumably for nutrients (Olsson, 1999).

In correspondence with microbial parameters density of testate amoebae was higher in Layer I than in Layer II (Krashevska et al., 2007). Parallel to microorganisms, the community structures of testate amoebae of Layer I and Layer II became more similar with increasing altitude. Generally, however, the community structure of testate amoebae in Layer I and Layer II was more similar than that of microorganisms (see Fig. 3.4 and Krashevska et al., 2007). Overall, the similarity in community structure but decline in density of testate amoebae from Layer I to Layer II suggests that with soil depth resources become scarcer but the quality of resources changes little.

Conclusions

The results suggest that compared to temperate forests decomposing litter materials in the studied tropical mountain rain forests support only small populations of microorganisms. Low contents of microorganisms at 1000 m presumably are due to mixing of litter materials into the mineral soil, i.e. bioturbation, whereas low concentration of microorganisms at 2000 m but in particular at 3000 m likely reflects declining litter quality and increasing nutrient limitation with altitude. These conclusions are supported by the fact that in particular in Layer I at higher altitude the microbial community changed from a bacterial-dominated system to a fungal-dominated system. Parallel to microbial parameters, the density of testate amoebae peaked at 2000 m, and with the decline in microbial biomass from Layer I to Layer II density of testate amoebae also decreased.

The data suggest that density and community structure of testate amoebae are driven by the availability of food resources (bacteria and fungi). Adverse abiotic factors such as high moisture and low pH at high altitude likely reduce the availability of food resources and thereby the density of testate amoebae, however, adverse abiotic conditions may also directly affect testate amoebae. For proving the relative importance of resources vs environmental factors as structuring factors for the density and community structure of testate amoebae experiments manipulating the availability of food resources and abiotic factors such as pH and humidity are needed.
Carbon and nutrient limitation of soil microorganisms and microbial grazers in a tropical montane rain forest
CHAPTER 4. CARBON AND NUTRIENT LIMITATION OF SOIL MICROORGANISMS AND MICROBIAL GRAZERS IN A TROPICAL MONTANE RAIN FOREST

4.1. Abstract

We investigated the role of carbon, nitrogen and phosphorus as limiting factors of microorganisms and microbial grazers (testate amoebae) in a montane tropical rain forest in southern Ecuador.Carbon (as glucose), nitrogen (as NH$_4$NO$_3$) and phosphorus (as NaH$_2$PO$_4$) was added separately and in combination at two month intervals to experimental plots for 20 months. By adding glucose and nutrients we expected to increase the biomass of microorganisms as major food resource of testate amoebae. The response of microorganisms to experimental treatments was determined by analysing microbial biomass (SIR), fungal biomass and microbial community composition as measured by phospholipid fatty acids (PLFAs). We hypothesized that the response of testate amoebae is closely linked to that of microorganisms. The addition of glucose strongly increased ergosterol concentration and less pronounced also the concentration of the fungal PLFA biomarker 18:2ω6c, suggesting that saprotrophic fungi are limited by carbon. Microbial biomass and ergosterol concentrations reached a maximum in the combined treatment with C, N and P indicating that both N and P also were in short supply. In contrast to saprotrophic fungi and microorganisms in total, testate amoebae suffered from the addition of C and reached maximum density by the addition of N. The results indicate that saprotrophic fungi in tropical montane rain forests are mainly limited by carbon whereas gram positive and gram negative bacteria benefit from increased availability of P. Testate amoebae suffered from increased dominance of saprotrophic fungi in glucose treatments but benefited from increased supply of N presumably by increased availability of high quality detritus, certain bacteria and increased performance of endosymbiotic algae. The results show that testate amoebae of tropical montane rain forests are controlled by bottom-up forces relying on specific food resources rather than the amount of bacterial biomass with saprotrophic fungi functioning as major antagonists. Microbial food webs in soil therefore may be much more complex than previously assumed with trophic links being rather specific and antagonistic interactions potentially overriding trophic interactions.

4.2. Introduction

Tropical montane rain forests are among the most diverse ecosystems of the world (Myers et al., 2000; Küper et al., 2004; Hilt and Fiedler, 2005). This has been documented in rain forests of southern Ecuador for plant (Spermatophyta, Homeier and Werner, 2007; Pteridophyta, Lehnert et al., 2007; Bryophyta, Gradstein et al., 2007; Kürschner and Parolly, 2007; Lichens, Nöske et al., 2007) and a number of animal taxa (Mammalia, Matt and Werner, 2007; Aves, Paulsch, 2007; Lepidoptera, Häuser et al., 2007). However, compared to aboveground biota, belowground communities received little attention (Anichkin et al., 2007); only mites (Illig et al., 2007) and testate amoebae (Krashevska et al., 2007) have been investigated in some detail.
Testate amoebae reach high diversity (ca. 135 species) and density (ca. 13,000 ind./g dry weight) in tropical montane ecosystems (Krashevska et al., 2007). They are trophically diverse with species consuming organic matter (Schönborn, 1982), microorganisms and also other soil animals (Laminger, 1978; Yeates and Foissner, 1995). Protist activity modifies microbial activity and thereby alters nutrient fluxes in the soil system (Stout, 1980; Griffiths, 1994; Couëteaux and Darbyshire, 1998). Despite their importance for decomposition processes, factors that regulate their density and diversity are little understood especially in tropical ecosystems. Commonly, the distribution of testate amoebae is explained by abiotic factors, in particular humidity and acidity (Geltzer et al., 1985; Schaefer and Schauermann, 1990), and UV-B radiation (Searles et al., 2001). However, the availability and quality of resources, i.e. bottom-up forces, likely also control the density and species composition of testate amoebae, but this has not been investigated yet.

Generally, bacteria, fungi and also saprophagous soil animals are assumed to be controlled mainly by resource availability (Hunt et al., 1987), and limited by the availability of carbon and nitrogen (Anderson and Domsch, 1978; Aber et al., 1991; Vitousek and Howarth, 1991; Gallardo and Schlesinger, 1994). However, studies on the role of bottom-up forces in decomposer communities are scarce. Scheu and Schaefer (1998) investigated the effects of increased energy and nutrient resources on microorganisms and soil invertebrates in a beechwood on limestone in Germany. They reported that litter microorganisms are limited by nitrogen but none of the animal groups studied (Lumbricidae, Diplopoda, Isopoda, and Chilopoda) responded parallel to that of microorganisms. Maraun et al. (2001) investigated the effects of carbon and nutrients on density and community structure of soil mesofauna (Collembola, Gamasida and Oribatida) and microfauna (Nematoda, amoebae and flagellates) and reported that the response of mesofauna, microfauna and microorganisms differed from each other suggesting differential limitation of soil biota. Most lowland tropical forests grow on highly weathered soils that are relatively rich in nitrogen (Martinelli et al., 1999) but have been depleted with time of rock-derived nutrients (Walker and Syers, 1976; Sanchez et al., 1982; Wardle et al., 2004). Previous studies (Vitousek, 1984; Vitousek and Sanford, 1986) suggest that phosphorus limits plant growth in tropical rain forests more often than nitrogen. However, rather than by a single factor Kaspari et al. (2008) suggest tropical forests to be limited by multiple nutrients and this may resolve the long standing debate on the relative importance of different nutrients as limiting factors in tropical forests (Tanner et al., 1998; Cleveland et al., 2006); unfortunately, there virtually is no information on limiting factors of soil microorganisms and other soil biota of tropical montane rain forests. Studies on the effect of resources on the density, diversity and species composition of testate amoebae in tropical ecosystems are lacking entirely, which probably is due to difficulties in determining them to species level. Generally, there is little information on food resources of testate amoebae.

By adding carbon (as glucose), nitrogen and phosphorus separately and in combination to the soil of a tropical montane rain forest in southern Ecuador we investigated the response of microorganisms and microbial grazers to increased supply of resources and investigated which element is the most limiting. We followed the response of testate amoebae and for relating changes to microbial food resources we
investigated microbial biomass, fungal biomass and microbial community composition as measured by phospholipid fatty acids (PLFAs). We expected the response of testate amoebae to be closely linked to that of microorganisms. More specifically we expected (1) microorganisms to be primarily limited by carbon and thereby also the density of bacterial and fungal feeding testate amoebae, (2) phosphorus to function as second limiting element for microorganisms and testate amoebae, and (3) nitrogen to be generally of little importance.

4.3. Material and Methods

Study site

The tropical mountain rain forest studied is located in southern Ecuador in the upper valley of the Rio San Francisco between the provincial capitals Loja and Zamora at the border of the Podocarpus National Park within the „Reserva Biológica San Francisco“ (3°58´ S, 79°5W). More details on the region and study site are presented in Beck and Müller-Hohenstein (2001) and Beck et al. (2008). The forests of the study site are largely undisturbed by man but frequent landslides result in a mosaic of mature and regeneration forests. The climate is warm humid with an annual precipitation of about 2,000 mm and an average annual temperature of 15.7°C (Beck et al., 2008; Moser et al., 2008). Precipitation is particularly high from April to July and less from September to December. The bedrock consists mainly of weakly metamorphosed Palaeozoic schists and sandstones with some quartz veins (Wilcke et al., 2001). The soil types are mainly Aquic and Oxaquic Dystropepts (Schrumpf et al., 2001).

The experiment was set up in the upper region of a small catchment („Quebrada 1“, Q1) at 1922 m a.s.l. Trees are generally small reaching a maximum of 12 m and are dominated by Euphorbiaceae, Lauraceae, Melastomataceae and Rubiaceae. The most abundant trees include species of the genera Heliocarpus, Miconia, Cecropia and Graffenrieda (Wilcke et al., 2001). The thickness of organic layers is about 30 cm. Soil pH ranges between 3.8 and 4.5. The mean C-to-N ratio of the litter layer averages 28-30 (Wilcke et al., 2002; Leuschner et al., 2007).

Experimental design and sampling

The experiment was set up in February 2004 and finished in October 2005. Experimental plots were established by digging plastic tubes of a diameter of 35 cm and a height of 40 cm ca. 10 cm into the organic layers of the soil. Cane sugar (C_{6}H_{12}O_{6}) for carbon (C), ammonium nitrate (NH_{4}NO_{3}) for nitrogen (N) and sodium dihydrogenphosphate (NaH_{2}PO_{4}) for phosphorus (P) were added together with water (0.5 L) to the experimental plots separately and in combination in a complete three factorial design resulting in eight treatments (Control, C, N, P, CN, CP, NP, CNP). Each treatment was replicated four times and treatments were arranged in four blocks (randomized complete block design).

The amount of nutrients added was equivalent to about five times the input of these elements in the litter entering the system per year. The amount of leaf litter at a comparable site in close vicinity is 862 g m^{-2}y^{-1} (Röderstein et al., 2005). Assuming that about 50% of the litter consists of carbon, 1% of nitrogen and
0.1% of phosphorus we added: 88.4 g cane sugar, 2.0 g NH$_4$NO$_3$ and 0.272 g NaH$_2$PO$_4$ per plot every second month. Control plots received the same amount of water without nutrients.

After 20 months and 10 fertilizer applications samples (L/F layer material) were taken from each plot to a depth of 5 cm using a corer (Ø 5 cm). Three corers were taken per plot and pooled. The material consisted of decomposing leaves, seeds, flowers, twigs, fine roots and some larger woody material. The samples were transferred to the laboratory at the nearby research station. Subsamples for the extraction of testate amoebae (ca. 100 g) were air dried prior to transport to Germany. Subsamples (ca. 100 g) for analysis of microbial biomass, ergosterol content and PLFAs were placed in plastic bags, stored in a refrigerator (5°C) for a maximum of two days and transported in cooling boxes to Germany where they were processes immediately. Short storage of soil in the refrigerator is known to affect microbial biomass and PLFA patterns only little (Zelles et al., 1991; Bååth et al., 2004).

**Microbial basal respiration and microbial biomass**

Soil respiration and microbial biomass were determined by measuring O$_2$ consumption using an automated respirometer system (Scheu, 1992). Microbial basal respiration of field moist samples was measured at 22°C and calculated as mean O$_2$ consumption during hours 10-20 after attachment to the respirometer. Microbial biomass C (C$_{mic}$; µg g$^{-1}$ dry wt) was assessed by measuring the maximum initial respiratory response (MIRR, µl O$_2$ g$^{-1}$ h$^{-1}$) after glucose addition at 22°C and calculated as 38 × MIRR (SIR-method, Anderson and Domsch, 1978; Beck et al., 1997). Glucose (80 mg g$^{-1}$ litter dry wt) was added as an aqueous solution (Scheu, 1987) adjusting the water content to 80-90 % of the water holding capacity of the L/F material (Joergensen and Scheu, 1999). The mean of the three lowest measurements during the first 10 h after glucose addition was taken as MIRR. Microbial specific respiration (metabolic quotient; qO$_2$) was calculated as µl O$_2$ mg$^{-1}$C$_{mic}$ h$^{-1}$ (Theenhaus and Scheu, 1996).

**Ergosterol content**

Ergosterol content was determined according to the method described in Djajakirana et al. (1996). Briefly, 1 g litter material was extracted with 100 ml ethanol in bay-coloured glass flagons for 30 min by shaking (250 rev min$^{-1}$ on a rocker). Then, samples were transferred into 50 ml screw-top centrifuge tubes and centrifuged at 3,700 g for 40 min. Immediately after centrifugation 20 ml of the supernatant was evaporated in a vacuum rotary evaporator at 40°C and 10 hPa. The residue of ca. 1 ml was diluted with 3 ml ethanol and evaporated again. The dried extract was collected in 0.5 ml methanol and subsequently filtered through a cellulose acetate membrane (0.45 µm). Since ergosterol is photosensitive all steps were carried out in darkness. The samples were stored at -18°C until analysis. Ergosterol concentrations were measured by reserved-phase high performance liquid chromatography (HPLC; Beckmann Coulter, System Gold 125, Fullerton, USA) using the following set up: main column 10 cm, pre-column 0.5 cm (Spherisorb ODS II, 5 µm diameter), mobile phase 100% methanol, flow rate 1.0 ml h$^{-1}$ and detection at 282 nm (Beckmann Coulter, System Gold 166 UV-detector, Fullerton, USA).
PLFAs

Lipid extraction was performed on 2 g of litter according to Frostegård et al. (1993). Lipid fractionation was performed with silica acid columns (0.5 g silicic acid, 3 ml; Varian Medical Systems, Palo Alto, USA). Methanolyis of phospholipid fatty acids (PLFAs) was conducted with 0.2 M methanolic KOH. The resulting fatty acid methyl esters (FAMEs) were kept at −20°C until analysis with iso-octane as solvent. FAMEs were identified by chromatographic retention time comparison with a standard mixture composed of 37 different FAMEs that ranged from C11 to C24 (Sigma-Aldrich, St Louis, USA). The analysis was performed by gas chromatography (GC) using an Auto System XL (Perkin Elmer Corporation, Norwalk, USA) equipped with a HP-5 capillary column (50 m x 0.2 mm i.d., film thickness 0.33 μm). The temperature program started with 70°C (hold time 2 min) and increased with 30°C per min to 160°C, and then with 3°C per min to 280°C and held for 15 min. The injection temperature was 260°C and helium was used as carrier gas.

The sum of all identified PLFAs was taken to represent the amount. Individual PLFAs were calculated as a percentage of total PLFAs (relative nmol g−1 dry mass soil). The ω notation (IUPAC-IUB, 1978) was used to classify fatty acids, where unsaturated acid are ascribed according to the number of carbon atoms from the terminal methyl group (ω end) to the nearest double bond, i.e. ω9, ω6, ω3 (IUPAC-IUB, 1978). The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω7, 17:0, i17:0, cy17:0, 18:1ω7 and cy19:0 were assumed to represent bacteria (Zelles, 1999); 16:1ω5c was used as an indicator for arbuscular mycorrhizal fungi (AMF) (Olsson et al., 1995). In addition to ergosterol, the concentration of the fungal specific fatty acid 18:2ω6c was used as a fungal marker (Frostegård and Bååth, 1996). The sum of 16:1ω7, cy17:0 and cy19:0 was used as marker for gram negative bacteria and the sum of i15:0, a15:0, i16:0 and i17:0 as marker for gram positive bacteria (Zelles et al., 1995; Zelles, 1997; Zelles, 1999). The sum of 20:2 and 20:4ω6 was taken as animal marker (Chen et al., 2001; Salomonová et al., 2003; Chamberlain et al., 2005), and 20:5ω3 as marker for algae (Dunstan et al., 1994; Boschker et al., 2005). The ratio of fungal-to-bacterial PLFAs (see above) was used as indicator for the relative abundance of these two microbial groups, i.e. as fungal-to-bacterial biomass ratio (Frostegård and Bååth, 1996). The ratios between the bacterial fatty acids cy17:0 and cy19:0 and their metabolic precursors, 16:1ω7c and 18:1ω7c, was used as indicator of the turnover and physiological response to environmental conditions (Guckert et al., 1985, 1986; Grogan and Gronan, 1997).

Extraction and analysis of testate amoebae

The samples were rewetted for 24 h with sterile tap water (250 ml per 5 g litter) to detach the tests from the air-dry litter. The next day the samples were filtered through a 500 μm sieve to separate coarse organic particles. Testate amoebae were subsequently collected from the filtrate on a 250 μm mesh, and small forms were recovered by a final filter step using a 25 μm sieve. Microscopic slides were prepared and tests were identified and counted at 200x and 400x magnification with an upright Leitz Ortholux II and a Nikon Inverted Microscope DIAPHOT-TMD. Additionally, we used an environmental scanning
electron microscope (ESEM®, Philips Electron Optics, Eindhoven, The Netherlands) to verify species identification. The following taxonomic references were used for identification: Bonnet (1964, 1965, 1974, 1975, 1980), Chardez (1967, 1969), Coûteaux et al. (1979), Decloitre (1962, 1978, 1981), Grospietsch (1965), Ogden and Hedley (1980), Schönborn et al. (1987) and Geltzer et al. (1995). For each sample a minimum of 150 individuals were inspected. The number of tests was expressed per gram of air dry litter material. The classification of species is based on morphological characters (morphospecies) according to recent publications (Cavalier-Smith, 2002; Meisterfeld, 2002a, b; Cavalier-Smith et al., 2004). No comprehensive phylogeny of testate amoebae is available until today and therefore we adopted a species level approach in this study. Full names of species are listed in Appendix 4.1, p. 52-53.

Statistical analysis

Data on microbial basal respiration, microbial biomass, PLFAs and ergosterol concentrations were analyzed by three-factor randomized complete block analysis of variance (ANOVA) with the fixed factors Carbon (with and without), Nitrogen (with and without) and Phosphorus (with and without) using SAS 9.13 (SAS Institute Inc., Cary, USA). Prior to the analyses the data were inspected for homogeneity of variances (Levene test). Ergosterol data were log-transformed to improve homoscedasticity. Data on density of testate amoebae were log(x+1) transformed. PLFA data (expressed as percentages of total, see above) were arcsin square-root transformed. PLFA patterns were analysed by discriminant function analysis (DFA) using eight treatments (Control, C, N, P, CN, CP, NP, CNP) as independent variables. Squared Mahalanobis distances between group centroids (treatments) and the reliability of sample classification were determined. Two significant canonical roots were derived and graphically presented in 2-dimensional space. DFA was carried out with STATISTICA 7.0 for Windows (StatSoft, Tulsa, USA). To evaluate which of the testate amoebae species reacted to carbon and nutrient additions the multivariate dataset consisting of 104 species of testate amoebae was reduced to four dimensions using non-metric multidimensional scaling (NMDS) in STATISTICA. Stress values in NMDS indicated that four dimensions represented most of the variation in the data. The four dimensions were analysed by discriminant function analysis (DFA) to identify treatment effects. Subsequently, DFA with all 104 testate amoebae species was carried out to evaluate which of them contributed to the treatment effects. As indicated by Pearson correlation 46 taxa were significantly affected by carbon and/or nutrient additions. To evaluate which of the factors affected testate amoeba species MANOVA with these species was carried out, and for the main factors that were significant in the MANOVA (C, N, P, NP, CNP; see results section) protected ANOVAs were carried out to investigate the effects of treatments on the respective species (Scheiner and Gurevich, 2001). In total, 24 species significantly responded to at least one of the treatment factors or their interactions.

Finally, data on testate amoebae species were analysed by canonical correspondence analysis (CCA) using CANOCO (Ter Braak, 1988). CCA was used to sort the sites (coded as dummy variables) to environmental conditions and to relate abundance patterns of testate amoebae species to environmental factors and to test for trends in ecological preferences of particular species. Only species occurring in at
least three independent samples were included in CCA. Environmental variables included \( C_{\text{mic}} \), ergosterol concentration, fungal PLFA marker, AMF marker, gram negative bacteria marker, gram positive bacteria marker, animal marker and algae marker (see above). Treatments (Control, C, N, P, CN CP, NP, CNP) were included as passive variables. Monte-Carlo tests (999 permutations) were performed to evaluate the significance of individual axes (Ter Braak ,1996).

4.4. Results

Response of microorganisms

Microbial basal respiration (BR) was at a minimum in treatments with P (23.3 ± 7.1 \( \mu l \) O\(_2\) g\(^{-1}\) litter dry wt) and at maximum in the treatments with CNP (41.0 ± 15.2 \( \mu l \) O\(_2\) g\(^{-1}\) litter dry wt) and was on average 27.8 ± 6.9 \( \mu l \) O\(_2\) g\(^{-1}\) litter dry wt. The addition of C significantly increased BR (+31 \%; \( F_{1.24} = 6.0, P = 0.022 \)). Microbial biomass (\( C_{\text{mic}} \)) was on average 7.0 ± 0.6 mg g\(^{-1}\) litter dry wt, but depended on nutrient addition treatments (Fig. 4.1a). The addition of C increased \( C_{\text{mic}} \) more than five fold (5.16; \( F_{1.24} = 495.73 \) \( P < 0.0001 \)). Further, \( C_{\text{mic}} \) increased by the addition of N, however, by factor of 3.6 only (\( F_{1.24} = 208.27, P < 0.0001 \)). Addition of N and C increased \( C_{\text{mic}} \) further (\( F_{1.24} = 151.31, P < 0.0001 \)). Also, the addition of P modified the response to C and N addition (\( F_{1.24} = 114.93 \) \( P < 0.0001 \) for the interaction of C, N and P), however, P only or together with C or N did not increase microbial biomass (Fig.4.1a).

Generally, both the addition of C (-55.3 \%) and N (-36.0 \%) significantly and strongly reduced the respiratory quotient (qO\(_2\); \( F_{1.24} = 19.16, P = 0.0002 \) and \( F_{1.24} = 6.24, P = 0.02 \), respectively), however, the effect depended on each other (\( F_{1.24} = 4.59, P = 0.04 \) for the interaction of C and N; Fig. 4.1b). The respiratory quotient was modified by the addition of P (\( F_{1.24} = 13.95, P = 0.001 \) for the interaction of C, N and P); it was most strongly reduced in the CNP treatment (90 \%) but remained unaffected by the addition of P and CP.

As indicated by ergosterol concentrations, fungal biomass was at a minimum in the treatment with P only and at a maximum in the CNP treatment (Fig. 4.1c). Generally, it increased by a factor of 5.2 after addition of C (\( F_{1.24} = 122.04, p < 0.0001 \)) and increased after addition of N (+40 \%; \( F_{1.24} = 7.04, P = 0.014 \)).
**Fig. 4.1.** Effects of the addition of carbon (glucose, C), nitrogen (N) and phosphorus (P) on microbial biomass (a), specific respiration (b), ergosterol concentration (c) and density of testate amoebae (d). Means with standard errors. Bars with different letters vary significantly (Tukey's HSD test, \( \alpha < 0.05 \)).

**PLFA**

In total, 24 PLFAs with a chain length up to \( C_{24} \) were detected and identified. The average amount of PLFA was at a minimum in treatments with P (158.2 ± 44.1 nmol g\(^{-1}\) litter dry wt) and at maximum in the treatments with CNP (1091.8 ± 230.4 nmol g\(^{-1}\) litter dry wt) and was on average 465.3 ± 125.7 nmol g\(^{-1}\) litter dry wt. Discriminant function analysis (DFA) of all PLFAs (Wilks' Lambda < 0.001, \( F_{140,43} = 2.9 \), \( P < 0.001 \)) separated the treatments with C from those without along Root1, and treatments with N from those without along Root2 (Fig. 4.2; Table 4.1.). Both the effect of C (Wilks' Lambda = 0.1588, \( F_{6,19} = 16.17 \), \( P < 0.0001 \)) and N (Wilks' Lambda = 0.3653, \( F_{6,19} = 5.50 \), \( P = 0.002 \)) were significant but depended on each other (Wilks' Lambda = 0.4352, \( F_{6,19} = 4.1 \), \( P = 0.008 \) for the interaction of C and N). Separate ANOVAAs of individual or grouped fatty acids (see Methods) indicated that these differences were mainly due to gram positive bacteria, which were significantly decreased by C (-19 %; \( F_{1,24} = 20.77 \), \( P = 0.0001 \)), and fungi, which were significantly increased by C (+20 %; \( F_{1,24} = 9.29 \), \( P = 0.006 \)).
Fig. 4.2. Separation of treatments according to their fatty acid composition as indicated by discriminant function analysis.

Table 4.1.
Squared Mahalanobis distances between group centroids and reliability of discrimination based on data of PLFA dominance structure.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N</th>
<th>P</th>
<th>NP</th>
<th>C</th>
<th>CN</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>201.2</td>
<td>_</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>47.7</td>
<td>279.1*</td>
<td>_</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>89.2</td>
<td>84.7</td>
<td>147.6</td>
<td>_</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>731.1**</td>
<td>648.5**</td>
<td>1029**</td>
<td>590.1**</td>
<td>_</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>482.1*</td>
<td>258.9*</td>
<td>709.8**</td>
<td>319.2*</td>
<td>186.7</td>
<td>_</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>401.6*</td>
<td>475.2*</td>
<td>589.7**</td>
<td>346.9*</td>
<td>125.8</td>
<td>201.9</td>
<td>_</td>
</tr>
<tr>
<td>CNP</td>
<td>400.9*</td>
<td>251.9*</td>
<td>578.6**</td>
<td>277.5*</td>
<td>177.4</td>
<td>34.5</td>
<td>135.2</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.005
Further, separate ANOVAs indicated that the addition of N significantly decreased the biomass of AMF (-12 %; F\textsubscript{1,24} = 5.38, P = 0.03). Addition of N and C decreased the concentration of gram negative bacteria in each of the treatments (F\textsubscript{1,24} = 6.95, P = 0.01 for the interaction between N and C) and decreased the concentration of the animal marker (F\textsubscript{1,24} = 7.46, P = 0.01). Gram positive and negative bacteria reached a maximum in treatments with P only, and a minimum in C and CP treatments. As a consequence of increased fungal biomass and decreased biomass of gram negative and positive bacteria the fungal-to-bacterial PLFA ratio significantly increased by addition of C (+80 %; F\textsubscript{1,24} = 14.43, P = 0.0009).

The ratio between cy17:0 and 16:1\omega7c was significantly reduced by the additional of C but only in treatments without N (-65 %; F\textsubscript{1,24} = 11.82, P = 0.002 for the interaction of C and N).

Density and diversity of testate amoebae

Density of testate amoebae was at a maximum in treatments with N (32,345 ± 887 ind. g\textsuperscript{-1}) and at a minimum in the treatments with C (6,920 ± 1,480 ind. g\textsuperscript{-1}). Generally, there was an overall negative effect of the addition of C (F\textsubscript{1,24} = 57.46, P < 0.0001) with the density being reduced by 38 % (Fig. 4.1d). In contrast, the addition of N generally increased the density of testate amoebae on average by 90% (F\textsubscript{1,24} = 92.59, P < 0.0001). However, the beneficial effects of N only occurred in treatments without additional C and/or P addition (F\textsubscript{1,24} = 30.33, P < 0.0001 for the interaction of C, N and P).

A total of 104 species/subspecies were identified (Appendix 4.1, p.52-53), of these 21 have not been found in Ecuador before. Species number was at a maximum in the CNP treatment (26.0 ± 12.6) and at a minimum in the CP treatment (19.0 ± 2.6). Generally, the addition of C negatively affected the diversity of testate amoebae reducing the number of species on average by 21 % (F\textsubscript{1,24} = 4.17, P = 0.048).

MANOVA suggests that the community structure of testate amoebae significantly responded to the addition of C (Wilks' Lambda = 0.395, F\textsubscript{4,21} = 8.04, P < 0.001), N (Wilks' Lambda = 0.564, F\textsubscript{4,21} = 4.06, P = 0.01) and P (Wilks' Lambda = 0.499, F\textsubscript{4,21} = 5.25, P = 0.004), however, the effect depended on each other (Wilks' Lambda = 0.233, F\textsubscript{4,21} = 17.19, P < 0.0001 for the interaction of C, N and P). Individual ANOVAs indicated that the density of 15 species was significantly affected by the three factor interaction between C, N and P, five species by the two-factor interaction between N and P, two species by N addition only and one by C addition only (for details see below; Fig. 4.3).

Relationships between microorganisms and testate amoebae

In the forward selection procedure of the CCA, four of the ten quantitative variables were significant when tested as a first variable in the model (P < 0.05; Fig. 4.3). Together the variables explained 61 % of
**Fig. 3.** Canonical correspondence analysis of testate amoebae and environmental variables. In closed box are significant factors. Variables that do not significantly contribute to the model are projected passively in the ordination (in bold). Gram–, gram negative bacteria; Gram+, gram positive bacteria; full species names are given in Appendix 4.1, p. 52-53.

the variation in species data. The trace was significant (F = 1.89, P = 0.001). Axes 1 and 2 explained 27% and 11% of the total variation of the data, respectively.

Including the explanatory variables one after the other using forward selection, fungal markers accounted for most of the variation of the data (15% of total; F = 3.94, P = 0.002). The second environmental variable with significant explanatory power was qO₂ (additional 9% of total; F = 2.40, P = 0.02), the third was Cmic (additional 8% of total; F = 2.47, P = 0.005) and the fourth was gram positive bacteria (additional 5% of total; F = 1.77, P = 0.04).

CCA ordination separated treatments with from those without C. CCA indicated close intercorrelation of microbial parameters (ergosterol, fungi marker, Cmic and BR), virtually all were associated with C treatments. In contrast, gram negative and positive bacteria and qO₂, animal and algae marker were negatively correlated with C treatments. Generally, few species grouped with C treatments (Archerella flavum, Tracheleuglypha acolla, Cyclopyxis eurystoma v. parvula); most species either clustered with the N and P only treatments (e.g. Schwabia terricola, Nebela tubulata, Trigonopyxis arcula) and only one with NP treatment (Trinema complanatum). Plagiopyxis labiata and Pl. callida v grandis clustered with the control, being negatively affected by the addition of any nutrient.
4.5. Discussion

The main hypothesis of this study was that testate amoebae in soils of tropical montane rain forests are bottom-up regulated. To prove this hypothesis, we added carbon and nutrients (N, P) to the soil and investigated the response of microorganisms and testate amoebae to the additional resource supply. We assumed that microorganisms will benefit from the additional resources and that this will propagate into the next trophic level, i.e. protists represented by testate amoebae, since microorganisms are the major food resources for testate amoebae.

Response of microorganism

In agreement to our expectation, microbial biomass in fact increased in plots supplemented with glucose and nutrients, especially in treatments with carbon and nitrogen (CN) and carbon, nitrogen and phosphorous (CNP). Our results are similar to those of Scheu and Schaefer (1998) from a temperate beechwood on limestone in Germany where microbial biomass in litter was at a maximum after addition of carbon, nitrogen and phosphorous. However, microbial biomass in our CP treatment was low, and the high metabolic quotient suggests that microorganisms in this treatment suffered from stress (Anderson, 1992; Blagodatskaya and Anderson, 1999). Potentially, mycorrhizal and saprotrophic microorganisms competed for P with the intensity of competitive interactions being increased by the addition of glucose resulting in increased respiratory activity. The assumption that mycorrhizal and saprotrophic fungi interacted antagonistically is further supported by ergosterol and PLFA data (see below).

Carbon limitation of microorganisms was expected as soil microorganisms are generally assumed to be primarily limited by carbon (Wardle, 1992; Vance and Chapin, 2001; Brooks et al., 2004; Demoling et al., 2007; Buckeridge and Grogan, 2008). However, additional supply of N in CN but in particular in CNP treatments further increased microbial biomass suggesting that in the studied tropical montane rain forest microbial biomass is not only limited by C but also by N. Vance and Chapin (2001) also found microorganisms to respond most to the combined addition of C and N and Joergensen and Scheu (1999) found microbial biomass in litter to be at a maximum after addition of glucose, N and P. Further, in soils from southern Sweden separate addition of C, N and P had little effect but adding them together increased microbial biomass considerably (Demoling et al., 2007), indicating that each of these elements limited microbial growth. Supporting our conclusion on the combined limitation by C and N in the studied tropical montane rain forest, low metabolic quotients in treatments with C and N indicate that microorganisms efficiently used the additional carbon for growth and spend less energy for respiration.

Fungal biomass measured as ergosterol content mainly increased in treatments with C and less in those with N addition. Ergosterol is the dominating sterol in cell membranes of ascomycetes and basidomycetes. Arbuscular mycorrhizal fungi (AMF) usually contain other sterols (Olsson et al., 2003); although minor amounts of ergosterol may be present in some AMF species (Frey et al., 1994). In the studied forest the community of mycorrhizal fungi virtually exclusively consists of AMF (Kottke et al., 2004) as is typical for tropical forest ecosystems (Smith and Read, 1997). Ectomycorrhiza therefore...
contributes little to the observed changes in ergosterol concentrations, rather they reflect the biomass of saprotrophic fungi. Therefore, the results suggest that the saprotrophic asco- and basidiomycetes benefited from additional C and N resources. On the contrary, as indicated by the fatty acid marker 16:1ω5c, AMF were detrimentally affected by the addition of N suggesting that saprotrophic asco- and basidiomycetes antagonistically affected AMF. Reduction of AMF by the addition of N may be explained by reduced importance of AMF to provide nitrogen to plants (Barea et al., 2005; Johnson and Gehring, 2007). Wallenda and Kottke (1998) reviewed the response of mycorrhizal fungi to nitrogen fertilization. They found that long-term nitrogen addition had the strongest negative effects on AMF fungi; the dominant species changed and diversity was reduced.

Fungal fatty acid marker (18:2ω6c) correlated with ergosterol content, confirming marker specificity. Correlation of these biomarkers with microbial biomass indicates that the majority of microorganisms in the upper soil layer in this forest consist of saprotrophic fungi. Additionally, the fungal-to-bacterial PLFA ratio generally increased by the addition of C suggesting that fungi disproportionately benefited from increased availability of C. Only in the CNP treatment the fungal-to-bacterial PLFA ratio was low suggesting that if carbon and the major macro-nutrients are present bacteria disproportionately benefit from increased resource supply. Presumably, decreasing N and P limitation combined with enhanced C supply increases the competitive strength of bacteria lacking the ability to translocate nutrients.

Using PLFA analysis we found gram positive and gram negative bacteria to be detrimentally affected by the addition of C, whereas they benefited from the addition of P indicating that bacteria were limited by P. Despite the availability of N has been found to stimulate bacteria if degradable C is available (Schröter et al., 2003), CN addition decreased contents of gram negative bacteria. As discussed above, in this treatment saprotrophic fungi dominated and antagonistically affected bacteria competing for carbon.

In general, the response of microorganisms to C and N application was more complex than we expected and suggests internal feedbacks among different groups of microorganisms. Saprotrophic microorganisms appear to be limited primarily by C with the response to additional nutrients differing markedly between saprotrophic fungi and bacteria with AMF potentially interfering. Fungi predominantly responded to N whereas bacteria responded to P. Differential limitation of fungi and bacteria by N and P presumably was responsible for maximum microbial biomass in the combination of C, N and P.

Response of testate amoebae

In contrast to our expectations soil microorganisms and testate amoebae differently responded to the addition of C and nutrients. Generally, testate amoebae suffered from the addition of C and from increased microbial biomass, whereas the density strongly increased in the treatment with N only. This suggests that the response of amoebae cannot be explained directly by the availability of food resources; obviously, they were unable to exploit the added glucose and the increased microbial biomass. Decreased density in treatments with C also contrasts the response of protozoa (flagellates and naked amoebae) to the addition of glucose in a temperate forest ecosystem (Maraun et al., 2001), suggesting that microbial food web interactions in soil of the studied tropical montane rain forest differ markedly from those in
temperate forests. A marked difference between the studied tropical forest and the temperate forest studied by Scheu and Schaefer (1998) and Maraun et al. (2001) is soil pH. Low soil pH at the studied tropical rain forest likely favoured the dominance of fungi over bacteria (Bååth and Anderson, 2003; Krashevska et al., 2008). As discussed above, increased microbial biomass by the addition of C likely was due to increased fungal biomass, with bacteria even suffering from additional C. Since testate amoebae predominantly feed on bacteria detrimental effects of the addition of C likely resulted from increased dominance of fungi, with fungi being low quality or unavailable prey; predator – prey relationships may even be reversed with fungi preying on testate amoebae (Pussard et al., 1994). According to Pussard et al. (1994) gram negative bacteria are more edible to protozoa than gram positive bacteria, mycobacteria and actinomycetes. Our data support this assumption as the density of testate amoebae correlated with PLFA markers of gram negative bacteria.

In contrast to our hypothesis that N is generally of little importance, the addition of N strongly increased the density of testate amoebae, and the concentration of animal (including testate amoebae) and algae PLFA markers. The correlations between algae and animal markers (in CCA) likely reflect trophic links, e.g. testate amoebae feeding on algae.

Some species were only found in the control, some only in N or C treatments suggesting differential response to changes in the amount and quality of food resources at the species level. Species specific responses are also indicated by close correlations of species with certain PLFA markers; e.g. Trinema lineare, T. complanatum v. elongata closely correlated with PLFA markers of gram negative bacteria suggesting that they predominantly feed on these bacteria which is consistent with previous findings (Coûteaux and Pussard, 1983). Further, close association between certain testate amoebae species point to predator-prey relationships, e.g. Hyalosphenia subflava and T. enchelys are likely to prey on T. lineare and Pseudodifflugia gracilis v. terricola which is consistent with earlier suggestions (Schönborn, 1965; Laminger, 1978).

The increase in density of testate amoebae in the N addition treatment was mainly due to humus and detritus feeders, such as e.g. Centropyxis constricta, Cyclopyxis kahli, Cy. ambigua C. eurystoma v. parvula, Sch. terricola, Tri. arcula (Bonnet 1964, 1981; Laminger, 1980), and some of their predators, such as species of the genus Nebela (Schönborn, 1965). Increased density of humus and detritus feeding testate amoebae in N addition treatments potentially was due to increased availability of detritus rich in N.

The increase in other species, such as Tracheleuglypha acolla and Archerella flavum, likely is related to mixotrophy with algal symbionts benefiting from increased N supply (Bonnet et al., 1981).

In conclusion, the results suggest that saprotrophic fungi in tropical montane rain forests are strongly limited by C whereas gram positive and gram negative bacteria mainly by P. In contrast to our expectations, the response of testate amoebae strikingly differed from that of these major groups of (mainly) saprotrophic microorganisms. Rather than benefiting from increased availability of C and P, testate amoebae in general benefited from increased availability of N and suffered from increased C supply. Beneficial effects of N, presumably were due to increased availability of humus and detritus rich in N and improved performance of symbiotic algae. Further, certain species profited by increased density
of gram positive and gram negative bacteria in P and NP treatments. Suffering of testate amoebae from increased supply of C, which resulted in strong increase in fungal biomass, suggests antagonistic effects of fungi on testate amoebae. Overall, the results suggest that testate amoebae of tropical montane rain forests are controlled by bottom-up forces with their density depending on the availability of high quality detritus resources, certain bacterial groups, the performance of endosymbiotic algae and antagonistic interactions with saprotrophic fungi. Control mechanisms therefore differ markedly from those of the major saprotrophic microorganisms suggesting that trophic interactions in microbial food webs are more specific than previously assumed and modulated markedly by antagonistic interactions.
Appendix. 4.1. Species list of testate amoebae in eight treatments. Nomenclature according to Meisterfeld, 2000a,b.
### Appendix 4.1 (continued)

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SD = Standard Deviation
Chapter 5

Micro- and macroscale changes in density and diversity of testate amoebae of tropical montane rain forests
CHAPTER 5. MICRO- AND MACROSCALE CHANGES IN DENSITY AND DIVERSITY OF TESTATE AMOEBAE OF TROPICAL MONTANE RAIN FORESTS

5.1. Summery

We investigated changes in diversity and density of testate amoebae in epiphytes of trees in tropical montane rain forests in the Andes of southern Ecuador. Local – microscale [tree height of 0 (base of tree trunk), 1 and 2 m; TH I, TH II and TH III, respectively] and regional – macroscale (forests at 1000, 2000 and 3000 m) changes were investigated. At the macroscale diversity and density of testate amoebae peaked at intermediate altitude (2000 m) with an average richness of 53 species and average diversity of 15,165 ind. g\(^{-1}\), exceeding that in litter and soil. At the microscale diversity of testate amoebae reached a maximum at TH I, whereas density of testate amoebae reached a maximum at TH III. The percentage of empty shells at the macroscale was at a maximum at 2000 m and at the microscale at TH I, whereas the percentage of live forms was at a maximum at 3000 m and at TH III. The diversity of testate amoebae in epiphytes found in the present study is high (115 species). However, only two to nine species were dominant representing 54-85 \% of total living testate amoebae. These consisted predominantly of cosmopolitan species occurring in humid habitats of low pH, such as Assulina muscorum and Euglypha strigosa.

Results of the present study suggest significant variations in density and diversity of testate amoebae at both the micro- and macroscale. However, for testate amoebae density the macroscale appears most important whereas changes in diversity of testate amoebae are more pronounced at the microscale. The decline in diversity with increasing tree height suggests that the diversity of testate amoebae predominantly depends on abiotic factors, i.e. constant moisture conditions. Maximum density at 2000 m along the studied altitudinal gradient suggests that testate amoebae density benefits from high diversity of other organisms, in particular that of plants. Maximum number of species at 2000 m suggests that this also applies to testate amoebae diversity indicating that at given abiotic conditions testate amoebae diversity is modulated by biotic factors such as the diversity of plants. To prove these assumptions experimental manipulations of major abiotic factors, such as the moisture regime, and biotic factors, such as the diversity of litter materials, are necessary.

5.2. Introduction

Testate amoebae are widespread and an ecologically important group of unicellular organism, with the cell contained wholly or partially within a protective covering (Clark, 2003). They have been shown to be good indicators of environmental conditions, such as humidity and acidity (Bonnet, 1961, 1978; Beyens et al., 1991; Warner and Chimielewski, 1992). Tropical montane rain forests are typically wet forests (Vance and Nadkarni, 1990) with a high abundance and diversity of plants and animals (Brehm et al., 2006; Liede-Schumann and Breckle, 2008). Due to the high precipitation and humidity in particular epiphytes are abundant and diverse (Gradstein et al., 2008). Epiphyte mats attract a variety of animal taxa
(Nadkarni and Longino, 1990; Yanoviak et al., 2007), especially small species that live in water films, such as testate amoebae (Bonnet, 1973; Meisterfeld, 1978; Bamforth, 2007). Moisture plays an important role for the density and diversity of testate amoebae (Bonnet, 1973; Coûteaux, 1976) and epiphyte habitats receive enough water and provide sufficient resources to support semi-aquatic life (Maguire, 1971). Analysing these microhabitats we addressed two fundamental questions regarding the diversity and density of free-living protists (testate amoebae) in epiphyte habitats: how do they respond to (1) altitudinal changes (macroscale) and (2) the location of epiphytes on trees (tree height; microscale)?

Altitudinal gradients recently attracted much interest in ecology (Körner, 2000; Lomolino, 2001; Rahbek, 2005; Beck et al., 2008). Abiotic conditions such as temperature, precipitation, humidity and soil conditions change profoundly with altitude (Tanner, 1977; Marrs et al., 1988; Grieve et al., 1990; Flenley, 1995). As a result species richness of most organisms also change, but the changes often are not in parallel to altitude and vary among taxonomic groups (Gentry, 1988; Lomolino, 2001; Rahbek, 2005; Gradstein et al., 2008). Recent studies in tropical montane rain forests of Ecuador suggest that the diversity and density of plants and animals (testate amoebae, geometrid moths, birds) peaks at intermediate altitude probably because of favourable abiotic conditions (Krashevska et al., 2007, 2008; Beck et al., 2008). Therefore, we hypothesised that total diversity and density of testate amoebae in epiphytes also peak at intermediate altitude irrespective of tree microhabitat (macroscale hypothesis, H 1).

Existing studies on testate amoebae in epiphytes focused on biogeographical issues and the structure of communities in different habitats (Nair and Mukherjee, 1969; Chardez et al 1972; Smith 1974, 1978; Bonnet, 1978; Meisterfeld, 1979; Beyens et al., 1992; Mitchell et al., 2004). Generally, however, compared to soil habitats testate amoebae in epiphytes on trees received little attention and this applies in particular to epiphytes of montane rain forests, e.g. no information is available on changes in diversity and density of testate amoebae in epiphytes with tree height. Bonnet (1973) investigated epiphytes growing on trees and found them to be colonized predominantly by ubiquitous species. Therefore, we hypothesised that diversity and density of testate amoebae varies little with tree height thereby contributing little to testate amoeba diversity (microscale hypothesis, H 2).

We quantified separately empty shells and live organisms to investigate factors that affect the turnover of testate amoebae. Low pH supports the conservation of empty shells (Schönborn, 1973; Geltzer et al., 1985) and soil pH decreases with increasing altitude in the studied montane rain forests (Moser et al., 2007). Further, low temperature slows down the turnover of testate amoebae (Meisterfeld and Heisterbaum, 1986) and temperature decreases with increasing altitude (Moser et al., 2007; Röderstein et al., 2005). In addition, the turnover of testate amoebae increases with increasing stress and stress conditions, such as variations in humidity, are likely to be more pronounced higher up in trees (Bonnet, 1973; Meisterfeld, 1978; Bohlman et al., 1995). Therefore, we hypothesised that the percentages of live forms decreases and empty shells increases with altitude and height of epiphyte location on trees (H 3).
5.3. Material and Methods

Study sites

The study area is located in southern Ecuador within the Eastern Cordillera of the Andes in the province of Zamora-Chinchipe. Along an altitudinal transect three study sites at 1000, 2000 and 3000 m a.s.l. were investigated. The maximum distance between the sites was 30 km. The study site at 1000 m was located in Bombuscaro near the province capital Zamora (04°06’ S, 78°58’ W), the one at 2000 m in the Reserva Biologica San Francisco (RBSF) in the valley of the Rio San Francisco (03°58’ S, 79°4’ W) and the one at 3000 m in the Cajanuma area at the north-west gate of Podocarpus National Park south of Loja (04°06’ S, 79°10’ W).

The sites are covered with mostly undisturbed mountain rain forest (Homeier et al., 2002). The climate is semihumid with 8 to 10 humid months per year. Accordingly, annual rainfall is high with ca. 2200, 3500 and 4500 mm at 1000, 2000 and 3000 m, respectively. The mean annual air temperature is decreasing with altitude from 14.9, to 12.3 to 8.9°C at 1000, 2000 and 3000 m, respectively. The coldest month on average is August, the warmest November (Röderstein et al., 2005); more details are given in Beck et al. (2008).

In the RBSF, a montane forest reserve of approximately 1000 ha in southern Ecuador, almost 2400 species of flowering plants, ferns, bryophytes and lichens have been recorded (Liede-Schumann and Breckle, 2008), about every second species is an epiphyte (for more details see Kürschner and Parolly, 2007).

Sampling

At each altitude a sampling area of 100-200 m$^2$ was selected and six trees with epiphytes were randomly chosen (macroscale). From each tree, three epiphyte samples at different tree height were taken (microscale): (1) at the bottom of the tree trunk (tree height 0 m, TH I), (2) at a tree height of 1 m (TH II) and (3) at a tree height of ca. 2 m (TH III). Therefore, a total of 54 samples were analysed. The samples included epiphytes and decomposing organic materials, such as abscised parts of epiphytes and bark of host trees; epiphytes at TH I and II consisted mainly of mosses, whereas at TH III they consisted mainly of bromelians. Samples were taken in March 2007.

Testate amoebae

Testate amoebae extraction was done by washing samples over filters of 500 µm and 250 µm mesh size, and than back-sieving the filtrate over a 20 µm mesh filter. The sieving over 500 and 250 µm was done with high water flow to maximize the extraction of tests (see Mitchell et al., 2004). Testate amoebae were investigated from the fraction between 250 and 20 µm and small forms were recovered from the filtrate on a 20 µm mesh filter. Microscopic slides were prepared and shells were identified and counted at 200x
or 400x magnification with an upright Leitz Ortholux II. Testate amoebae were divided into live forms (live organisms and organism in cyst) and empty shells after staining with aniline blue (Merck, Darmstadt). For staining testate amoebae on microscopic slides one drop of aniline blue solution (2 %) was added (see Schönborn, 1968; Wanner, 1989). Testate amoebae were determined to species (morphospecies) level and numbers were expressed per gram of air dry material (see Krashevska et al., 2007). The full names of species are listed in Appendix 5.1, p. 63-64.

Statistical analysis

Data on diversity and density of testate amoebae taxa were analyzed by MANOVA to evaluate the effects of the factors Altitude and Tree height. If significant protected ANOVAs were carried out for individual taxa to evaluate which of the taxa contributed to the significant MANOVA effect. Statistical analyses were performed using SAS 9.13 (SAS Institute Inc., Cary, USA). Data of density were log-transformed to improve homoscedasticity. Percentage data of live forms and empty shells were arcsine square-root transformed.

5.4. Results

Species diversity

A total of 115 testate amoebae taxa were identified and counted (see Appendix 5.1, p. 63-64). Generally, the number of species was at a maximum at 2000 m (Fig. 5.1), at a minimum at 3000 m and intermediate at 1000 m ($F_{2,45} = 21.08$, $P < 0.0001$), and varied with tree height with a maximum at TH I, a minimum at TH II and intermediate diversity at TH III ($F_{2,45} = 33.63$, $P < 0.0001$). However, species number at TH II

![Fig. 5.1. Average species number of testate amoebae in epiphytes at three altitudes (1000, 2000 and 3000 m) and three tree heights (0, 1, and 2 m labelled TH I, TH II and TH III, respectively). Means with standard errors. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).]
varied less than at TH I and TH III with the number at 1000 and 2000 m being similar (significant Altitude × Tree height interaction $F_{2,45} = 5.24$, $P = 0.0015$).

**Dominance**

**Live forms:** At 1000 m 9 (TH I) and 7 (TH II and TH III) species dominated (contributing > 5% of total abundance) and accounted for 70, 80 and 71% of the total abundance, respectively (Fig. 5.2a,b,c). At 2000 m 6 (TH I and TH II) and 7 species (TH III) dominated and accounted for 83, 85 and 54% of the total abundance, respectively. At 3000 m 6 (TH I), 2 (TH II) and 7 species (TH III) dominated and accounted for 81, 69 and 81% of the total abundance. *Euglypha strigosa* was the most dominant species at eight sites, *Assulina muscorum* at seven sites and *Centropyxis euristoma v. parvula* at six.

**Empty shells:** The dominance of empty shells generally resembled that of live forms but with *C. euristoma v. parvula* dominating at eight sites, *E. strigosa* at six sites and *A. muscorum* at only five sites (Fig. 5.2d,e,f).

**Fig. 5.2.** Dominance of live forms (a, b, c) and empty shells (d, e, f) of testate amoebae in epiphytes at three altitudes (1000, 2000 and 3000 m) and three tree heights (0, 1, and 2 m labelled TH I, TH II and TH III, respectively).
Density

MANOVA suggested that the density of testate amoebae significantly responded to changes in altitude (Wilks’ Lambda 0.1033, $F_{6,86}=30.26$, $P < 0.0001$) and tree height (Wilks’ Lambda 0.5011, $F_{6,86}=5.91$, $P < 0.0001$) and the interaction of both (Wilks’ Lambda 0.4049, $F_{12,114}=3.87$, $P <0.0001$). The density of live forms increased in the order $1000 < 3000 < 2000$ m ($F_{2,45} = 31.30$, $P < 0.0001$; Fig. 5.3), and that of empty shells in the order $3000 < 1000 < 2000$ m ($F_{2,45} = 58.63$, $P < 0.0001$; Fig. 5.4). At each of the altitudes the density of live forms significantly increased from TH I to TH II to TH III ($F_{2,45} = 16.23$, $P < 0.0001$ for Tree height). The interaction between Altitude and Tree height was significant for empty shells, reflecting the strong increase in the number of empty shells in the order TH II < TH I < TH III at 2000 m ($F_{2,45} = 4.97$, $P = 0.002$; Fig. 5.4).

**Fig. 5.3.** Density of live forms of testate amoebae in epiphytes at three altitudes (1000, 2000 and 3000 m) and three tree heights (0, 1, and 2 m labelled TH I, TH II and TH III, respectively). Means with standard errors. Bars with different letters vary significantly (Tukey’s HSD test, $\alpha < 0.05$).

**Fig. 5.4.** Density of empty shells of testate amoebae in epiphytes at three altitudes (1000, 2000 and 3000 m) and three tree heights (0, 1, and 2 m labelled TH I, TH II and TH III, respectively). Means with standard errors. Bars with different letters vary significantly (Tukey’s HSD test, $\alpha < 0.05$).
Percentage of live forms were not similar to density and decreased in the order 3000 > 1000 > 2000 m ($F_{2,45} = 53.09, P < 0.0001$). However, changes in the percentage of empty shells resembled that in density increasing in the order 3000 < 1000 < 2000 m ($F_{2,45} = 53.79, P < 0.0001$). With tree height the percentage of live forms increased in the order TH I < TH II < TH III ($F_{2,45} = 12.08, P < 0.0001$), whereas the percentage of empty shells increased in the order TH II < TH III < TH I ($F_{2,45} = 12.11, P < 0.0001$). The percentage of live forms was at a maximum (74 %) whereas that of empty shells was at a minimum (26 %) at 3000 m TH III ($F_{2,45} = 9.82, p < 0.0001$ for the Altitude × Three height interaction), and the percentage of empty shells was at a maximum (85 %) and that of live forms at a minimum (15 %) at 2000 m in TH I ($F_{2,45} = 9.58, P < 0.0001$ for the Altitude × Tree height interaction).

5.5. Discussion

Abiotic factors in particular acidity and humidity are assumed to be the main determinants of the diversity and density of testate amoebae (Bonnet, 1973; Couteaux, 1976; Searles et al., 2001). In addition, testate amoebae have been assumed to be sensitive to stress, e.g. variations in humidity (Bonnet, 1973; Meisterfeld, 1978). Further, testate amoebae are likely to depend on the availability and diversity of resources and therefore may respond parallel to that of other organisms (Schönborn, 1992; Couteaux and Darbyshire, 1998; Lousier and Parkinson, 1984) in particular plants, providing the basal resources for decomposer systems, plant litter material. Along the studied altitudinal gradient in tropical rain forests of Ecuador acidity, humidity and UV-radiation increase, and temperature and tree height decrease. With tree height the variability of humidity and temperature increases likely resulting in increased stress for testate amoebae. In contrast to abiotic conditions, the diversity of plants and animals does not uniformly change with altitude but often peaks at intermediate altitude, i.e. 2000 m (Beck et al., 2008; Liede-Schumann and Breckle, 2008). Investigating testate amoebae in epiphytes at the microscale (tree height of 0, 1 and 2 m) and macroscale (altitudes of 1000, 2000 and 3000 m) we expected their density and diversity to peak at 2000 m (parallel to plants and other animals; H 1) and to decline with tree height (due to increased variability of abiotic factors; H 2).

A total of 115 testate amoebae taxa were found with the mean species richness of 25 per sampling location. Other studies investigating species richness of testate amoebae in epiphytes (mosses, bromelians) reported a maximum of ca. 80 species (Bartoš, 1963; Bonnet, 1973; Beyens and Chardez, 1994; Bamforth, 2007). By comparison, a total of 135 species of testate amoebae were found in litter and soil of the studied montane tropical rain forests in Ecuador (Krashevska et al., 2007). Therefore, the diversity of testate amoebae in epiphytes found in the present study is high. However, only two to nine species were dominant representing 54-85 % of total living testate amoebae. Notably, these consisted predominantly of cosmopolitan species occurring in humid habitats of low pH, such as Assulina muscorum and Euglypha strigosa confirming earlier findings (Bonnet, 1964; Schönborn, 1973).
In agreement to our expectation (H 1) diversity and density of testate amoebae peaked at intermediate altitude (2000 m) with an average richness of 53 species and average diversity of 15,165 ind. g\(^{-1}\). Remarkably, the density of testate amoebae in epiphytes exceeded that in litter and soil (Krashevska et al., 2007).

We expected that diversity and density of testate amoebae in epiphytes decline with increasing tree height (H 2). The results supported this hypothesis for testate amoebae diversity but not for density. Irrespective of altitude the diversity of testate amoebae indeed reached a maximum at TH I, whereas density of testate amoebae reached a maximum at TH III. High density at TH III at least in part may be due to the combined effect of favourable microclimatic conditions, e.g. high humidity combined with high UV-radiation (Beck et al., 2008), both are known to beneficially affect testate amoebae (Bonnet, 1973; Couteax, 1976; Searles et al., 2001). High frequency of species with acrostomy, e.g. species of the genera *Euglypha*, *Assulina* and *Nebela*, supports the conclusion that humidity is a major structuring force for testate amoebae at TH III. Aboveground species experience greater extremes in temperature, wetting and drying cycles than species on the forest floor (Bohlman et al., 1995). Therefore, only species adapted to variable environmental conditions are able to survive, e.g. species with short life cycles such as *Trinema complanatum* and *T. enchelys* which already start to reproduce after ca.1-3 days (Schönborn, 1975).

Maximum diversity at TH I presumably results from the close vicinity to the forest floor where the number of species is even higher (Krashevska et al., 2007) and some of these species presumably colonize epiphytes at the base of trees, e.g. species of the genus *Plagiopyxis*. Furthermore, more constant abiotic conditions at the base of trees as compared to TH II and TH III presumably contributed to the high diversity of species at TH I.

Increasing acidity and decreasing temperature along the altitudinal gradient and more pronounced stress conditions higher up in trees led us to expect that percentages of live forms decreases and empty shells increases with altitude and height of epiphyte on trees (H 3). In contrast to this expectation, the percentage of empty shells along the altitudinal gradient was at a maximum at 2000 m and on trees at TH I. With the data available, these patterns are difficult to explain but suggest caution of relating the turnover of empty shells to environmental conditions such as pH. Further, in contrast to our expectations, the percentage of live forms was at a maximum at 3000 m and at TH III. Potentially, the high precipitation at this altitude (4500 mm y\(^{-1}\); Röderstein et al., 2005) results in accelerated decay of shells of testate amoebae and this is most pronounced higher up in trees were the physical forces of rain presumably are most pronounced.

Results of the present study suggest significant changes in density and diversity of testate amoebae along both the micro- and macroscale. However, for testate amoebae density the macroscale appears most important whereas changes in diversity of testate amoebae are more pronounced at the microscale. The decline in diversity with increasing tree height suggests that the diversity of testate amoebae predominantly depends on abiotic factors, in particular on the water regime of the habitat, e.g. constant moisture conditions. Maximum density at 2000 m along the studied altitudinal gradient suggests that testate amoebae density benefits from high diversity of other organisms in particular that of plants (Beck,
et al., 2008; Liede-Schumann and Breckle, 2008). Maximum number of species at 2000 m suggests that this also applies to testate amoebae diversity indicating that at given abiotic conditions testate amoebae diversity is modulated by biotic factors such as the diversity of plants. To prove these assumptions experimental manipulations of major abiotic factors, such as the moisture regime, and biotic factors, such as the diversity of litter materials, are necessary. These investigations are currently under way at our study site.
Appendix 5.1. Species list of testate amoebae in epiphytes at the three altitudes (1000, 2000 and 3000 m) and three tree heights (0, 1, and 2 m labelled epiphyte 0 m, epiphyte 1 m and epiphyte 2 m, respectively).

Nomenclature according to Meisterfeld 200a,b.
### Appendix 5.1. (continued)

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**SD** = Standard Deviation
General Discussion
CHAPTER 6. GENERAL DISCUSSION

6.1. Diversity and density of testate amoebae

Mountain rain forests of southern Ecuador at the eastern slopes of the Andes are among the most species-rich ecosystems on Earth (Küper et al., 2004; Hilt and Fiedler, 2005). The diversity of plants (especially trees and bryophytes) and animals (especially birds, bats, arctiid and geometrid moths) has been investigated in detail, indicating that the region indeed is a biodiversity hotspot (Liede-Schumann and Breckle, 2008).

Testate amoebae are widespread and an ecologically important group of unicellular organisms. They colonize almost any habitat but are most abundant and diverse in soils with high organic content and slow rates of decomposition (wetlands, mosses, peats, as well as montane and forest soils; Geltzer et al., 1985). In acidic forest soils which are typical for many tropical regions, litter accumulates on the forest floor forming ectorganic matter (humus forms moder and mor). These soils typically harbour few decomposer macrofauna species, such as earthworms, millipedes and isopods. On the contrary, decomposer meso- and microfauna flourish (Schaefer, 1991). The protozoan community of moder and mor soils is dominated by testate amoebae, which contrasts with mull soils where naked amoebae and flagellates are most abundant (Schaefer and Schauermann, 1990). The density and diversity of testate amoebae in these soils therefore reflect biotic and abiotic conditions and form a major component of the bacterial energy channel (Wardle, 2002; Scheu et al., 2005).

This study evaluated (Chapters 2 and 5): (1) if south Ecuador is a biodiversity hotspot for testate amoebae; (2) if testate amoebae diversity and density in tropical acidic forests soils is particularly high as is the case in respective forests of the temperate and boreal zone; (3) if the diversity of testate amoebae of the studied region is typical for tropical southern hemisphere. In total, 135 species and subspecies of testate amoebae were found with the density in the litter and soil reaching up to 12,500 ind. g\(^{-1}\) dry weight. Rarefaction plots suggested that only few more species are to be expected (Chapter 2). A similar number of species (104) but higher density (up to 367,000 ind. g\(^{-1}\) dry weight) has been found in a rain forest of Puerto Rico (Bamforth, 2007), whereas in a temperate forest in Germany Wanner (1989) reported 62 species and densities up to 23,000 ind. g\(^{-1}\) dry weight. In aboveground epiphyte habitats 115 testate amoebae taxa were found (Chapter 5). Other studies found a maximum of 80 species in epiphytes of tropical trees (Bartoš, 1963; Bonnet, 1973; Beyens and Chardez, 1994; Bamforth, 2007). Despite the diversity of testate amoebae in epiphytes was lower than in litter, they reached higher density (15,200 ind. g\(^{-1}\) dry weight).

These results suggest that the diversity but not the density of testate amoebae in tropical mountain forests indeed is high exceeding that in temperate forests. However, there is a large overlap in species between temperate and tropical forests of similar soil pH and humus form. Most of the species found were cosmopolitan, however, nine species (i.e. 6.7 %) are considered tropical and some of these species likely represent Gondwana relicts.
There are contrasting views on the global distribution and diversity of protists (Finlay, 2002; Foissner, 2006). In part, results of the present study support the view that „everything is everywhere“ since the great majority of the recorded testate amoebae morphospecies in our study also occurs in very different geographical regions, such as the temperate and boreal zone of the Northern hemisphere. Some species claimed to be endemic to certain geographical regions, e.g. *Playfairina valkanovi* (Indonesia, Australia), *Lamptopyxis* spp. (Africa) and *Distomatopyxis* spp. (Laurasia) (Foissner, 2006), were also recorded at our study sites, suggesting that they in fact are widespread in the Southern hemisphere. The results further suggest that primarily the conditions of the local habitat rather than history select for testate amoebae species, although small but distinct differences occur between the Southern and Northern hemisphere. In general however, the community composition of the moist and acidic tropical forest investigated closely resembles habitats with similar abiotic environment at very different geographical regions, such as moist and acidic forests of the Northern hemisphere (Korganova, 1997; Rakhleeva, 2002).

6.2. Altitudinal gradient

The sites investigated in this study were located in the Ecuadorian Andes at an altitude between 1000 and 3000 m. With altitude and decreasing air temperature in tropical mountains tree height (Whitmore, 1998), aboveground biomass (Röderstein et al., 2005), leaf litter production and soil pH decrease, whereas the thickness of organic layers, litter C-to-N ratio, annual rainfall and soil moisture increase (Leuschner et al., 2007; Moser et al., 2007). Additionally, abiotic factors change with soil depth and with the location of epiphytes on trees (Chapters 3 and 5). These different environmental conditions are likely to affect the density and diversity of testate amoebae either directly or indirectly, through changes in the availability of resources (Chapters 2, 3 and 5).

The aim of this work was to investigate if density and diversity of testate amoebae decline with altitude, and to evaluate if these changes are related to changes in abiotic factors. Further, altitudinal changes in density and diversity of testate amoebae were related to changes in microbial biomass and microbial community composition to evaluate relationships between microorganisms and testate amoebae.

The results presented in Chapters 2 and 5 suggest that density and diversity of testate amoebae in the litter/soil and epiphyte habitats does not decrease continuously with elevation; rather, it peaks at intermediate elevation (2000 m). A similar pattern was found for moth species richness by Brehm et al. (2006). The pattern, however, contradicts the study of Bonnet (1978) reporting a decrease in species richness and an increase in cosmopolitan testate amoebae species with altitude (Chapters 2 and 3). Above the ground testate amoebae diversity reached a maximum in epiphytes located at the bottom of tree trunks, however, density of testate amoebae reached a maximum with increasing height of epiphytes on trees irrespectively of altitude (Chapter 5).
The data suggest that increasing humidity and decreasing pH and temperature with altitude did not directly affect the density and diversity of testate amoebae, however, community structure and morphological features of testate amoebae were affected. High humidity at the study sites was reflected by morphological features of testate amoebae e.g. species with cryptostomy became rare, whereas the diversity and density of acrostome species exceeded that typically occurring in non-permanent moist soils (Chardez, 1968). Higher acidity at the study sites was reflected by higher frequency of „acidophilic“ morphospecies in community structure, e.g. *Trigonopyxis* spp, *Assulina* spp and *Corythion* spp. (Chapters 2 and 5). Greater extremes in temperature, wetting and drying cycles in aboveground epiphyte habitats was reflected by higher frequency of testate amoebae with short life cycle, such as *Trinema complanatum* and *T. enchelys* (Chapter 5).

Testate amoebae primarily feed on bacteria (Bonnet, 1964; Stout and Heal, 1967), but also on fungal hyphae, spores and yeasts (Coûteaux and Devaux, 1983; Ogden and Pitta, 1990), algae and other protists (Bonnet, 1964; Laminger, 1978; Laminger, 1980); some even feed on small metazoans (Yeates and Foissner, 1995), others on humus particles (Schönborn, 1965, 1982). Testate amoebae prefer certain prey-species and reject others (Laminger and Bucher, 1984). Selective grazing may be an important factor determining the taxonomic composition and metabolic activity of microbial communities (Sherr et al., 1992; Bonkowski, 2004).

Microbial biomass and concentration of ergosterol generally declined with soil depth and peaked at 2000 m. Compared to temperate forests decomposing litter materials in the studied tropical mountain rain forests support only small populations of microorganisms (Chapter 3). Low biomass of microorganisms at 1000 m presumably is due to mixing of litter materials into the mineral soil, i.e. bioturbation, whereas low biomass of microorganisms at 2000 m but in particular at 3000 m likely reflects declining litter quality and increasing nutrient limitation with altitude. These conclusions are supported by the fact that in particular in the upper litter layer at higher altitude the microbial community changed from a bacterial-dominated system to a fungal-dominated system. Parallel to microbial parameters, the density of testate amoebae peaked at 2000 m (Chapters 2 and 3), and with the decline in microbial biomass with soil depth density of testate amoebae also decreased.

The data presented in Chapter 3 suggest that density and community structure of testate amoebae are driven by the availability of bacteria and fungi (bottom-up forces). Adverse abiotic factors such as high moisture and low pH at high altitude likely reduce the availability of food resources and thereby the density of testate amoebae, however, adverse abiotic conditions may also directly affect testate amoebae (Chapters 2 and 5).

### 6.3. Nutrient limitation

The main hypothesis of the study presented in Chapter 4 was that testate amoebae in soils of tropical montane rain forests are bottom-up regulated. To prove this hypothesis, we added carbon (C) and nutrients (N, P) to the soil and investigated the response of microorganisms and testate amoebae to the
additional resource supply. We hypothesised that microorganisms will benefit from the additional resources and that this will propagate into the next trophic level, i.e. protists represented by testate amoebae, since microorganisms are the major food resources for testate amoebae.

The results suggest that saprotrophic fungi in tropical montane rain forests are strongly limited by C whereas gram positive and gram negative bacteria are limited mainly by P. In contrast to our expectations, the response of testate amoebae strikingly differed from that of these major groups of (mainly) saprotrophic microorganisms. Rather than benefiting from increased availability of C and P, testate amoebae in general benefited from increased availability of N and suffered from increased C supply. Beneficial effects of N presumably were due to increased availability of humus and detritus rich in N and improved performance of symbiotic algae. Further, certain species profited by increased density of gram positive and gram negative bacteria in P and NP treatments. Suffering of testate amoebae from increased supply of C, associated with which a strong increase in fungal biomass, suggests antagonistic effects of fungi on testate amoebae. Overall, the results suggest that testate amoebae of tropical montane rain forests are controlled by bottom-up forces with their density depending on the availability of high quality detritus resources, certain bacterial groups, the performance of endosymbiotic algae and antagonistic interactions with saprotrophic fungi. Control mechanisms therefore differ markedly from those of the major saprotrophic microorganisms suggesting that trophic interactions in microbial food webs are more specific than previously assumed and modulated markedly by antagonistic interactions.

In conclusion, testate amoebae in Ecuador are highly diverse (166 species in 124 samples), compared with other tropical forests and ecosystems of the temperate zone, however, their density is low. Testate amoebae of tropical montane rain forests are controlled by bottom-up forces with their density depending on the availability of high quality detritus resources, certain bacterial groups, the performance of endosymbiotic algae and antagonistic interactions with saprotrophic fungi. The availability of food resources decrease at higher altitude, with increasing moisture and decreasing pH, resulting in maximum density and diversity at an intermediate elevation.

Results of the present study, in particular the results of the experimental manipulation of resources, allowed considerable progress in understanding direct and indirect effects of abiotic factors on testate amoebae, and in the feeding biology and trophic niche differentiation of testate amoebae. However, more laboratory and field experiments are needed to gain further insight into regulatory forces of testate amoebae communities, e.g. field and laboratory experiment with manipulation of humidity, acidity, temperature and UV-radiation. Further, laboratory and field experiment are needed which help to understand food preferences, e.g. the development of model systems with one species and few different food resources.

No comprehensive phylogeny of testate amoebae is available until to date, therefore, we used the traditional classification based on morphological features of shell and pseudopodia. In addition to
investigations of the general phylogeny of testate amoebae, studies investigating the genetic structure of morphospecies of testate amoebae are needed. In particular cosmopolitan species may consist of genetically very different lineages. Further, there is evidence that species may locally consist of different lineages/subspecies, e.g. at the studied montane rain forests lineages of *Trigonopyxis arcula* and *Hyalosphenia subflava* differ in body size which likely represents genetically different lineages. Therefore, studies investigating the local, regional and global genetic diversity of testate amoebae species are urgently needed. These studies may ultimately resolve the hotly debated controversy whether „everything is everywhere“ or whether locally adapted species predominate.
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Publications


In preparation


Part in conference

Krashevska, V., Bonkowski, M., Maraun, M., Scheu, S. Changes in microbial and protist (testate amoebae) community structure along an elevational gradient in tropical mountain rain forests of Ecuador.

Krashevska, V., Illig, J., Bonkowski, M., Maraun, M., Scheu, S. Diversity of testate amoebae (Protozoa, Testacea) in different litter types of a mountain rain forest in southern Ecuador.

Krashevska, V., Illig, J., Maraun, M., Scheu, S., Bonkowski, M. Diversity of testate amoebae (Protozoa, Testacea) in different litter types of a mountain rain forest in southern Ecuador.

Krashevska, V., Bonkowski, M., Maraun, M., Scheu, S., Diversity of testate amoebae (Protozoa, Testacea) of a mountain rain forest in southern Ecuador.
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Tropical ecosystems
Soil ecology
Microbial ecology
Soil science

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Determination of diversity, density and community structure of protists (testate amoebae, ciliates, naked amoebae), identification to species level (testate amoebae) by:
- light microscope, ESEM, electron microscope
- staining, impregnation methods
- cultivation
- PCR techniques

Determination of microbial activity, biomass and community composition by:
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- Phospholipid Fatty Acid Analysis (PLFA)
- Ergosterol extraction

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Eidesstattliche Erklärung

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Valentyna Krashevs’ka
„Wissenschaft ist wie Sex. Manchmal kommt etwas Sinnvolles dabei raus, das ist aber nicht der Grund, warum wir es tun.“

Richard P. Feynman