Protists in soil ecology and forest nutrient cycling

M.S. Adl and V.V.S.R. Gupta

Abstract: Recent progress in protistology has shown that these organisms (protists) are far more diverse than traditionally assumed by soil ecologists. Most studies have grouped these into motility groups, as amoebae, flagellates, and ciliates. Unfortunately, these do not represent functionally useful groups and do not have any ecological relevance to food web processes and community structure. Typically, abundance values have relied on the most probable number estimate based on bacterivore cultures. In fact, there are many functional groups of protists besides the bacterivores. These other functional groups are very much part of the forest soil decomposition food web, but they remain unaccounted for in models. Modelling studies have shown repeatedly that protozoan bacterivores are responsible for much of the nutrient turnover and flux through the soil food web, as they are in the aquatic microbial loop. The contribution of other protist functional groups to this nutrient cycling remains to be quantified. To this end, new sampling strategies are required, and functional diversity needs to be considered in future studies. We consider both temporal and spatial stratification as contributing factors, to explain the apparent redundancy of function. Finally, drawing on data from agricultural fields, we consider new ideas on rates of recovery after disturbance.

Résumé : Les progrès récents accomplis dans l'étude des protistes ont montré que ces organismes sont beaucoup plus diversifiés que l'ont traditionnellement assumé les écologistes du sol. La plupart des études ont regroupé ces organismes en fonction de leur motilité : les amibes, les flagellés et les ciliés. Malheureusement, ces regroupements ne sont pas utiles du point de vue fonctionnel et n'ont aucun rapport du point de vue écologique avec les processus du réseau trophique et la structure des communautés. Les valeurs d'abondance ont généralement été établies à partir d'estimations du nombre le plus probable basées sur la culture des protistes qui se nourrissent de bactéries. En fait, il y a plusieurs groupes fonctionnels de protistes en plus de ceux qui se nourrissent de bactéries. Ces autres groupes fonctionnels contribuent très activement à la décomposition des sols forestiers dans le réseau trophique mais ne sont pas considérés dans les modèles. Les travaux de modélisation ont montré à maintes reprises que les protozoaires qui se nourrissent de bactéries sont responsables de la majeure partie du recyclage et du flux des nutriments dans le réseau trophique du sol, comme ils le sont dans la boucle microbienne aquatique. La contribution des autres groupes fonctionnels de protistes à ce recyclage des nutriments doit encore être quantifiée. À cette fin, de nouvelles stratégies d'échantillonnage sont nécessaires et la diversité fonctionnelle doit être considérée dans les études futures. Les auteurs considérent que la stratification spatiale et temporelle sont des facteurs qui contribuent à la compréhension de la redondance apparente de fonction. Finalement, ils explorent de nouvelles idées au sujet du taux de récupération après une perturbation à partir de données provenant de terres agricoles.

Soil protists and sampling the forest soil

Over the past three decades, since the convergence of molecular phylogenies with the morphological descriptions of species based on both ultrastructure and biochemistry, the modern classification of protists has been dramatically transformed. Recent reviews of the new organisation of eukaryotes agree on the basic structure of the phylogeny at the highest ranks and of most lineages through to the lower ranks (Baldauf et al. 2000; Cavalier-Smith 2002, 2003; Simpson and Roger 2002). In the new classification of eukaryotes (Adl et al. 2005), the terms "algae" and "protozoa" no longer have any taxonomic sense (Table 1). The new classification accepts "protists" as a common term for unicellular eukaryotes (single cells, colonial, or filamentous, as in many algae and fungi). The traditional categorization of protozoa into motility groups (flagellates or amoeboid), on the basis of the Bütschli (1880–1889) classification, has no relevance to phylogeny or to ecologically useful functional groups. Species with eukaryotic flagella (cilia), amoeboid locomotion, or both occur in many distinct lineages.

Forest soil samples in general contain 10^4–10^7 active protist individuals per gram of dry soil and litter. Abundances of other soil organisms vary with depth through the profile, along gradients of organic matter (OM) and physical properties. These values fluctuate daily with changes in moisture, temperature, and food abundance (Adl and Coleman 2005). In drier conditions, abundances of active protists may decrease to zero as cells form cysts. The cyst stage is necessary in terrestrial protists: it provides a mechanism for survival in dry periods that are frequent and cyclical. The duration and frequency of wet–dry cycles in the surface litter may differ

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Table 1. Higher ranks of the new classification of eukaryotes.

First rank	Example of groups included
Amoebozoa	Acanthamoebidae, Eumycetozoa, Flabellinea, Tubulinea
Archaeplastida	Chloroplastida, Plantae, Rhodophyceae
Chromalveolata	Haptophyta; stramenopiles: all brown algae, Bacillariophycea (diatoms), Peronosporomycetes (Oomycetes); Alveolata: Apicomplexa, Ciliophora, Dinoflagellata
Excavata	Euglenozoa, Heterolobosea, Parabasalia, Preaxostyla
Opisthokonta	Animalia, Choanomonada, Fungi, Mesomycetozoa
Rhizaria	Cercozoa, Foraminifera, Radiolaria

Note: Based on Adl et al. (2005).

from those in deeper soil. Encystment involves partial dehydration of the cytoplasm and the secretion of a cellulosic, chitin, or protein cell wall outside the cell membrane. Cysts are also resistant to temperatures outside the range tolerated by the active cells, because of the stabilization of proteins and nucleic acids. Cysts also offer a last resource for survival in periods with insufficient food, rather than suffering starvation. Excystment requires rehydration of the habitat but is insufficient as a stimulus on its own. Adequate abiotic parameters such as temperature and solution chemistry, as well as food, must be present.

A consequence of cyst formation is that at any one time, the habitat is occupied by many active individuals and by many inactive (encysted) individuals (Bamforth 2001). Because different species have specific food preferences and tolerate a unique range of abiotic conditions, groups of active individuals are at any one time dominated by certain species, while other species remain encysted. Therefore, the local microenvironment or microclimate is an important factor in species activity and abundance. This fundamental fact determines which protocols and storage procedures would be adequate for analysing soil and litter samples for protist dynamics. Incubation conditions in the laboratory must be defined and varied enough to provide realistic estimates of the diversity of species and functions. Unfortunately, the most probable number (MPN) technique, routinely used to describe soil protozoa, fails to distinguish between these, as discussed below.

If the aim is to measure the active species at the time of sampling, then samples must be observed within 1–2 days and kept under conditions similar to the sample temperature and moisture. With time, the abundance and species composition of the active populations change, and depending on the conditions during storage some species encyst and others excyst. Both encysted and active species can be observed by culture-based methods. However, once in culture, different species excyst under different conditions, and some require longer times (days to weeks) to excyst than the faster *r*-selected species. Therefore, depending on when cultures are observed, different abundances and different species will be enumerated and identified. It is not possible to obtain initial abundance values from cultured samples. For this reason, the MPN technique is generally not recommended by bacteriologists or protistologists for enumerating in situ active populations (Foissner 1987, 1999; Lüftenagger et al. 1988; Berthold and Palzenberger 1995; Adl 2003), and alternative protocols are used (Adl and Coleman 2005; Adl et al. 2006*b*). Frequency of encountering species in cultured samples is a suitable alternative to determining the abundance of species in the habitat (Krebs 1999). Finally, success at extracting or culturing individuals depends on the care taken in storing samples and on the researcher's ability with culture techniques. Care must be taken in handling samples if protists are to be extracted: these cells are fragile, with often just the cell membrane between the cytoplasm and the physical environment. Procedures that are suitable with bacteria or species with cell walls are not suitable with "naked" species without cell walls. Friction against soil particles will lyse cells. Similarly, rapid air-drying during soil handling will lyse cells before cysts form.

It is worth considering when dynamics of active species should be described and when total species diversity should be compared. First, if one field site is being studied, it is fair to assume that species composition at the site will not vary from day to day or even over several months. However, the active species from the pool of total encysted species at that site will vary as conditions change. In this situation, the dynamics of populations through active–inactive cycles are more informative. Variations in active species through time reflect changes in the local microclimate, abiotic factors, and species community structure. This is an important consideration when relating protist activity to specific ecosystem functions. Second, in an experimental situation where different field sites are being compared, differences in active species reflect differences in microclimatic conditions and abiotic parameters between sites. In this situation, species present at each site, as well as the total number of species, can be different. The correct measurement then depends on the hypothesis. Third, in situations in which soil is used in laboratory pot or mesocosm experiments, total species present is determined by the origin of the soil. Therefore the species that are active depend on the conditions when the experiment is sampled. It is important to remember that in this situation, all pots or mesocosms have the same background species composition. All these species are then amenable to being excysted by cultivation, but which ones are active depends on the experimental conditions. Finally, in situations where species diversity is required — for example, in comparing successional stage — the total number of species based on molecular data and culture studies would be required. The latter is probably more useful than total abundance estimates, which fail to discriminate between the number of species contributing. A recent study found that total abundance values failed to discriminate between very different sites, but a measure of the diversity per gram of soil was much more effective (Adl et al. 2006*a*).

Table 2. Protist groups common in soil, including Fungi, with locomotion and trophic functional groups represented.

Taxonomic group	Example	Locomotion, morphology	Functional groups
Amoebozoa	Acanthamoebidae	Amoeboid	Bacterivory
	Flabellinea	Amoeboid	Bacterivory, cytotrophy, detritivory, fungivory, invertebrate consumers
	Tubullinea	Amoeboid, flagellate stages	Bacterivory, cytotrophy, detritivory, fungivory
	Mastigamoebidae	Amoeboid, flagellates	Bacterivory
	Eumycetozoa	Amoeboid, flagellate stages	Bacterivory
Fungi	Ascomycota	Filamentous and yeasts	Primary saprotrophs, lichens, mycorrhizae
	Basidiomycota	Filamentous	Primary saprotrophs, ectomycorrhizae
	Chytridiomycetes	Cilium, thallus	Predators, primary saprotrophs
	Glomeromycota	Filamentous	Root symbionts
	Urediniomycetes	Filamentous and yeasts	Endophytes, pathogens, rhizosphere species
	Ustilaginomycetes	Filamentous and yeasts	Parasites
	Zygomycota	Filamentous	Predators, primary saprotrophs, endomycorrhizae
Parabasalia	Cristamonadida	Flagella	Symbionts
	Spirotrichonymphida	Amoeboid, flagella	Symbionts
	Trichomonadida	Amoeboid, flagella	Symbionts
	Trichonymphida	Amoeboid, flagella	Symbionts
Preaxostyla	Oxymonadida	Flagella	Symbionts
Euglenozoa	Euglenida	Flagella	Bacterivory, cytotrophy, photosynthetic
	Kinetoplastea	Flagella	Bacterivory, parasites
Heterolobosea	Acrasidae	Amoeboid	Bacterivory
	Gruberellidae	Amoeboid	Bacterivory
	Vahlkampfiidae	Amoeboid, flagella	Bacterivory, cytotrophy, Detritivory, fungivory
Cercozoa	Cercomonadidae	Amoeboid with flagella	Bacterivory
	Silicofilosea	Amoeboid	Bacterivory, cytotrophy, Detritivory, fungivory
Peronosporomycetes		Flagella, thallus	Predators, primary saprotrophs
Ciliophora		Flagella	Bacterivory, cytotrophy, detritivory, fungivory
Apicomplexa		Dispersal spores	Parasites

Functional role of soil protists in the forest ecosystem

Soil protists contribute to OM decomposition and mineralization, or to the detritus food web, through several trophic functional groups. The structure and function of the soil food web were recently reviewed (Adl 2003; Coleman et al. 2004), and the ecology of soil protists was discussed previously (Foissner 1987; Darbyshire 1994; Gupta and Yeates 1997). Protist groups important to soil ecology and their functional groups are summarized in Table 2.

The cells of primary saprotrophs secrete enzymes to digest and absorb soluble nutrients from substrates. Most primary saprotrophs are bacteria and fungal (Ascomycota and Basidiomycota) species, but they also include some protists. Notably, the slime moulds (Amoebozoa: Mycetozoa: Eumyxa) represent about 600 species that digest woody debris, bark, and cellulosic dung. Certain testate amoebae (Amoebozoa: Testacealobosia) are abundant in surface litter and organic horizons (10^5–10^7 individuals per gram), where they contribute to tissue decomposition (Schönborn 1965; Chardez 1985). The extent of their role as primary saprotrophs remains undetermined. The Peronosporomycetes flagellates (old name, Oomycetes) (Chromalveolata: Stramenopiles) contribute to the digestion of leaves, woody debris, dung, and cadavers (Fuller and Jaworski 1987). Most soil species belong to the order Saprolegniales; and some, to the order Peronosporales. The chytrid flagellates (Opisthokonta: Fungi) contain many species that contribute to the digestion of pollen, chitin, keratin, and cellulosic debris (Adl 2003). In general, most species are selective about their substrate preference and have habitat or seasonal preferences. For example, local slime mould species are found during certain months on particular substrates.

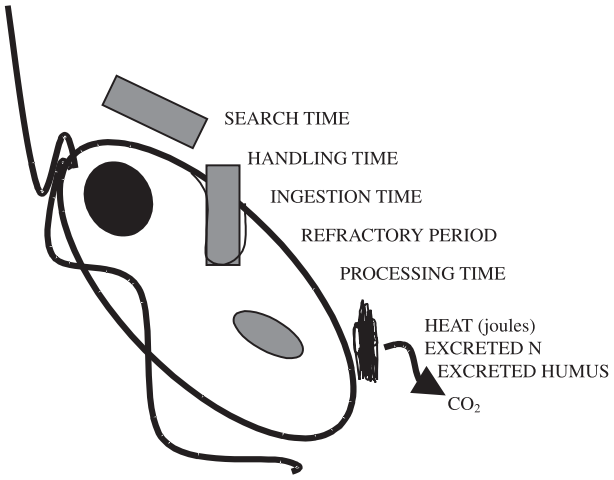
Secondary saprotrophs are detritivores, bacterivores, fungivores, cytotrophs, and osmotrophs. Each functional group includes a diversity of specialized protists. Detritivores ingest fragments of microdetritus for digestion inside food vacuoles. These include many Testacealobosia and occasionally larger gymnamoebae (Amoebozoa). The gut of wood-eating insects (lower termites, wood-eating cockroaches) harbours a complex community of bacteria and specialized anaerobic protists that cooperate in the digestion of wood microdetritus (Breznak and Brune 1994; Rother et al. 1999). These involve species of trichomonads, oxymonads, and hypermastigotes (Excavata: Parabasalia and Preaxostyla) that tend to be specific to the insect host species (Lee et al. 2001). The protists harbour endo- and ecto-symbiotic bacteria. The hypermastigotes ingest the cellulosic microdetritus into food vacuoles for partial digestion, and the remains are excreted. The digestion of the cellulosic debris is completed in the gut by the bacteria with the protists through syntrophic metabolism.

Some ecological aspects of protist bacterivory were re-

viewed previously (Griffiths 1994). Many soil protists are bacterivorous. The main bacterivores belong to the bodonids (Excavata: Euglenozoa: kinetoplastids); the Vahlkampfiidae (Excavata: Percolozoa); the gymnamoebae, Testacealobosia and dictyostelids (Amoebozoa); the cercomonads, which comprise most soil flagellates and the filose testate amoebae (Rhizaria: Cercomonadida and Silicofilosea); and the ciliates (Alveolata) (Adl 2003; Adl et al. 2005). About half of soil ciliate species belong to the class Colpodea; and about 37%, to surface-associated Stichotrichia (Foissner 1987). Bacterivores include species that ingest bacteria by phagocytosis. In selecting bacteria as prey, some bacterivores are less discriminating than others. These less discriminating species are usually larger organisms that can ingest 10^2–10^3 bacteria per food vacuole; others are much more selective and will avoid or even expel certain bacteria (Boenigk et al. 2001; Ronn et al. 2001). Selective grazing of prey species can be attributed to the size, cell wall chemistry, nutritional value, and growth properties of the prey and their ability to produce toxic or inhibitory compounds (Simek et al. 1997). For example, *Pseudomonas* and *Burkholderia* spp. have been shown to produce compounds that inhibit the growth of protist species (Gupta et al. 1995; Caine et al. 2000). In some cases, ingested prey bacteria contain toxins that cause lysis of consumers such as amoebae (Singh 1945). The mechanism of ingestion is pseudopodium phagocytosis in the amoeboid species. Phagocytosis occurs at the cytostome in flagellates and ciliates. The body size of protists restricts access to prey located in smaller pore spaces in the soil matrix. For example, pores of <6 μm diameter are considered inaccessible to the smallest ciliates, but pseudopods of amoeboid species can still extend into these pores. Filopodia of the Cercozoa are fine pseudopodia 0.6–2 μm in diameter that can access many of the smallest spaces in peds to find bacteria and dissolved nutrients.

Cytotrophic species prey on and consume other protists. They ingest prey by phagocytosis, as the bacterivores do, or by external digestion of prey cytoplasm, absorbing the dissolved nutrients. They include certain gymnamoebae, such as *Mayorella*; many Stichotrichia ciliates, such as the ubiquitous *Oxytricha*; some colpodids, such as *Bresslaua* and *Sorogena*; and numerous testate amoebae in the Testacealobosia and Silicofilosea (Foissner and Foissner 1984; Chardez 1985; Foissner 1987; Bardele et al. 1991; Adl 2003). Some of the ciliates with larger or more flexible cytostomes, such as *Frontonia*, *Loxodes* and *Oxytricha*, can ingest active testate amoebae. Similarly, many testate amoebae will ingest smaller ones or extend a pseudopodium into another individual to absorb the cytoplasm. Studies on the prey preference of cytotrophic and bacterivorous species have contributed to our understanding of ingestion rates and growth rates of protists. These studies clearly showed that not all prey are ingested, different prey do not have the same nutritional value, and this affects growth rate (Adl 1998; Pfister and Arndt 1998; Boenigk and Arndt 2000). The studies described an optimal foraging behaviour, based on prey abundances, prey preference hierarchy, and temperature, that aims to maximize the rate of growth (Fig. 1). Feeding is divided into time budgets that measure search time, handling time, ingestion time, processing time, and refractory period. Capture efficiency and retention times vary with prey species

Fig. 1. Optimal foraging by a bacterivorous protozoa on bacteria prey. Prey selection and several measurable parameters are indicated. Undigested remains are excreted as humus along with nitrogenous wastes, CO_2, and energy.



and temperature. Therefore, in cases in which a single prey is handled, both bacterivory and cytotrophy are conceptually the same as predation in animals.

Fungivorous protists ingest fungal cytoplasm from hyphae or fungal spores by puncturing a 2–6 μm hole through the cell wall (a <1 μm hole if with filopodia) and extending pseudopodia into the cytoplasm (Old and Chakraborty 1986). Not many genera of soil protists are known to engage in this form of feeding. Some of the genera involved in fungivory include gymnamoebae, *Leptomyxa* and the Vampyrellidae (Rhizaria: Cercozoa: Filosea), and the colpodid Grossglockneriididae (Adl 2003). However, in some cases, the abundance of fungivores has been found sufficiently high to reduce the abundance of spores of plant pathogens (Chakraborty 1985; Chakraborty and Warcup 1985) or to reduce ectomycorrhizae root colonization in pot experiments (Chakraborty et al. 1985). Higher populations of fungivores were found to be one of the main reasons for reduced survival of the pathogen *Rhizoctonia solani* in a "disease suppressive" soil in South Australia (Gupta and Neate 1999). The amoebae known to engage in fungivory are not necessarily obligate fungivores. Many are facultative fungivores that engage in other forms of feeding, such as bacterivory or detritivory. Notably, since many soil invertebrate cuticles also consist of chitin, amoebae with chitinase also decompose shed cuticles from moults or dead organisms, as well as preying on invertebrates and puncturing fungal spores and hyphae (Old and Darbyshire 1978; Anderson and Patrick 1980; Homma and Kegasawa 1984). This aspect of chitin decomposition may have been neglected or underdescribed.

Cysts and spores of protists and fungi are protected by combinations of chitin, protein, or cellulosic cell wall, depending on taxonomic lineage. Some fungal spores can be digested by fungivorous amoebae through a hole, as observed for the hyphae. In some cases, fungal spores were reported to have been digested inside food vacuoles by species with chitinase. However, ingestion of spores and cysts by most species that do not have chitinase or cellulase leads to

excretion of viable spores at a distance (Wolff 1909; Heal 1963; Chakraborty and Old 1982). Cysts of protists are dehydrated and tend to be more resistant to digestion. Other researchers have also reported that a fraction of the spores and cysts ingested and then excreted by amoebae, ciliates, or mites remains viable (Behan and Hill 1978; Anderson 1988; Pussard et al. 1994; Hubert et al. 1999, 2000). This may contribute to species dispersal at a smaller scale within the soil habitat. The ecological significance of this dispersal of soil protists in terms of diversity measurement at microsites has not been fully considered.

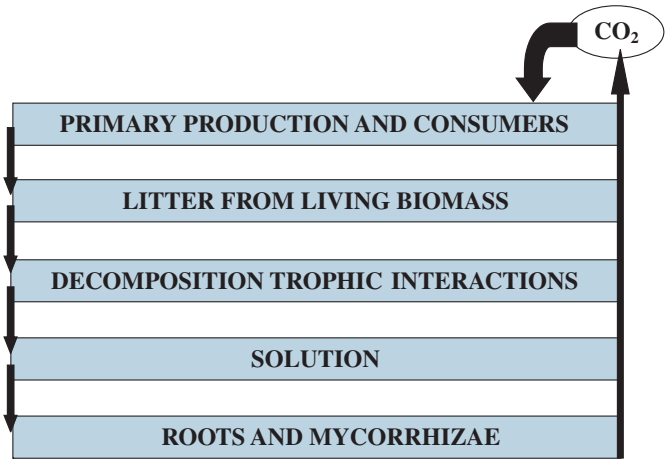
Some protists are responsible for predation on invertebrates, particularly on nematodes, rotifers, and tardigrades (Barron 1977; see Adl 2003). The cytotrophic filose testate amoeba *Nebela* (Cercozoa: Silicofilosea) was reported to sometimes capture nematodes (Yeates and Foissner 1995). It is not known how widespread predation on invertebrates by amoebae, with or without a test, is. Certainly, many have chitinase activity that could enable them to prey on invertebrates, as discussed above for fungivory. Certain chytrids, such as *Catenaria*, swim toward their invertebrate prey by chemotaxis, attach to the cuticle or penetrate an orifice, and extend a filamentous thallus into the prey tissues, which are then digested. The predacious Peronosporomycetes, such as *Myzocytium*, form sticky cysts that attach to passing prey. The attachment stimulates excystment and the extension of a filamentous thallus into the prey tissues. Several genera of Fungi engage in predation on small invertebrates, as well as on amoebae and other protists, using sticky segments of hyphae, looped hyphae, or nematotoxic substances (Barron 1977; Thorn et al. 2000). In saprotrophic species, the prey cytoplasm is an important source of organic nitrogen, especially for cellulolytic and ligninolytic species that grow on nitrogen-poor substrates.

Protists in the forest soil food web

The microbial loop has been described as a link between available nutrient turnover rate through microbes, transformation of OM through microbial metabolism and digestion, and transfer of biomass to other trophic levels (Pomeroy 1970, 1974; Coleman 1994) (Fig. 2). An important component of the original idea was the distinction between (*i*) available nutrients that were dissolved and available for transfer through cell membranes (e.g., of primary saprotrophs) and (*ii*) the immobilized biomass in cells or in indigestible OM that was not immediately available. The role of primary saprotrophs in soil is to utilize the polymers in dead tissues accumulated as litter and to transform them into new living biomass. A diversity of primary saprotrophs is necessary to supply a diversity of enzymes, to occupy a diversity of substrates and microhabitats, and to supply a diversity of species to fill the same niche under a variety of environmental conditions. If that were the end of the pathway, much of the litter biomass would then accumulate into bacteria and fungi, especially as immobilized in their cell walls as chitin and murein.

The second role of the microbial loop is to provide organic nutrients to consumers that feed on primary saprotrophs. Fungivory, bacterivory, and cytotrophy are necessary to digest primary saprotrophs and transform these

Fig. 2. Decomposition of litter from the primary production subsystem through the soil microbial loop recycles dissolved nutrients and CO_2 for photosynthesis and plant growth.



cells and their cell walls into new biomass. The process is required to transfer the nutrients into living biomass in subsequent trophic levels. It is also necessary to transform the cell wall polymers by digestion into monomers and to assimilate these into cytoplasmic molecules. The transformation of polymers into soluble forms and their assimilation into new cytoplasm prevents their accumulation (or immobilization) in the soil. Organic molecules not digested by any species become an end product that is not recycled and accumulates in the soil.

It has been shown repeatedly in field studies and in microcosms that the protist grazers stimulate nutrient (C, N, P, and S) mineralization, enhance nutrient availability and uptake by plants, and improve plant growth (Coleman 1994 (and references therein); Griffiths et al. 1999; Bonkowski et al. 2000; Bonkowski 2004). It has also been demonstrated that the contribution of protists to nutrient cycling and respiration far exceeds that of other groups of organisms per unit mass (Anderson et al. 1978; Paustian et al. 1990; Coleman 1994; de Ruiter et al. 1995; Griffiths et al. 1999). Bacterivory, particularly by protists and nematodes, enhances overall nutrient turnover and growth rates of bacteria, as well as enhancing the specific decomposition of pollutants (Mattison and Harayama 2001; Selph et al. 2003). These observations in the soil environment are similar to those of more extensive studies in the aquatic microbial loop (Sherr and Sherr 2000). The role of protist grazers in the food web was compared with that of an enzyme in a substrate solution (Adl 2003). One enzyme molecule (or cell) will process a large amount of substrate (or prey), and the functional response curves are mathematically the same as the curves for Michaelis–Menten equations, except that their derivation is different (Williams 1980). For any biological metabolic reaction, substrate is transformed into new biomass as cytoplasm and cell wall components, with a loss of thermal energy and with gaseous, liquid, and solid by-products (Figs. 1, 2). The average efficiency of protists in processing prey was estimated to be 30% (Hunt et al. 1977). Therefore, protists in the food web help return CO_2 to the air for photosynthesis, return soluble nutrient molecules to the soil solution for root uptake, and excrete undigested food vacuole contents as hu-

mus for further digestion in the soil food web. In forest soil, where active protists are usually abundant, their contribution to litter recycling must not be underestimated. Per unit mass, their contribution is calculated to exceed that of other soil organisms (Anderson et al. 1978; Paustian et al. 1990; Coleman 1994; de Ruiter et al. 1995; Griffiths et al. 1999), and they remain a key component of the decomposition food web in setting C and nutrient flux rates (C, N, P, S) through the forest system.

Species, niches, and the biodiversity conundrum

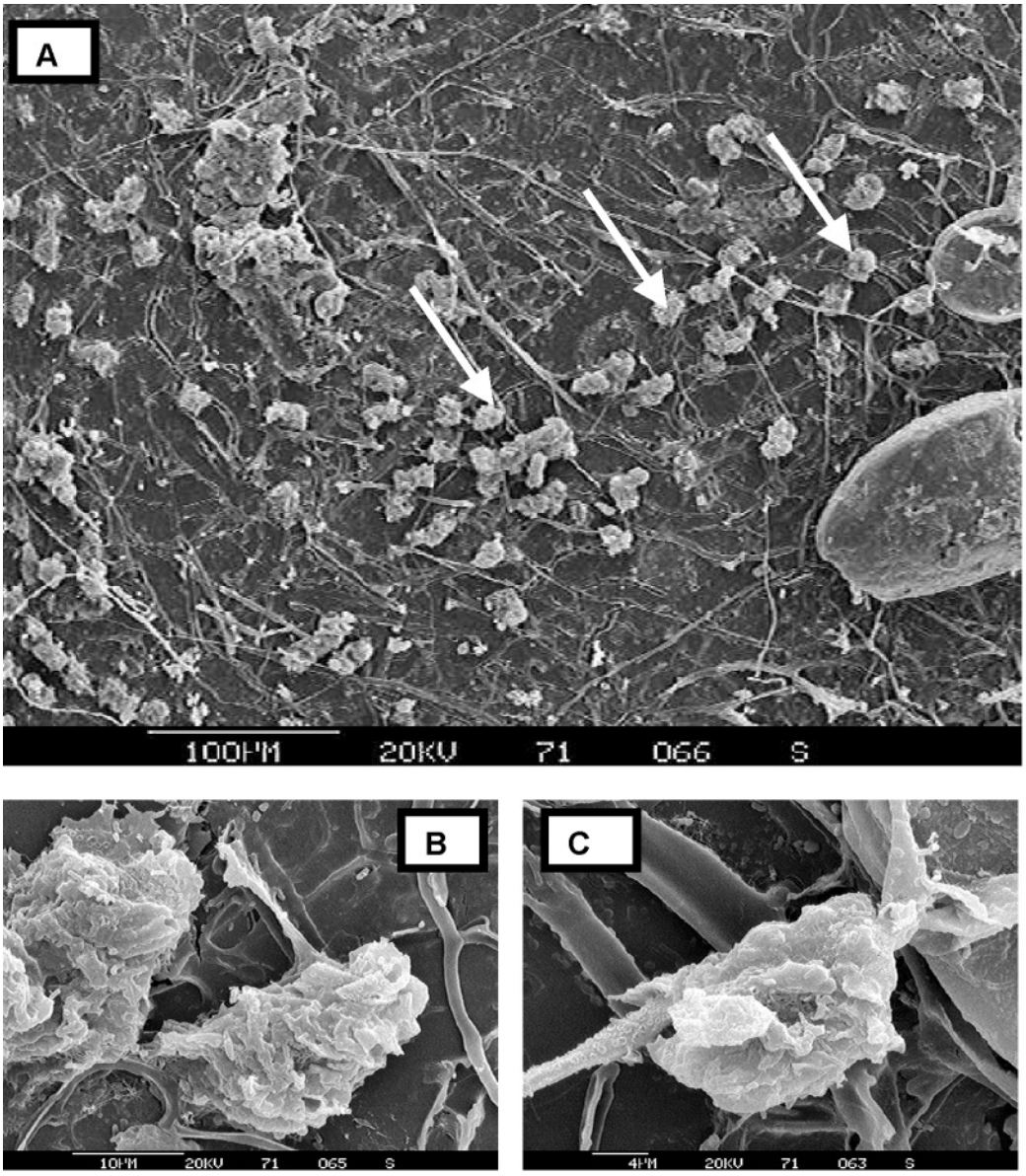
Species in ecology occupy a functional niche, compete for their niche with other species, and are adapted to live that role (Krebs 2001). Yet, on average, forest soils contain probably 50–100 species of protists, an equivalent number of fungi, 30–80 invertebrate species, and a much greater diversity of bacteria per gram. Any 1 g sample of soil contains many species of primary saprotrophs, bacterivores, and other secondary saprotrophs and consumers. Moreover, protist species composition varies between 1 g samples such that only a small fraction of the forest species is represented in any one sample. Are there sufficient niches at this small scale to support so many apparently competing species? Are there sufficient resources to partition between species and enough spatial heterogeneity to accommodate all the species? These questions need satisfactory answers if ecological theory is to hold true. Below we first consider spatial stratification, then temporal stratification at different time scales, to seek an understanding of this apparently overwhelming biodiversity.

The soil matrix provides a reticulum of pore spaces through and between peds. Soil aeration, drainage, moisture retention, and temperature changes are all affected by the tortuosity and porosity of the soil. Throughout the profile are gradients of oxygen, CO_2, temperature, moisture, and OM quality. Edaphic species are affected by these gradients of organic composition, physical structure, and chemical properties (Adl 2003). Organisms are adapted in their habitat to a range of physical–chemical parameters and food quality that limit their distribution and periods of activity — this is known in microbial ecology as Shelford's law of tolerance. For example, species vary in their tolerance to soil drying and encyst accordingly (Cowling 1994; Neher et al. 1999). Organism growth rate is also limited by the most limiting nutrient in their diet — this is known as Liebig's law of the minimum. In the forest soil habitat, there is a heterogeneous landscape extending horizontally and vertically through the profile, at a submillimetre scale, and above to larger scales. At this scale, protist species could be affected by the effects of the plant rhizosphere, depending on plant species and distance from the rhizosphere (Foster et al. 1983; Rodriguez-Zaragoza et al. 2005*a*). Besides changes through the profile, the microhabitat is affected by the type of litter (cuticle, twig, leaf, dead root, etc.) serving as a resource patch, by shade on the surface, by the presence of a pebble, by the aspect of the slope, and so on. A multitude of microclimates and microhabitats can be imagined at all scales, both horizontally and vertically. Therefore, it is conceivable that many species are spatially stratified. In fact, there are many examples of substrate preference, prey preference, and depth preference, with some species tolerating narrow or wider ranges through the profile (Foissner 1987; Adl 2003). These ideas have been verified in the field for nematode spatial distribution, which confirmed that patch dynamics occurred, as well as spatial segregation between species (Ettema 1998; Ettema et al. 2000). Amoeboid, cercomonad, and many other flagellates are surface-attached species that explore surfaces and pores that pseudopodia and filopodia can reach into. Film water and moist surfaces are sufficient for their activity, which can persist into drier soil conditions. For certain flagellates and most ciliates, water-filled pores and capillary water are required. The size of species further limits their habitable pore space. When substrate or prey preferences are also taken into account, as well as spatial stratification preferences, it becomes possible to delineate niches on the basis of morphotypes, food preferences, and other requirements. When seasonal or temporal patterns of activity are also taken into account, even more niches become possible. Key in this discussion is that despite the number of species that can be described from forest soil, only a small fraction is active at any one time, with the remainder encysted or in spores.

Numerous studies have described changes in the abundance of invertebrate species in litter bags through 1 year or more. For example, a succession of activity through 1 year was obtained by monitoring leaf litter bags for microbial enzymatic activity and microarthropods (Dilly and Irmler 1998; Dilly et al. 2001). Another study that followed succession patterns in litter decomposition identified a functional shift in the primary saprotroph community after 21 months (Osono and Takeda 2001). In one study, amoebae were the most abundant (by population and species number) protist fungivores and bacterivores on wheat residues in a litter bag study conducted in South Australia (V.V.S.R. Gupta and S. McClure, unpublished data). The Mediterranean climate in South Australia provides short periods of moist conditions separated by long periods of dry conditions, resulting in low levels of active protist populations in the soil at any one time. However, large populations of amoebae were seen on the surface of decomposing litter (Fig. 3). To complement these, microinvertebrate and protist families and abundances were monitored in litter bags through 1 year in a North Carolina forest and in a Puerto Rico forest (Hunter et al. 2003; Adl et al.[2]). The results showed a succession of types of protists in the leaf litter that was affected both by the season and by the state of tissue decomposition (Table 3). At a shorter time scale, protist abundances have been shown to vary over several days with weather, with rain, and with soil drainage (Adl and Coleman 2005). Ciliates were active only infrequently, when both moisture levels and bacteria or flagellate prey abundances were high. The abundance of bacterivorous flagellates closely followed the abundance of bacteria. Certain gymnamoebae persisted through dry periods, when few other species remained active. The pattern observed with protists was not dissimilar to the activity and seasonal patterns observed with other soil organisms. De-

[2] M.S. Adl, A. Maharning, M.D. Hunter, and D.C. Coleman. Manuscript in preparation.

Fig. 3. Scanning electron micrographs showing protozoan feeding of *Rhizoctonia solani* hyphae on wheat stubble incubated in a disease-suppressive soil at Avon, South Australia. (A) *Aphalyx* amoebae (arrows) feeding on the *R. solani* hyphal network and perforations and broken cell walls of fungal hyphae. (B) Bacteria-feeding amoeba. (C) Mycophagous amoeba attached to *R. solani* hypha (V.V.S.R. Gupta and S. McClure, CSIRO Land and Water).



spite the large number of coexisting cysts at any one time, a small number of species were active. These species probably compete for resources and space until the resources are utilized or until the abiotic conditions are outside the tolerance range for some species. Further evidence for differences in protist stratification and activity patterns as affected by treatment or rhizosphere of different plant species was provided by Rodriguez-Zaragoza et al. (2005*a*), who studied a desert soil with well-separated plant rhizospheres.

The most plausible consensus on soil biodiversity is that there are sufficient microhabitats, substrates, prey varieties, microclimates, and changes throughout the seasons to accommodate the high number of species. Co-occurring species that appear to share the same niche probably do so only transiently when the resources are not limiting. Detailed studies on the feeding behaviour of protists, as described above, suggest that optimal foraging behaviour and prey preferences are important components of species interactions on similar prey or substrates. These interactions were already well established for fungal species that are known to compete for space and substrate through growth rates and chemical secretions (Renvall 1995; Boddy 2000). Similar chemical interactions between coexisting or competing populations of protists are possible. The best known example is that of *Paramecium* strains (Alveolata: Ciliophora) that have a killer trait: toxic particles in the cytoplasm eliminate competition from nonkiller populations (Quackenbush 1988; Paracer and Ahmadjian 2000). This type of interaction between species in soil is poorly studied.

If ecological theory is correct — that there are sufficient

Table 3. Abundance of protozoa in litter bags of different plant leaf species at Coweeta, in a North Carolina temperate forest, and at Luquillo, in a tropical forest, through four seasons.

		Individuals·(g dry litter)$^{-1}$			
	Date	Gymnamoebae	Testate amoebae	Large flagellates	Small flagellates
Coweeta	Apr.	0	0	0–100	$<10^4$
	July	10^2, patchy	10^4	10^2–10^3	$>10^4$
	Oct.	10^3	10^3	10^3	$2 \times 10^4 - 10^3$
	Jan.	$2 \times 10^4 - 10^3$	$<10^3$, patchy	10^3–10^2	2×10^4
Luquillo	Apr.	10^3, patchy	0	10^3	0
	July	10^3–10^4	10^4	10^2–10^3	10^4
	Oct.	10^3–10^4, patchy	10^3–10^4	10^2–10^3	10^4
	Jan.	0	10^3–10^4	10^3	10^4–10^5

Note: Large and small flagellates represent sizes larger or smaller than 12 μm.

niches in the soil to account for the observed biodiversity — then we can consider whether this holds true at a larger scale, between ecosystems and across ecoregions. Studies of soil microinvertebrates usually show that most species are regional and only a few species are ubiquitous (Deharveng and Lek 1995; D.A. Crossley, Jr., personal communication, 2002). It has been known for a long time that a nematode morphotype may not identify the same species, especially when isolated from different regions (Maupas 1900, 1919; Osche 1952). Fungal isolates are notoriously difficult to identify without reproductive structure and biochemistry. There is a limit to what can be identified by microscopy and morphology. It has been argued, on the basis of morphology, that many protist genera are found to occur everywhere and that it is plausible that all protist species are present everywhere there is a suitable habitat, across continents (Finlay et al. 1999, 2001).

This hypothesis proposes that if one looks hard enough, any morphospecies (as they are identified now) will be found in a suitable habitat on any continent, although it might be rare in some regions and more common in others. The evidence rests on morphological identification of species at the microscope. Experience and the molecular evidence warn us to be more cautious. Selected examples include all protist species that were domesticated in the laboratory for cell biology or sampled multiple times from different environments. For example, in ciliates, it is known that *Tetrahymena* refers to a cluster of more than 20 morphologically indistinguishable species (Strüder-Kypke et al. 2001). Several *Euplotes* species and *Paramecium aurelia* are identical and could be distinguished only by mating-type clusters (Nyberg 1988; Caprette and Gates 1994; Jones and Gates 1994). In oxytrichids, which include several ubiquitous soil and aquatic genera, the genera are difficult to distinguish (Foissner and Berger 1997, 1999). Identical isolates of *Sterkiella histriomuscorum* (also referred to as *Oxytricha fallax* or *Histriculus muscorum* in the literature) from different geographical regions were found after careful study to have different feeding preferences, different cyst morphology, different mating types and sexuality, and differences in the position and sequence of introns[3] (Table 4). Similarly, a study of marine ciliates revealed specialization within morphotypes (Dini and Nyberg 1999). Similarly, molecular sequence data and biochemistry indicate that the green algal genus *Chlamydomonas* and the brown algal genus *Nannochloropsis* consist of many morphologically similar or identical species (Proshod et al. 2001; Suda et al. 2002). Isolates of the common soil gymnamoeba *Acanthamoeba* genus were investigated through molecular phylogenies, which revealed a cluster of almost identical but related species (Chung et al. 1998; Stothard et al. 1998). The same was true for many parasitic species, such as the entamoebae (Verweij et al. 2001) and *Trypanosoma* blood parasites (Stevens and Gibson 1999; Barnabe et al. 2003). There does not seem to be any molecular evidence of single morphotypes from a broad geographical distribution representing a single species of protist. Because of the facts, one must accept that a suitable working hypothesis is that morphotypes ("morpho species") within genera probably represent a cluster of similar species. These species have adaptations for prey or habitats that set them apart. These species, if sexual, usually have differences in conjugation, autogamy, and mating type.

From a biogeography perspective, the topic remains pertinent. A recent insightful statistical analysis of metadata (Hillebrand et al. 2001) cautioned against claims that microbial species are ubiquitous in distribution. Are the same genera and species encountered in all ecoregions? This answer depends on whether one is describing morphotypes or mo-

Table 4. Feeding preferences and sexuality of morphologically similar or identical isolates of *Oxytricha* (or *Sterkiella*) isolated from different geographic regions.

Isolate	Sex	Food size range (μm)
Oxytricha bifaria	>8 mating types	1–20
Oxytricha nova (DMP)	Autogamy	1–35
Oxytricha fallax (JRB)	>4 mating types	1–20
Oxytricha fallax (AF1)	Zygocyst	1–35
Oxytricha fallax (AF2)	Zygocyst	1–25
Oxytricha fallax (JK)	None known	5–35
Sterkiella mytilus	>4 mating types	1–20

Note: *Stylonichia mytilus* is a related aquatic species. Prey size of 1 μm indicates ability to grow on bacteria; >20 μm, ability to ingest *Tetrahymena*; 20–25 μm, ability to grow on *Colpoda steinii*. Intermediate prey size ranges are for flagellates.

[3] M.S. Adl and D.M. Prescott. Manuscript in preparation.

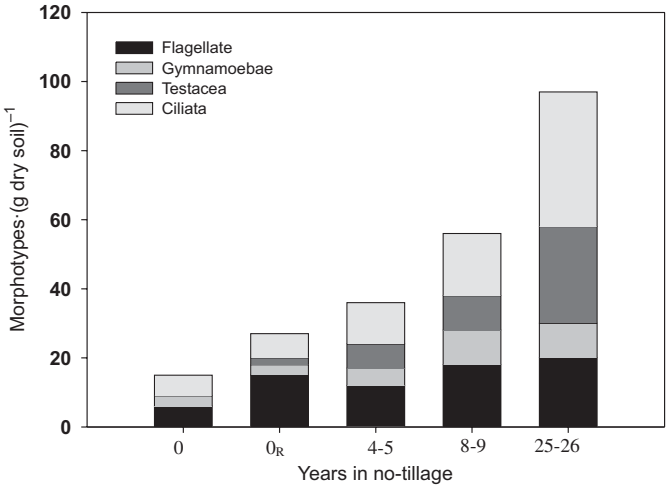
lecularly identifiable species and to what extent the species have been studied and reisolated. The current debate leans toward the existence of different species, limited in biogeography, without excluding the possibility that some species can be ubiquitous to many regions. However, clearly, the morphological descriptions alone are insufficient for identifying most microscopic species.

Disturbance and recovery

Plant succession and soil profile development through time are well known to ecologists. Plant and animal litter deposition is followed by decomposition and incorporation in the soil profile. Therefore, aboveground species composition has a direct effect on belowground species composition. Interactions and feedback between the aboveground and belowground components of the ecosystem were reviewed recently (Wardle 2002). Agricultural fields provide useful disturbed field sites for comparison with natural and managed forest (Neher 1999). It is not always clear why in some cases, aboveground diversity and food web structural complexity do not correlate with belowground patterns of species gradients, whereas in other cases there are clear patterns over time (Wanner and Xylander 2005; Hassall et al. 2006). Several explanations can be brought forth, but none is entirely satisfactory. One is that different sites are to some extent arbitrarily assigned to a successional stage, such as a selection of pastures, meadows, and forests, without fully accounting for the site history or seasonal cycles and weather-related changes belowground at the time of sampling. These plots may have different land-use histories and recent disturbances that would affect the successional stage of species through the profile. A second possibility is that succession belowground is to some extent dependent on species of plant litter input from leaves and roots but is mostly determined by the existing OM through the profile. Although there is expected to be a rhizosphere effect from exudates and root decomposition, it is unclear at this time to what extent this affects belowground protist species composition (Bonkowski et al. 2000; Phillips et al. 2003; Rodriguez-Zaragoza et al. 2005*b*). A third possibility is that succession through the profile does not change at the same time scale as aboveground plant succession. It could take several years for new plant species and surface litter to affect the decomposition food web deeper in the profile.

There are, however, good recent examples of clear succession in soil biota. One study, based on reclaimed mining sites, described colonization and the subsequent increase in biodiversity at recultivated mining dumps 4–46 years old (Wanner and Dunger 2001). The older sites had a testate amoebae diversity similar to that of local undisturbed sites, but species composition between these two types of sites were different. Another study of recovering old mine sites described an increase in species number for all soil invertebrates with age of plot (Frouz et al. 2001). Species replacement with time was observed for some groups. Despite site differences, an overall pattern of succession was discernable, and this pattern was comparable to that at other sites in the region. In another study, chronosequences of agricultural fields that were in no tillage for 0–25 years were compared (Adl et al. 2006*a*). The fields showed similar abundances of protists, nematodes, and microarthropods across sites. The abundances were compared at both spring and fall sampling in two consecutive years and showed little if any statistical difference, despite dramatic differences in the profiles and OM content. However, when protist diversity was compared between sites, there was a very clear trend of increasing diversity per gram of soil with time in no-tillage management (Fig. 4). This trend correlated with an increase in the light fraction and in OM through the profile. This result is in agreement with laboratory experiments showing that the total abundance of protists did not vary as species richness increased (Lawler 1993). The reason for this may be associated with soil OM content, prey biomass, and the carrying capacity of the soil for biological activity.

Fig. 4. Increase in protozoan species diversity along a no-tillage chronosequence in south Georgia, United States. Field sites represented as years in no-till management. Two conventionally tilled sites were also sampled as control plots. The 0_R field differed from the 0-year field in having the roots left to decompose in the soil.



In these studies, species observed in disturbed soils (tillage agriculture, remediation site recolonization stages) were dominated almost exclusively by *r*-selected species (Foissner 1994, 1999). These tend to be many colpodid ciliates, certain stichotrich ciliates, bodonid species, and characteristic gymnamoebae, such as *Acanthamoeba*. In general, with disturbance one tends to observe an increase in the colpodid/ciliate ratio, a decrease of active testate amoebae, and losses of many gymnamoebae and cercozoan species. Fungal abundance declines and bacterial species composition shifts with an increase in dominance of Actinobacteria. It is possible to describe soil protists along *r–k–a* gradients based on species adaptations for a particular life history and abiotic conditions (Bamforth 2001). The *k*-selected species appear in later field successional stages in undisturbed soils, whereas the *r*-selected species persist in these soils but have reduced species dominance and restricted activity periods. The *a*-selected species are probably active only during periods of extreme abiotic conditions for that habitat. Attributing species to *r* or *k* status on the basis of when they excyst in Petri plates is probably not relevant to species *r–k* status in the field. Certain issues need to be treated with caution, such as the ability of coexisting cysts to survive the same extreme

environmental conditions at the same field site. Should only those cysts that survive be *a*-selected? Or only those species that can be active under locally extreme conditions? More fieldwork with succession chronosequences that focus on species inventories, accounting for species stratifications in the profile and their activity patterns, would be most useful in relating species *r–k–a* adaptations to field successional history.

Conclusions

Most forest soil studies on protozoa used the MPN technique, which is discredited by protistologists, and obtained values for bacterivory based on species cultured under narrow conditions. These studies ignored the diversity of functional groups represented in soil protists. Future studies need to accept this diversity, limit sampling to fewer taxonomic groups of protists, and assay the contribution of each functional group and of the diversity of species within these groups to nutrient cycling. This will require methodological adaptation so that samples collected are limited to the number that can be analysed. The microscopy must be supplemented with molecular identification, and the MPN approach should be abandoned in favour of modern and molecular techniques. Molecular techniques used for bacteria and fungi are also suitable for protists. These techniques include differential gradient gel electrophoresis and fluorescent in situ hybridization (Jezbera et al. 2005). A vast database of suitable oligonucleotides that was generated for reconstruction of protist phylogenies remains underutilized by ecologists. These tools will be needed to address diversity and to identify species more rapidly and more precisely than by microscopy.

There are exciting opportunities ahead to consider the role of microbial diversity in ecosystem function. Experimental manipulations of biodiversity tend to focus on plant taxonomic diversity and its effect on ecosystem function and productivity (Loreau et al. 2001). Many studies have relied on short-term effects that do not necessarily reflect the long-term effects under fluctuating conditions, but the latter would test the stability of the system with varying complexity. Microcosm experiments with protists have been popular for testing hypotheses about diversity and function (Lawler 1993; Naeem et al. 2000). It remains to be seen to what extent general theories about plants and animals are applicable to unicellular species and to what extent scaling issues need to be accounted for (Lawton 1999; Hillebrand et al. 2001; Loreau et al. 2001). Further attention to linkages between the primary production subsystem and the decomposition subsystem would allow more rigorous testing of these theories.

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