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## The role of fungi in carbon and nitrogen cycles in freshwater ecosystems

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### Introduction

Fungi are adapted to a diverse array of freshwater ecosystems. In streams and rivers, flowing water provides a mechanism for downstream dispersal of fungal propagules. The dominant group of fungi in these habitats, aquatic hyphomycetes, have conidia that are morphologically adapted (tetra- and sigmoid) for attachment to their substrates (leaf litter and woody debris from riparian vegetation) in flowing water (Webster, 1959; Webster & Davey, 1984). In freshwater wetlands and lake littoral zones, production of emergent aquatic macrophytes is often extremely high, resulting in an abundance of plant material that eventually enters the detrital pool. The dead shoot material of these macrophytes (leaf blades, leaf sheaths and culms) often remains standing for long periods of time before collapsing to the sediments or water. This plant matter is colonized by fungi that are adapted for surviving the harsh conditions that prevail in the standing-dead environment (Kuehn *et al.*, 1998). There are a number of other freshwater ecosystems where fungi are present and exhibit interesting adaptations, e.g. aero-aquatic fungi in woodland ponds, zoosporic organisms (Chytridiomycota and Oomycota) in a variety of habitats including the pelagic zones of lakes, and Trichomycetes that inhabit the guts of a variety of aquatic insects. Despite the well-known occurrence of these fungal groups in aquatic habitats, virtually nothing is known concerning their roles in biogeochemical processes. Overall, the contributions of fungi to biogeochemical cycles have been understudied in most freshwater ecosystems.

Most studies examining fungal participation in biogeochemical cycles in freshwater ecosystems focused on the role of fungi in the decomposition of plant litter. Historically, the lack of appropriate methods to accurately quantify

fungus biomass and rates of biomass production was a major reason for the paucity of knowledge concerning the role of fungi in litter decomposition (Gessner *et al.*, 1997). However, a growing body of evidence has emerged over the last two decades on the usefulness of the fungal sterol, ergosterol, in the quantification of fungal biomass within decaying plant litter and the technique for measuring *in situ* instantaneous growth rates of fungi from rates of [<sup>14</sup>C]acetate incorporation into ergosterol (Gessner & Newell, 2002 and references therein). Both of these methodological developments are increasingly used today. This has been particularly useful in allowing quantitative assessment of the magnitude of fungal contributions to the cycling of carbon and the flow of energy in freshwater ecosystems. Results of these studies have indicated that fungi are significant decomposers of particulate detritus and play key roles in detrital food webs of freshwater ecosystems.

During plant litter decomposition, fungi are involved in a variety of processes that result in the conversion of plant carbon into fungal biomass and also into CO<sub>2</sub> as a result of their respiratory activities (Gessner *et al.*, 1997). In addition, fungal decomposition of plant litter, as well as feeding activities of detritivore consumers (invertebrates) on this microbially colonized plant litter facilitates the export of plant carbon as either fine particulate organic matter (FPOM) or dissolved organic matter (DOM). This chapter will examine the role of fungi in carbon and nitrogen cycles during plant litter decomposition in two freshwater ecosystems, streams and wetlands. The use of quantitative methods has led to a greater appreciation of the impact of these organisms on biogeochemical processes within both of these ecosystems.

### **Freshwater streams**

Riparian vegetation shades woodland streams and limits the magnitude of primary production occurring within these ecosystems. This vegetation also contributes the bulk of organic matter inputs to the stream, primarily in the form of leaves and woody debris. Woodland streams have been shown to receive up to 99% of their organic carbon from the riparian vegetation (Fisher & Likens, 1972; Webster & Meyer, 1997). Since woodland streams are dependent on leaf litter as a major source of carbon and energy, considerable attention has focused on the decomposition of this detritus and its links to higher trophic levels (Webster & Benfield, 1986; Suberkropp, 1998b).

#### *Carbon cycle*

In temperate streams, leaf resources enter as a pulse during autumn leaf fall (Fig. 17.1a) as temperatures are declining. Once leaf litter enters a stream, it is rapidly colonized by aquatic fungi and bacteria.

Aquatic hyphomycetes are ubiquitous in streams and concentrations of their conidia in the water typically reach annual maxima shortly after peaks of leaf inputs in the autumn (Fig. 17.1b, Iqbal & Webster, 1973; Bärlocher, 2000; Gulis & Suberkropp, 2004). As aquatic hyphomycete hyphae penetrate and grow in leaf litter, they secrete an array of extra-cellular enzymes (e.g. cellulases, xylanases, pectinases) that digest leaf

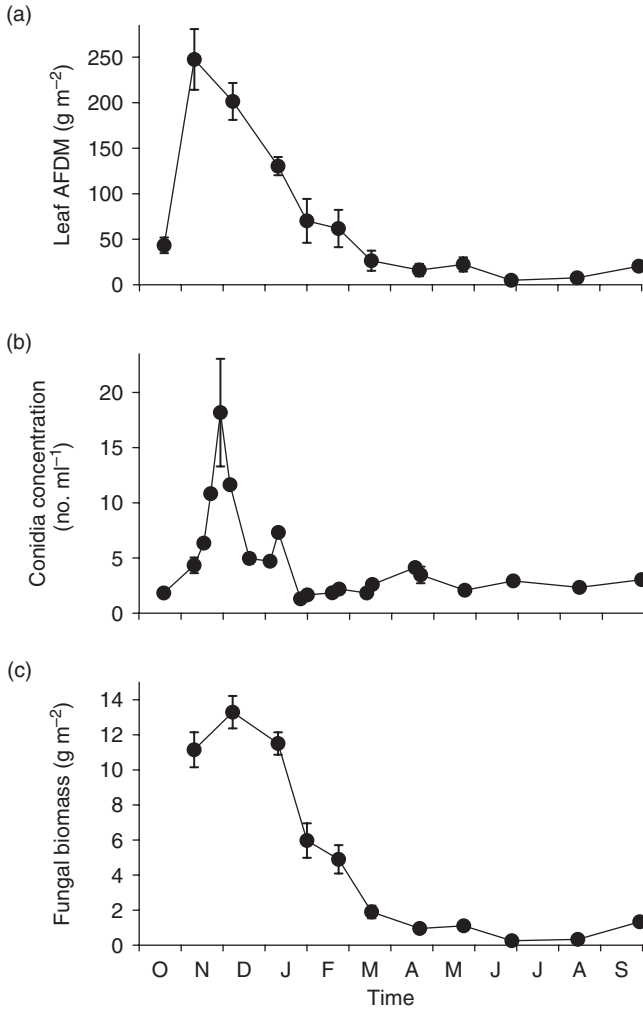


Fig. 17.1. Leaf litter standing crop as ash free dry mass (AFDM) per unit of stream bottom area (a), conidia concentration of aquatic hyphomycetes in water (b) and fungal biomass associated with leaves as dry mass (DM) per unit of stream bottom area (c) in a headwater southern Appalachian stream. Data from Suberkropp (1997). Symbols indicate means  $\pm 1$  SE ( $n = 3-10$ ).

polysaccharides, thereby allowing organic carbon to be assimilated by fungi (Suberkropp *et al.*, 1983; Chamier, 1985; Shearer, 1992). Fungal growth and enzymatic digestion also cause softening or maceration of leaf tissue (Suberkropp & Klug, 1980; Chamier & Dixon, 1982), contributing to the production and release of fine particulate organic carbon in streams.

*Fungal biomass* During the decomposition of leaves, fungal biomass typically increases to a maximum and then stabilizes or declines as conidia are released and hyphae senesce (Gessner & Chauvet, 1994). Fungal biomass can account for as much as 18%–23% of the total mass of detritus (Gessner & Chauvet, 1994; Methvin & Suberkropp, 2003). Fungal biomass associated with leaf detritus expressed on an areal basis reaches annual maximum values after leaf litter enters a stream and becomes colonized (Fig. 17.1c). Maximum fungal biomass of up to 27 g m<sup>-2</sup> of stream bed has been found associated with naturally occurring leaves (Suberkropp, unpublished results). Fungal biomass associated with decaying leaves enclosed in litter bags typically reaches values that are one to two orders of magnitude greater than bacterial biomass found on the same leaves (Table 17.1). Likewise, microbial biomass inhabiting naturally occurring leaves in streams is also dominated by fungi (Table 17.1)

Table 17.1. *Fungal and bacterial biomass associated with decomposing leaves in streams. All values are maximum biomass estimates from litter bag decomposition studies except the study by Findlay et al. (2002b) where average microbial biomass from randomly collected leaves was estimated*

Leaf species	Biomass as percent of detritus		Reference
	Fungal	Bacterial	
<i>Platanus hybrida</i>	4.8	0.5	Baldy <i>et al.</i> (1995)
<i>Populus nigra</i>	9.9	0.3	Baldy <i>et al.</i> (1995)
<i>Salix alba</i>	7.8	0.3	Baldy <i>et al.</i> (1995)
<i>Liriodendron tulipifera</i>	14.0	0.08	Weyers & Suberkropp (1996)
<i>Populus nigra</i>	8.0	0.06	Baldy <i>et al.</i> (2002)
<i>Alnus glutinosa</i>	7.7	0.4	Hieber & Gessner (2002)
<i>Salix fragilis</i>	7.0	0.3	Hieber & Gessner (2002)
<i>Acer rubrum</i>	17.4	0.6	Gulis & Suberkropp (2003c)
<i>Rhododendron maximum</i>	10.8	0.2	Gulis & Suberkropp (2003c)
Leaf detritus from 9 streams	1.5	0.1	Findlay <i>et al.</i> (2002b)

Factors such as chemical characteristics of the leaf species (Gessner & Chauvet, 1994) and concentrations of nutrients (N and P) in the water (Grattan & Suberkropp, 2001; Niyogi *et al.*, 2003) can affect the amount of fungal biomass associated with leaves as they decompose. The strong correlation between exponential decay rates of different leaf species and measures of fungal biomass and activity (e.g. maximum ergosterol concentrations, net mycelial production and sporulation rates of aquatic hyphomycetes) indicate that fungi play a key role in regulating the decomposition of leaves (Gessner & Chauvet, 1994). Significant correlations of ergosterol concentrations with mass loss of wood veneers (Fig. 17.2) and decay rates of grass leaves (Niyogi *et al.*, 2003) suggest that this conclusion holds true for various types of plant detritus decomposing in streams.

After colonization and growth, one major fate of fungal biomass includes the production of conidia that are carried downstream. Conidia may provide an inoculum for leaves entering the stream, may be captured by filter-feeding invertebrates or may undergo decomposition by bacteria. Fungal biomass within leaf litter also serves as an important food resource for detritivores that consume leaf detritus. Microbially colonized detritus

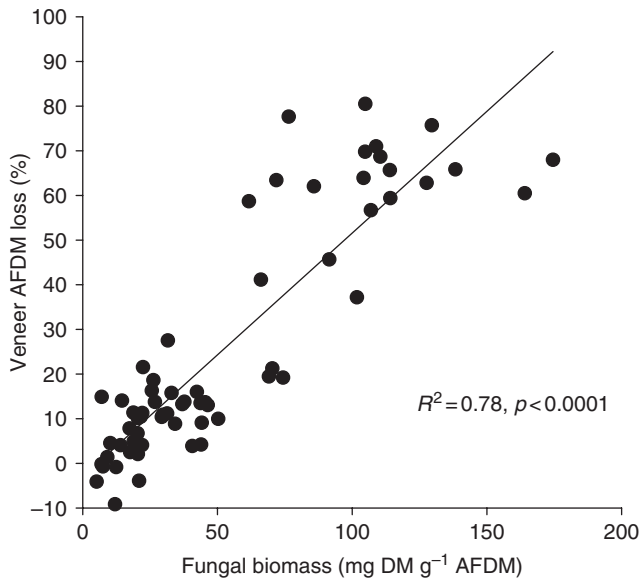


Fig. 17.2. Correlation between associated fungal biomass and mass loss of wood veneers decomposing in headwater streams. Data from Gulis *et al.* (2004).

is a more palatable and nutritious food source than dead leaves for invertebrate detritivores (Bärlocher, 1985; Suberkropp, 1992). Microbial growth and enzymatic decay of leaf polymers increases the nutritional quality of leaf detritus since microbial biomass is more digestible for detritivore consumers than recalcitrant leaf tissue (i.e. lignocellulose). In addition, microbial enzymes can partially degrade leaf polymers making them more digestible and microbial enzymes may remain active in the digestive tract of some detritivores (Bärlocher, 1985). Since the biomass of fungi associated with leaf detritus is much higher than that of bacteria, fungal biomass should provide a larger portion of the nutrition in the diets of invertebrate detritivores than bacterial biomass. Consequently, fungi occupy a central position in the trophic structure of stream food webs and mediate the cycling of carbon and flow of energy to higher trophic levels. However, fungal species that decompose leaf litter cannot be placed in a single black box within the carbon cycle, since species are not identical in their food quality for detritivore consumers. Fungal species are known to differ in their overall palatability (Bärlocher & Kendrick, 1973a; Suberkropp *et al.*, 1983) and nutritional value (Bärlocher & Kendrick, 1973b; Arsuffi & Suberkropp, 1986, Graça *et al.*, 1993) for these animals.

*Fungal production* Recently, production of fungi associated with decomposing plant material has been estimated by determining rates of incorporation of radiolabelled acetate into ergosterol. This method, introduced by Newell and Fallon (1991) for fungi decomposing *Spartina* litter in salt marshes, has been applied to the fungi decomposing leaves in freshwater streams (Suberkropp & Weyers, 1996; Gessner & Chauvet, 1997). Determination of fungal production allows estimation of the rate at which carbon from decomposing plant litter is converted into fungal biomass. This technique is particularly useful when losses in fungal biomass from leaf detritus are occurring (e.g. to sporulation, detritivore consumption, hyphal senescence and death) and biomass accumulation does not give a good indication of total fungal production.

Fungal production is typically higher than bacterial production associated with decomposing leaves. For example, maximum fungal production associated with decomposing *Liriodendron tulipifera* leaves was  $7 \text{ mg g}^{-1} \text{ d}^{-1}$  compared to  $0.3 \text{ mg g}^{-1} \text{ d}^{-1}$  for maximum bacterial production (Weyers & Suberkropp, 1996). Similarly, fungal production associated with *Populus nigra* leaves in a large river reached maximum values of  $1.3\text{--}1.4 \text{ mg g}^{-1} \text{ d}^{-1}$  whereas bacterial production achieved a maximum

of  $0.4 \text{ mg g}^{-1} \text{ d}^{-1}$  (Baldy *et al.*, 2002). Only when green, non-senescent *Alnus glutinosa* leaves were placed in a stream during the summer were bacterial rates of production ( $1.2 \text{ mg g}^{-1} \text{ d}^{-1}$ ) slightly higher than those of fungi ( $1 \text{ mg g}^{-1} \text{ d}^{-1}$ ) even though fungal biomass still accounted for 95%–99% of the total microbial biomass (Baldy & Gessner, 1997).

Although annual fungal production associated with naturally occurring leaves has only been estimated in a limited number of streams, it exhibits a wide range (Table 17.2). The amount of leaf litter present throughout an annual cycle appears to be an important factor controlling fungal production in a stream as annual fungal production is significantly correlated with the annual mean leaf standing crop (Fig. 17.3). The mean standing crop of leaf detritus is a function of both the input of leaf litter and the retentiveness of the stream. Most of the streams that have been examined exhibit relatively low retention of leaf litter, since winter storms (January to February) generally wash the bulk of autumn leaf litter from the stream (Suberkropp, 1997; Methvin & Suberkropp, 2003; Carter & Suberkropp, 2004). However, in streams that retain leaf detritus very efficiently (e.g. Coweeta 53, Table 17.2), the mean standing crop of leaf detritus is high and annual fungal production on areal basis is correspondingly high. Typically most fungal production per  $\text{m}^2$  occurs in the autumn and winter when the greatest amount of leaf detritus is present in the stream even though temperatures are at annual minima (Suberkropp, 1997; Methvin & Suberkropp, 2003). During the summer, when temperatures are higher, there is generally little leaf detritus remaining in streams. This may account for the relatively long turnover times calculated for fungi based on annual production to biomass ratios (18–44 days, Table 17.2).

Rates of fungal production can be used to estimate the fraction of leaf detritus that is assimilated by fungi (for mycelial biomass, sporulation, respiration). Net production efficiencies (production/production + respiration) determined for two aquatic hyphomycete species growing on leaf litter in microcosms ranged from 24% to 46% (Suberkropp, 1991). A third species exhibited production efficiencies of 32% and 60% depending on the nutrient concentrations in the water, and production efficiencies tended to decrease when bacteria were present (Gulis & Suberkropp, 2003b). Using the lowest and highest values together with estimates of leaf litter inputs to the streams, the percentage of leaf input to streams that is assimilated by fungi can be calculated (Table 17.2). In most cases the percentage of leaf inputs assimilated by fungi is significant. For streams in which leaf detritus is washed out by January–February (the first five streams in Table 17.2), fungi are estimated to assimilate only 5%–40%

Table 17.2. Annual production, production to biomass ( $P/B$ ) ratios and turnover times of fungi with annual leaf litter inputs and the percentage of the leaf input that was assimilated by fungi in different streams

Stream	Annual fungal production ( $\text{g m}^{-2}$ )	Annual $P/B$	Turnover time (d)	Annual leaf input ( $\text{g m}^{-2}$ )	% of leaf input assimilated	Reference
Payne Creek	16 $\pm$ 6	11.5	32	492 $\pm$ 19	5–14	Carter & Suberkropp (2004)
Hendrick Mill Branch	27 $\pm$ 9	20.8	18	422 $\pm$ 23	11–27	Methvin & Suberkropp (2003)
Basin Creek	32 $\pm$ 11	13.3	27	379 $\pm$ 26	14–35	Methvin & Suberkropp (2003)
Walker Branch	37 $\pm$ 8	8.2	44	460 (estimate)	13–33	Suberkropp (1997)
Lindsay Spring Branch	46 $\pm$ 25	13.0	28	478 $\pm$ 37	16–40	Carter & Suberkropp (2004)
Coweeta 53	193 $\pm$ 54	15.6	23	617–826	39–97	Suberkropp <i>et al.</i> (unpublished)

of leaf litter inputs. In contrast, in streams that retain leaf detritus more efficiently (e.g. Coweeta 53), fungi may assimilate as much as 39% to 97% of leaf litter inputs.

*Respiration* In studies examining leaf decomposition using litter bags or leaf packs, 17%–56% of the carbon loss from leaves has been found to be released as CO<sub>2</sub> by the microbial assemblages (Elwood *et al.*, 1981; Baldy & Gessner, 1997; Gulis & Suberkropp, 2003c). Both the whole-stream nutrient enrichment with nitrogen and/or phosphorus (Gulis & Suberkropp, 2003c; Ramírez *et al.*, 2003) and microcosm nutrient additions (Gulis & Suberkropp, 2003a, b) caused significant increases in the amount of leaf carbon lost through microbial respiration in comparison with detritus decomposing at ambient nutrient levels. In microcosms, fungi have been found to convert similar amounts of organic carbon into CO<sub>2</sub> to that observed for leaves colonized in streams, i.e. 14%–48% of the leaf carbon that is lost (Suberkropp, 1991; Gulis & Suberkropp, 2003b). Lower estimates have been reported for naturally decomposing leaves in two Alabama streams (Carter & Suberkropp 2004) where respiration by

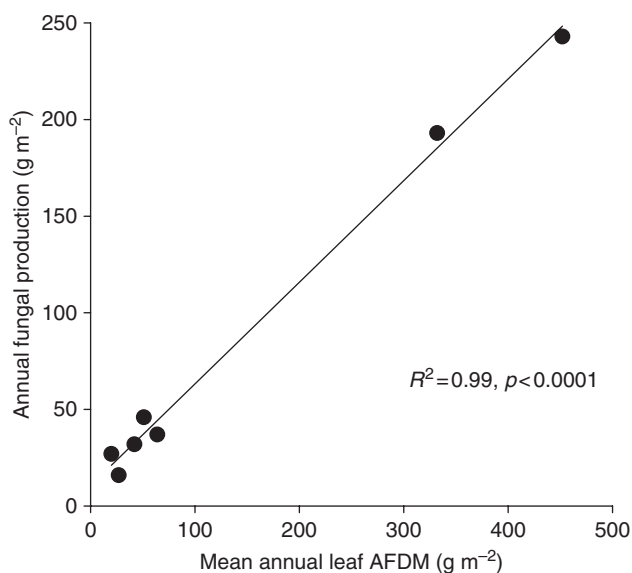


Fig. 17.3. Correlation between mean annual leaf litter standing stock and mean annual fungal production in streams. Based on data from Suberkropp (1997), Methvin and Suberkropp (2003), Carter and Suberkropp (2004) and Suberkropp *et al.* (unpublished).

microbial communities accounted for 7%–13% of the leaf litter input. However, both of these streams were not retentive and most of the leaf litter was thought to have been washed downstream before it decomposed.

Microbial respiration also accounts for a considerable mass loss of decomposing wood in streams (7%–44%, Collier & Smith, 2003). Respiration rates reported from submerged wood are generally lower than those from leaf litter if calculated per unit of mass (or volume) of detritus (Tank *et al.*, 1993; Fuss & Smock, 1996) because of lower surface to volume ratio of sticks versus leaves and fungal activity restricted mostly to the outer layers of wood. However, if expressed on a surface area basis, respiration rates from submerged wood are higher than those from leaves (Tank *et al.*, 1993; Fuss & Smock, 1996). Respiration rates from submerged decomposing thin wood veneers are comparable to those from leaf litter (Simon & Benfield, 2001; Stelzer *et al.*, 2003) and together with relatively high fungal biomass estimates (Simon & Benfield, 2001; Stelzer *et al.*, 2003; Gulis *et al.*, 2004) suggest that fungi are important players in the decomposition of submerged wood.

#### *Nitrogen cycle*

Fungi have lower C/N ratios than the substrates they colonize; consequently they should either retain substrate nitrogen more efficiently than carbon or acquire nitrogen from exogenous sources. Aquatic fungi are capable of utilizing nitrogen from both organic substrates and the overlying water (Suberkropp, 1995). These fungi are often nitrogen (and phosphorus) limited due to relatively low nitrogen concentrations of submerged substrates (leaf litter and especially wood) and also of water. The relative importance of each nitrogen source depends on organic substrate qualities and water chemistry.

*Nitrogen in water* Concentrations of dissolved inorganic nutrients (e.g. N and P) vary dramatically across aquatic ecosystems and, along with other factors, determine the level of fungal activity. The concentration of dissolved inorganic nitrogen in stream water (ammonium, nitrite and nitrate) depends on watershed characteristics, such as bedrock and soil chemical properties, land use, stream hydrology and biotic activity. Nitrate predominates in well-oxygenated waters while ammonium (and nitrite) concentrations can be high in anoxic waters, often as a result of human activities. Most aquatic hyphomycetes (and presumably ascomycetes) can use both organic and inorganic nitrogen (Thornton, 1963, 1965), while some chytrids and oomycetes are unable to utilize nitrate. Ammonium can be

assimilated directly while all other nitrogen sources should be first transformed into ammonium either with nitrate and nitrite reductases or through deamination.

There is a wealth of evidence that aquatic fungi obtain substantial amounts of their nitrogen from the water column. Fungal biomass accrual and sporulation of aquatic hyphomycetes are stimulated by dissolved nitrogen in both laboratory (Suberkropp, 1998a; Sridhar & Bärlocher, 2000; Bärlocher & Corkum, 2003) and field experiments (Fig. 17.4; Suberkropp & Chauvet, 1995; Tank & Dodds, 2003). Leaf litter nitrate uptake rates are correlated with decomposition rates, leaf toughness and respiration (Quinn *et al.*, 2000), which are functions of microbial activity. Increases in absolute nitrogen content of leaf litter have been reported in many studies (Hynes & Kaushik, 1969; Kaushik & Hynes, 1971; Suberkropp *et al.*, 1976; Triska & Sedell, 1976; Chauvet, 1987; Molinero *et al.*, 1996), suggesting that nitrogen is actively taken up from the water column by litter-inhabiting micro-organisms. Selective antibiotic experiments (Kaushik & Hynes, 1971) and laboratory studies with pure cultures growing on submerged wood (Gunasekera *et al.*, 1983) indicate that fungi are responsible for the majority of this uptake. Enrichment of stream water

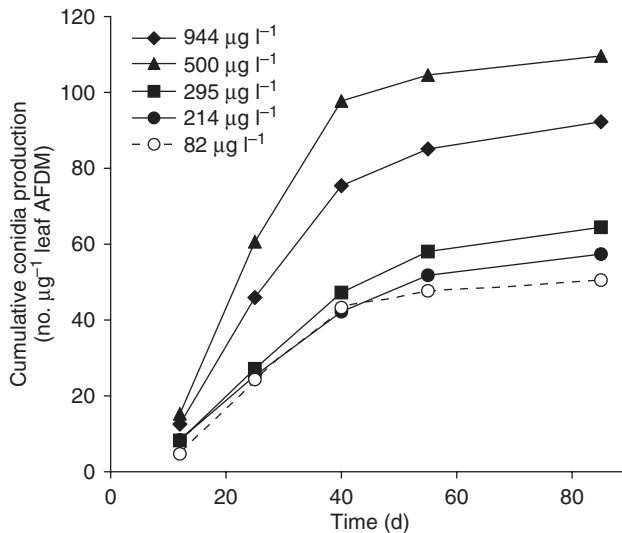


Fig. 17.4. The effect of whole-stream nitrate addition on sporulation of aquatic hyphomycetes on oak leaves. Average stream water  $\text{NO}_3\text{-N}$  concentrations at leaf bag stations during the study period are given (Ferreira, Gulis & Graça, unpublished).

with [ $^{15}\text{N}$ ]ammonium and subsequent comparison of  $\delta^{15}\text{N}$  values for bulk detritus and microbial nitrogen associated with decaying leaves and wood indicate microbial nitrogen immobilization (Mulholland *et al.*, 2000; Tank *et al.*, 2000; Sanzone *et al.*, 2001). Since fungi contribute 95% to over 99% of total microbial (fungi plus bacteria) biomass associated with decomposing leaf litter in streams (Baldy *et al.*, 1995; Hieber & Gessner, 2002; Gulis & Suberkropp, 2003c) it is likely that fungi, not bacteria, are largely responsible for the observed nitrogen immobilization.

On an ecosystem scale, Qualls (1984) estimated that 25% of dissolved inorganic nitrogen inflow can be immobilized by leaf litter in a blackwater swamp stream within a 1 km reach. Hamilton *et al.* (2001) found that 29% of stream water ammonium was taken up by heterotrophs associated with organic detritus. Estimates of the actively cycling fraction of nitrogen in leaves and small wood during  $^{15}\text{N}$  enrichments are almost identical to the measured microbial nitrogen fraction (Tank *et al.*, 2000; Hamilton *et al.*, 2001). Because of fungal dominance on coarse particulate organic matter in streams, these nitrogen transformations were likely to have been controlled by fungal activities.

Ample supply of nitrogen from stream water often leads to increases in microbial respiration (Stelzer *et al.*, 2003) and leaf litter decomposition (Fig. 17.5; Suberkropp & Chauvet, 1995; Hury *et al.*, 2002) that generally correlates with measures of fungal activity (Fig. 17.2, Gessner & Chauvet, 1994; Niyogi *et al.*, 2003). This stimulation of microbial carbon utilization demonstrates a tight coupling of carbon and nitrogen cycles in freshwater ecosystems. Consequently, eutrophication due to human activity may alter both nitrogen and carbon cycling within these habitats.

*Nitrogen in organic substrates* Initial nitrogen content was first suggested to affect microbially mediated leaf litter decomposition in water (Kaushik & Hynes, 1971). Among leaves of common riparian trees, alder leaves have a high nitrogen concentration because of nitrogen fixation by tree symbionts, decompose quickly and typically support high fungal biomass and conidia production of aquatic hyphomycetes soon after submergence (Gessner & Chauvet, 1994). However, it is apparent that leaf breakdown is also affected by fibre content, chemical inhibitors (phenolics) and physical barriers (Webster & Benfield, 1986). Gessner and Chauvet (1994) found that leaf litter decomposition rate, maximum fungal biomass, mycelial production and sporulation rate of aquatic hyphomycetes were negatively correlated with leaf litter initial lignin content but were not affected by nitrogen concentration. It appears that breakdown and fungal activity

depend on complex interplay of factors, e.g. lignin/nitrogen ratio as suggested by Melillo *et al.* (1982) for leaf litter and also external factors such as stream water nutrient concentrations (Gessner & Suberkropp, unpublished). A similar relationship was suggested for wood decomposing in streams (Melillo *et al.*, 1983). Decomposition rate of wood was also linked to the activity of enzymes involved in nutrient sequestration (Sinsabaugh *et al.*, 1993), which depends on nitrogen and phosphorus availability.

The nitrogen concentrations of autumn-shed leaves of deciduous trees vary between 0.5%–3% averaging around 1% (e.g. Gessner & Chauvet, 1994; Ostrofsky, 1997) while the nitrogen content of fungal mycelium is about 3%–10% (e.g. Paul & Clark, 1989; Högberg & Högberg, 2002). Since fungi can contribute up to 18%–23% of total detrital mass of submerged decaying leaves (Suberkropp, 1995; Methvin & Suberkropp, 2003), it is not surprising that leaf litter nitrogen concentration typically increases during decomposition and frequently doubles (e.g. Kaushik & Hynes, 1971; Suberkropp *et al.*, 1976). Increases in leaf litter nitrogen

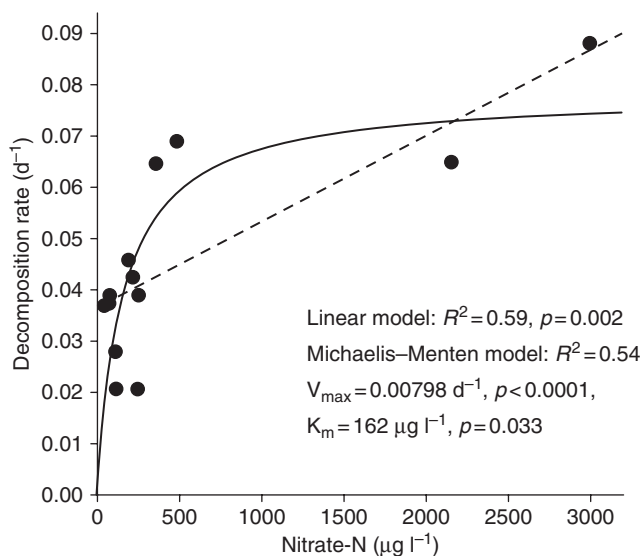


Fig. 17.5. Decomposition rates of alder leaf litter in streams differed in nitrate concentration in water. Both linear and Michaelis–Menten model  $V = (V_{\max} \cdot [S]) / (K_m + [S])$ , where (here)  $V_{\max}$  is the maximum decomposition rate,  $K_m$  is the nitrate concentration at which half rate of decomposition is achieved,  $[S]$  is nitrate concentration, gave fairly good fit (Gulis, Ferreira & Graça, unpublished).

content correlate well with fungal biomass accrual (Fig. 17.6). Rier *et al.* (2002) reported that fungal biomass was negatively correlated with detrital C/N ratio (i.e. inverse of %N) in a study comparing decomposition of leaf litter grown under ambient and CO<sub>2</sub>-enriched atmosphere and hence having different initial C/N ratios. Increases in nitrogen content during decomposition in streams also occur in wood (Sinsabaugh *et al.*, 1993; Gulis *et al.*, 2004). Overall, nitrogen increases are thought to coincide with increases in protein concentration due to fungal growth, thereby rendering plant litter a more palatable and nutritious food source for invertebrates (Kaushik & Hynes, 1971; Bärlocher, 1985; Suberkropp, 1992) and facilitating transfer of nutrients to higher trophic levels.

*Dissimilatory nitrogen transformations* Nitrification and denitrification are important processes of the global nitrogen cycle. Traditionally, bacteria are thought to be responsible for these transformations (including in aquatic ecosystems; Allan, 1995). However, nitrification has been reported for a wide range of terrestrial fungi (e.g. Falih & Wainwright, 1995) and fungi were confirmed to be capable of denitrification, reducing nitrate or nitrite to nitrous oxide or ultimately N<sub>2</sub> (e.g. Shoun *et al.*, 1992). Furthermore, fungi were found to dominate both microbial nitrification

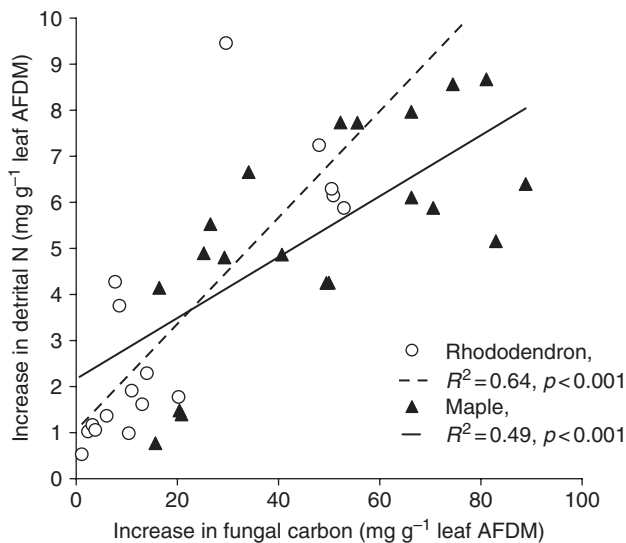


Fig. 17.6. The relationship between increases in fungal biomass and leaf litter nitrogen content of two species of leaves decomposing in a headwater stream. Based on data from Gulis and Suberkropp (2003c).

and denitrification in soil (Laughlin & Stevens, 2002). The potential for nitrification or denitrification has not been demonstrated specifically in aquatic fungi, but it is reasonable to expect that some species may be capable of denitrification, especially since it has been reported for *Fusarium*, *Cylindrocarpon* and *Nectria* (Hypocreales, Ascomycota) (Shoun *et al.*, 1992) and these genera are commonly found in aquatic environments. Low oxygen concentrations in benthic sediments are a prerequisite for denitrification by aquatic fungi. Recently, Guest and Smith (2002) proposed using fungi for biological nitrogen reduction of wastewater, because of their greater denitrification rate and other advantages over bacteria.

*Stoichiometric perspective* Even though carbon and nitrogen cycles in aquatic ecosystems are coupled, different stoichiometric ratios of water, detritus and consumers lead to different incorporation ratios of carbon and nitrogen (Frost *et al.*, 2002). Initial atomic C/N ratios were estimated to be around 20–96 for leaves entering aquatic ecosystems (calculated from %N in Melillo *et al.* (1982) and Gessner & Chauvet (1994); assumed carbon content of 50%) and 140–1100 for wood (Melillo *et al.*, 1984; Stelzer *et al.*, 2003). Carbon/nitrogen ratios have been estimated at 4.5–22 for terrestrial fungi (Paul & Clark, 1989; Högberg & Högberg, 2002; Wallander *et al.*, 2003, 2004) and 8–13 for stream fungi associated with leaf litter in two temperate streams (Sanzone *et al.*, 2001). Despite a certain plasticity of C/N ratios in microbes, considerable disparity between the elemental composition of the substrate and fungal mycelium would be likely to affect the growth rate of fungi. Thus, substrates with similar elemental composition to fungal biomass (i.e. lower C/N ratio or higher %N, see above) would support higher fungal activity. Elemental imbalance between the substrate and fungi can also be compensated by nitrogen uptake from the water column. Consequently, it is not surprising that the response of fungi (biomass, respiration and hence decomposition rate) to experimental nitrogen and phosphorus whole-stream enrichments was much higher for wood having an extremely high C/N ratio rather than for leaf litter (Stelzer *et al.*, 2003; Gulis *et al.*, 2004).

*Spiralling* In stream ecology, nutrient cycling is best described through the nutrient spiralling concept (Newbold *et al.*, 1981) that takes into account the unidirectional downstream movement of water in streams and rivers. It implies that an atom (e.g. nitrogen) is transported some distance downstream in an inorganic form before being incorporated into biota (which can also be transported downstream) and eventually released in inorganic

form. With respect to aquatic fungi, nitrogen assimilated at a given stream location can be transported downstream as mycelia growing within a substrate or as fungal propagules where fungal nitrogen can be transferred to higher trophic levels through invertebrate feeding, remain within the fungal compartment through mycelial growth or released as inorganic nitrogen. Notably, aquatic hyphomycetes can convert up to 80% of fungal production (or 8%–12% of leaf mass loss) into conidia (Suberkropp, 1991) that are transported downstream. Aquatic hyphomycetes also contribute to maceration of leaf litter (Suberkropp & Klug, 1980) and generation of dissolved and fine particulate organic matter and, consequently, facilitate in the transport of nutrients downstream. On the other hand, fungal growth on high C/N substrates and nitrogen immobilization from the overlying water column may affect dissolved nitrogen uptake length and nitrogen recycling.

### Freshwater Wetlands

Wetlands have been described as among the most biologically diverse and productive ecosystems on earth. These ecosystems represent a unique transition between the terrestrial and aquatic environment, have extensive food webs and provide a variety of habitats for a wide diversity of flora and fauna (Mitsch & Gosselink, 2000). A distinguishing feature of many freshwater wetlands and lake littoral zones is the presence of emergent vascular plants, such as *Phragmites*, *Typha* and *Juncus*. These plants frequently constitute a major fraction of the organic matter produced in wetlands, with estimates of annual net above-ground primary production often exceeding  $2 \text{ kg m}^{-2} \text{ yr}^{-1}$  (Wetzel & Howe, 1999; Mitsch & Gosselink, 2000).

Very little of the living plant biomass is consumed by herbivores during the growing season (Dvorák & Imhof, 1998), since much of the carbon within macrophyte tissues resides in the structural material of cell walls (i.e. lignocellulose), which is not readily digested and assimilated by animal consumers (Mann, 1988; Kreeger & Newell, 2000). As a consequence, most of the plant matter produced eventually enters the detrital pool following plant senescence and death, where it is transformed by microbial decomposers (bacteria and fungi) and detritivore consumers (invertebrates). Microbial assemblages, particularly fungi, associated with decaying wetland plant litter are recognized as an important food source for detritus feeding consumers (Newell & Bärlocher, 1993; Bärlocher & Newell, 1994; Graça *et al.*, 2000; Newell & Porter, 2000; Silliman & Newell, 2003), thereby serving as important intermediaries in the flow of both carbon and nutrients

to higher trophic levels. Similar to the stream systems described above, many characteristics related to nutrient cycling and energy flow in wetland ecosystems are regulated by the metabolic activities of microbial assemblages associated with this decaying plant matter.

Despite the overwhelming importance of plant detritus in wetlands, plant decomposition and the role of associated micro-organisms, particularly fungi, is a seldom appreciated or investigated component of wetland processes (Newell, 1993). Previous studies of emergent plant decomposition in wetlands have often focused on litter that had been prematurely harvested and placed at or buried within the surface sediments (e.g. Moran *et al.*, 1989; Thormann *et al.*, 2001). As a result, litter decomposition was thought to be (1) primarily restricted to the sediment–water interface and (2) predominantly mediated by sediment-associated bacterial assemblages (Moran *et al.*, 1988). However, in many emergent macrophytes the collapse of above-ground plant matter and incorporation of litter into the sediments does not typically occur following senescence and death of the plant shoot (Newell, 1993). Much of the dead plant litter remains attached to the parent plant in an aerial standing-dead position for extended periods of time, where it undergoes initial microbial decay prior to its entry into the aquatic environment (Newell, 1993; Newell *et al.*, 1995; Bärlocher & Biddiscombe, 1996; Kuehn & Suberkropp, 1998a; Kuehn *et al.*, 1999; Gessner, 2001; Findlay *et al.*, 2002a; Welsch & Yavitt, 2003).

Fungi extensively colonize and reproduce on and within standing and submerged litter of emergent macrophytes. For example, over 600 species of fungi have now been recorded from *Phragmites australis* (e.g. Gessner & van Ryckegem, 2002). However, despite the wealth of qualitative evidence showing extensive fungal colonization, few investigators have attempted to quantify the role of fungi in litter decay in freshwater wetlands (Findlay *et al.*, 1990, 2002a; Newell *et al.*, 1995; Kuehn & Suberkropp, 1998a; Kuehn *et al.*, 1999, 2000; Gessner, 2001; Newell, 2003; Welsch & Yavitt, 2003) or their overall contribution to total ecosystem metabolism (Kuehn & Suberkropp, 1998b; Kuehn *et al.*, 2004).

### *Carbon cycle*

*Fungal biomass and production* Recent studies conducted in subtropical and temperate freshwater wetlands provide compelling evidence that fungal participation in the aerial standing-dead litter phase can contribute significantly to overall carbon and nutrient cycling in wetlands (Kuehn & Suberkropp, 1998a, b; Kuehn *et al.*, 1999). Kuehn and Suberkropp (1998a)

reported that living biomass of fungi associated with standing leaf litter of the rush, *Juncus effusus*, accounts for *c.* 5% of the total detrital mass. Once established, a relatively constant level of living fungal biomass was maintained for over 800 days and over 30 species of fungi were identified from standing *J. effusus* litter. Seasonal estimates of above-ground detrital mass of *J. effusus* at the wetland study site (Wetzel & Howe, 1999) ranged from 0.6 to 2.3 kg AFDM m<sup>-2</sup> (Fig. 17.7a). When integrated on an areal basis, fungal biomass associated with standing *J. effusus* litter ranged from 24 to 116 g m<sup>-2</sup> (Fig. 17.7b) emphasizing the quantitative significance of fungal decomposers in this ecosystem. Likewise, when viewed on a whole ecosystem scale, mean annual living fungal biomass within this relatively small wetland (15 ha, *J. effusus* 64.8% coverage) equals 2–11 tons.

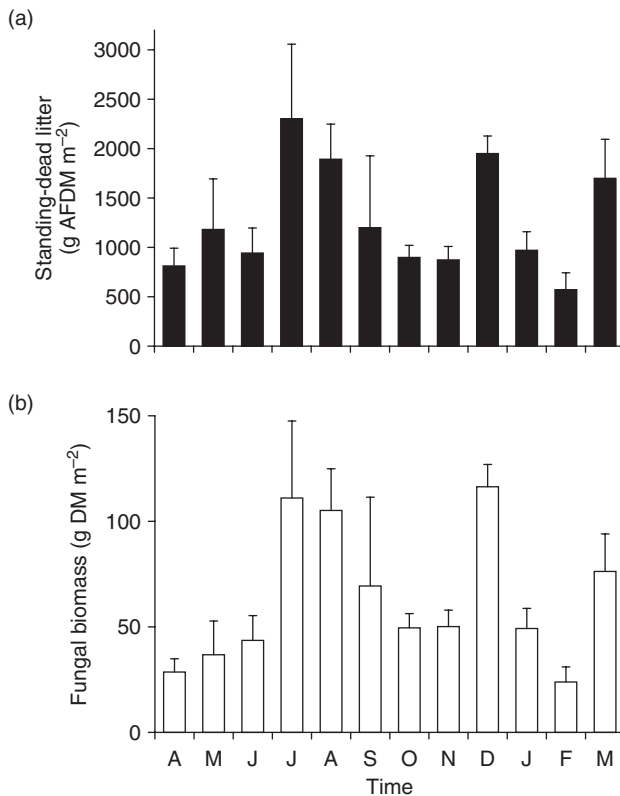


Fig. 17.7. Seasonal estimates of standing-dead litter of *J. effusus* (a) and litter-associated fungal biomass (b) at the Talladega Wetland Ecosystem, Alabama. Data from Wetzel and Howe (1999) and Kuehn and Suberkropp (1998a). Vertical lines indicate +1 SE (n = 6).

In addition to accumulating large quantities of biomass in decaying standing litter, fungal decomposers also exhibit high rates of biomass production. Newell *et al.* (1995) reported that microbial biomass and production associated with naturally standing and fallen litter of the freshwater sedge, *Carex walteriana*, were dominated by fungal decomposers, with bacterial biomass and production often accounting for less than 0.5% that of fungi. Rates of fungal biomass production associated with standing-dead litter of *J. effusus* accounted for >94% of the total microbial production, averaging  $42 \mu\text{g C g}^{-1} \text{ AFDM h}^{-1}$  (Kuehn *et al.*, 2000). Microbial biomass and rates of biomass production were also dominated by fungi (>99% of the total microbial production) in both standing and submerged litter of *Typha angustifolia* and *P. australis* in a tidal freshwater wetland of the Hudson River (Findlay *et al.*, 2002a).

The collapse of emergent plant litter into the water often leads to fungal succession and distinct changes in the biomass and activity of associated fungi. Kuehn *et al.* (2000) reported that fungal and bacterial biomass and production decrease rapidly following submergence of *J. effusus* litter, suggesting that the resident microbiota associated with decaying standing litter could not adapt to or survive the abrupt changes in conditions from an aerial to an aquatic environment. The initial decline is typically followed by an increase in fungal biomass and production during later stages of submerged decomposition. Similar changes in the activities of microbial assemblages were observed following submergence of standing-dead *P. australis* litter in a temperate lake littoral wetland (Kominková *et al.*, 2000).

After submergence of *J. effusus* litter and decreases in litter-associated microbial biomass, fungal decomposers continued to comprise the major microbial assemblage on/within decaying litter (Kuehn *et al.*, 2000). Estimates of fungal biomass and production greatly exceeded corresponding estimates of bacterial biomass and production throughout submerged litter decay. The comparison of the contribution of fungi and bacteria to carbon loss of *J. effusus* litter under submerged conditions (Table 17.3) indicates that fungi could explain a substantial portion (68%) of the litter mass loss observed. Similar findings of fungal dominance in other freshwater wetlands (Findlay *et al.*, 1990, 2002a; Newell *et al.*, 1995; Sinsabaugh & Findlay, 1995) suggest that fungi are an important microbial assemblage involved in submerged macrophyte decay. These findings contrast sharply with previous studies that have reported a more predominant role of bacteria in wetland carbon cycling (Benner *et al.*, 1986; Moran *et al.*, 1988; Buesing, 2002).

Table 17.3. Net production, production to biomass ( $P/B$ ) ratio, turnover time and contribution of fungal and bacterial decomposers to submerged *J. effusus* litter decay (from Kuehn et al., 2000)

Parameter	Fungi	Bacteria
Total net production (mg C g <sup>-1</sup> initial leaf C)	44	3
Mean biomass (mg C g <sup>-1</sup> initial leaf C)	16	0.2
P/B ratio	2.8	15
Turnover (d)	68	13
% of initial leaf C assimilated	13	2
% contribution to overall leaf C loss	68	11

*Respiration* Fungi associated with standing and submerged plant litter convert a considerable portion of the plant carbon into CO<sub>2</sub> as a result of their respiration. The microbiota, particularly fungi, associated with standing-dead wetland plant litter are well adapted to the fluctuating environmental conditions of the aerial habitat (Kuehn & Suberkropp, 1998b; Kuehn et al., 1998, 1999; Kuehn et al., 2004). Rates of microbial respiration (CO<sub>2</sub> evolution) from standing-dead litter exhibit pronounced diel periodicity (Fig. 17.8) and are positively correlated with night-time increases in plant litter water potentials (Kuehn & Suberkropp, 1998b; Kuehn et al., 2004). Temperature-driven increases in relative humidity and subsequent dew formation is the primary mechanism underlying night-time increases in water availability and microbial activities within standing-dead litter. In contrast, respiratory activities virtually cease during the day as a result of increased desiccation stress.

Large differences can occur among plant litter types (species and organ) in terms of microbial colonization and metabolic response of microorganisms to water availability. Microbial respiration rates associated with different *P. australis* litter fractions vary considerably (Kuehn et al., 2004). Maximum respiration rates from leaf blades were higher (24%–42%) than those from sheath litter under the same conditions (Fig. 17.8a) while maximum respiration rates from culm litter were consistently an order of magnitude lower than rates from both leaf and sheath litter. The observed differences in rates of respiration among *P. australis* litter fractions were consistent with differences in litter water absorption patterns, known structural characteristics among litter fractions (e.g. lignocellulose) and degree of fungal colonization (Fig. 17.9, Kuehn et al., 2004). Microbial respiration correlates well with litter-associated fungal biomass (ergosterol) (Fig. 17.9), providing convincing evidence that ergosterol is a good indicator of living

fungus mass (but see Mille-Lindblom *et al.*, 2004) and that fungi are likely to be responsible for most of the respiratory carbon release from standing litter in wetland habitats.

A rough budget also suggests that a considerable portion of plant carbon is likely to be converted into  $\text{CO}_2$  under standing-dead conditions (Table 17.4). Only a small portion of the  $\text{CO}_2$  flux from standing litter is due to wetting via precipitation, with most being accounted for by recurring night-time dew formation. In contrast to *P. australis* leaf and sheath litter, very little culm material is degraded under standing-dead conditions, perhaps because culms of *Phragmites* contain more recalcitrant and water-repellent tissues (Rodewald-Rudescu, 1974) and have low concentrations of fungal biomass. Hence, culm material appears to undergo more extensive microbial decay once shoots have collapsed to the sediments or water.

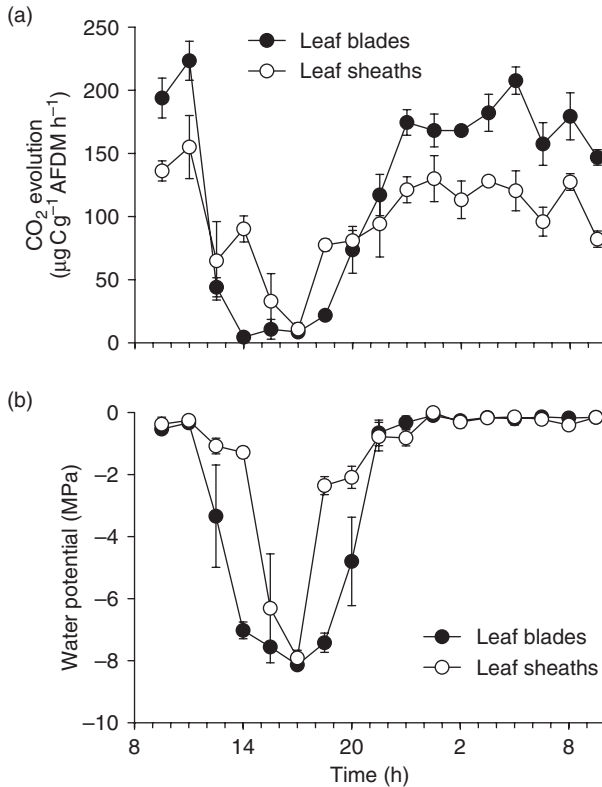


Fig. 17.8. Diel changes in (a) rates of  $\text{CO}_2$  evolution from standing-dead leaf and sheath litter of *P. australis* and (b) plant litter water potential during field studies conducted in a littoral reed stand of Lake Hallwil, Switzerland. Data from Kuehn *et al.* (2004). Symbols indicate means  $\pm 1$  SE ( $n = 3$ ).

How common is the diel pattern in fungal respiration and what are the potential implications for wetland carbon cycling on an ecosystem scale? Remarkably similar diel patterns in respiration have been reported from standing-dead *J. effusus* litter in Alabama, USA (Kuehn & Suberkropp, 1998b) and *P. australis* litter from a temperate littoral reed stand in Switzerland (Kuehn *et al.*, 2004), indicating that this phenomenon is not restricted to subtropical regions, but is common even at northern latitudes where most wetlands occur on a global scale. When integrated on an areal and temporal basis, diel fluctuations in microbial respiration rates are a potentially significant source of CO<sub>2</sub> from wetlands and may represent a pathway of carbon flow that has gone largely unnoticed in prior chamber-based estimates and models of total wetland CO<sub>2</sub> flux (Yavitt, 1997; Updegraff *et al.*, 2001; Chimner *et al.*, 2002; Larmola *et al.*, 2003). Kuehn and Suberkropp (1998b) estimated daily fluxes of 1.37 to 3.35 g C m<sup>-2</sup> d<sup>-1</sup> from microbial (fungal) assemblages inhabiting standing *J. effusus*, which were equal to or exceeded sediment CO<sub>2</sub> flux rates from the same wetland site (0.12 to 2.43 g C m<sup>-2</sup> d<sup>-1</sup>; Roden & Wetzel, 1996). Carbon dioxide flux rates reported for microbial assemblages inhabiting standing *P. australis* litter (Kuehn *et al.*, 2004) were lower

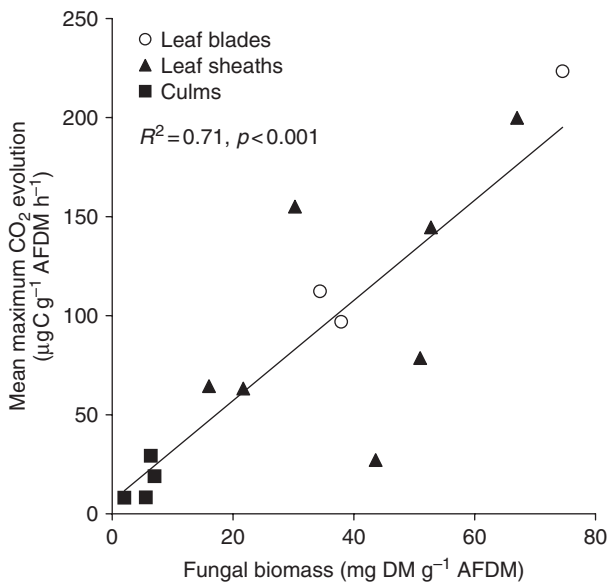


Fig. 17.9. The effect of litter-associated fungal biomass on the mean maximum rate of CO<sub>2</sub> evolution from standing-dead *P. australis* leaf litter. Data from Kuehn *et al.* (2004).

Table 17.4. Estimate of the annual above-ground production of *J. effusus* and *P. australis* that is lost as  $\text{CO}_2$  due to microbial, primarily fungal, respiration while in the standing-dead litter phase. Modified from Kuehn et al. (2004). Data for *J. effusus* were taken from Kuehn and Suberkropp (1998b)

Site	Plant	Plant organ	Plant production ( $\text{g C m}^{-2} \text{ yr}^{-1}$ )	Microbial respiration ( $\text{g C m}^{-2} \text{ yr}^{-1}$ )	Lost due to microbial respiration (%)	
					Total	During rainfall
Talladega, Alabama Neuchatel, Switzerland	<i>J. effusus</i>	Leaves	2086*	579	27.7	0.8
		Leaves	272	23	8.5	1.0
		Sheaths	191	57	29.8	5.7
Hallwil, Switzerland	<i>P. australis</i>	Culms	631	13	2.0	0.4
		Leaves	193	15	7.8	1.2
		Sheaths	88	25	28.0	3.9
		Culms	308	11	3.6	0.6

\*Estimates of standing-dead biomass for *J. effusus* were taken from Wetzel and Howe (1999).

(0.05–0.57 g C m<sup>-2</sup> d<sup>-1</sup>) but still within the range of CO<sub>2</sub> flux estimates reported for wetland sediments in northern temperate climates (e.g. Scanlon & Moore, 2000).

Under submerged conditions, rates of microbial respiration associated with macrophyte litter are similar to maximum rates reported above for standing-dead litter under water-saturating conditions. Komínková *et al.* (2000) reported respiration rates from submerged *P. australis* leaves ranging from 29 to 127 µg CO<sub>2</sub>-C g<sup>-1</sup> AFDM h<sup>-1</sup>, with fluctuations in rates following changes in lake water temperature. In addition, as observed in standing litter, significant differences in fungal biomass and rates of respiration were also noted between *P. australis* leaf and culm litter, with submerged culm litter having considerably lower fungal biomass and rates of respiration than corresponding leaf litter. These comparisons with respiration rates from standing litter reveal that microbial respiration associated with submerged emergent macrophyte litter can also be a sizeable contribution. A partial decomposition budget of submerged *P. australis* leaves (Komínková *et al.*, 2000), indicated that *c.* 65% of detrital carbon loss could be accounted for by microbial respiration. It is likely that a sizeable portion of this respiratory carbon loss was due to the metabolic activities of fungal decomposers, since total litter-associated microbial biomass was predominantly fungal (>90%).

#### *Nitrogen cycle*

Research examining the quantitative contribution of microorganisms to total detrital nitrogen pools in freshwater wetlands is limited. Previous studies have suggested that litter-inhabiting microbial assemblages (bacteria and fungi) account for only a minor fraction of the total nitrogen content in decaying plant matter (Mann, 1988). However, these conclusions were often based on methods that underestimated microbial biomass within decaying plant litter, particularly fungal. Studies by Newell (1993 and references therein), using improved methods for estimating fungal biomass (i.e. immunoassay and ergosterol), were among the first to recognize that fungi could account for a substantial portion of the total plant litter nitrogen. Fungal assemblages (mainly ascomycetes) associated with standing-dead litter of the saltmarsh cordgrass, *Spartina alterniflora*, immobilized nearly all (99%) of the available litter nitrogen (assuming 4% N in fungal biomass, see Newell & Statzell-Tallman, 1982). Similar studies in freshwater wetlands reveal that fungal contributions to total detrital nitrogen can also be significant (Newell *et al.*, 1995; Kuehn & Suberkropp, 1998a; Kuehn

*et al.*, 2000; Findlay *et al.*, 2002a); however, estimates are considerably lower than those obtained from saltmarsh systems.

Newell *et al.* (1995) reported that living fungal biomass accounted for 6%–35% of the total nitrogen in decaying standing and fallen litter of the freshwater sedge *C. walteriana*. Similar findings were reported for standing and submerged litter of *J. effusus* (Kuehn & Suberkropp, 1998a; Kuehn *et al.*, 2000), *T. angustifolia* and *P. australis* (Findlay *et al.*, 2002a), with fungal nitrogen accounting for as much as 40% of the total litter nitrogen content. However, note that in these studies, fungal contribution to total detrital nitrogen reflected only estimates of nitrogen within living fungal biomass and thus may be an underestimate. Residual non-living fungal biomass within decaying litter (e.g. N-acetylglucosamine) may account for a substantial portion of the total detrital nitrogen. Total fungal biomass (living plus dead hyphae) may be two-fold greater than living fungal biomass alone (Newell & Porter, 2000).

### Conclusions

In freshwater ecosystems where fungi have been studied, particularly in plant litter decomposition subsystems, they have been found to play key roles both in the cycling of carbon and nitrogen and in mediating energy flow to higher trophic levels. Fungi undoubtedly have a number of other roles in these ecosystems, as pathogens, symbionts and decomposers of other types of organic matter. As methods are developed, adapted and applied to other subsystems of aquatic ecosystems and particularly when fungal activities become amenable to quantification, the roles played by fungi in aquatic ecosystems will become better understood.

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### References

- Allan, J. D. (1995). *Stream ecology: Structure and Function of Running Waters*. London: Chapman & Hall.
- Arsuffi, T. L. & Suberkropp, K. (1986). Growth of two stream caddisflies (Trichoptera) on leaves colonized by different fungal species. *Journal of the North American Benthological Society*, **5**, 297–305.

- Baldy, V. & Gessner, M. O. (1997). Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus de l'Academie des Sciences – Series III – Sciences de la Vie*, **320**, 747–58.
- Baldy, V., Gessner, M. O. & Chauvet, E. (1995). Bacteria, fungi and the breakdown of leaf litter in a large river. *Oikos*, **74**, 93–102.
- Baldy, V., Chauvet, E., Charcosset, J. Y. & Gessner, M. O. (2002). Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. *Aquatic Microbial Ecology*, **28**, 25–36.
- Bärlocher, F. (1985). The role of fungi in the nutrition of stream invertebrates. *Botanical Journal of the Linnean Society*, **91**, 83–94.
- (2000). Water-borne conidia of aquatic hyphomycetes: seasonal and yearly patterns in Catamaran Brook, New Brunswick, Canada. *Canadian Journal of Botany*, **78**, 157–67.
- Bärlocher, F. & Biddiscombe, N. R. (1996). Geratology and decomposition of *Typha latifolia* and *Lythrum salicaria* in a freshwater marsh. *Archiv für Hydrobiologie*, **136**, 309–25.
- Bärlocher, F. & Corkum, M. (2003). Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. *Oikos*, **101**, 247–52.
- Bärlocher, F. & Kendrick, B. (1973a). Fungi and food preferences of *Gammarus pseudolimnaeus*. *Archiv für Hydrobiologie*, **72**, 501–16.
- (1973b). Fungi in the diet of *Gammarus pseudolimnaeus* (Amphipoda). *Oikos*, **24**, 295–300.
- Bärlocher, F. & Newell, S. Y. (1994). Growth of the saltmarsh periwinkle *Littoraria irrorata* on fungal and cordgrass diets. *Marine Biology*, **118**, 109–14.
- Benner, R., Moran, M. A. & Hodson, R. E. (1986). Biochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of prokaryotes and eukaryotes. *Limnology and Oceanography*, **21**, 89–100.
- Buesing, N. (2002). Microbial productivity and organic matter flow in a littoral reed stand. Unpublished Ph.D. thesis, Swiss Federal Institute of Technology, Zurich, Switzerland.
- Carter, M. D. & Suberkropp, K. (2004). Respiration and annual fungal production associated with decomposing leaf litter in two streams. *Freshwater Biology*, **49**, 1112–22.
- Chamier, A. C. (1985). Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. *Botanical Journal of the Linnean Society*, **91**, 67–81.
- Chamier, A. C. & Dixon, P. A. (1982). Pectinases in leaf degradation by aquatic hyphomycetes: the enzymes and leaf maceration. *Journal of General Microbiology*, **128**, 2469–83.
- Chauvet, E. (1987). Changes in the chemical composition of alder, poplar and willow leaves during decomposition in a river. *Hydrobiologia*, **148**, 35–44.
- Chimner, R. A., Cooper, D. J. & Parton, W. J. (2002). Modelling carbon accumulation in Rocky Mountain fens. *Wetlands*, **22**, 100–10.
- Collier, K. J. & Smith, B. J. (2003). Corrigendum to 'Role of wood in pumice-bed streams II: breakdown and colonization' [For. Ecol. Manage. 177 (2003) 261–276]. *Forest Ecology and Management*, **181**, 375–90.
- Dvorák, J. & Imhof, G. (1998). The role of animals and animal communities in wetlands. In *The Production Ecology of Wetlands*, ed. D. F. Westlake, J. Květ & A. Szezepanski. Cambridge: Cambridge University Press, pp. 211–318.
- Elwood, J. W., Newbold, J. D., Trimble, A. F. & Stark, R. W. (1981). The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. *Ecology*, **62**, 146–58.
- Falih, A. M. K. & Wainwright, M. (1995). Nitrification in-vitro by a range of filamentous fungi and yeasts. *Letters in Applied Microbiology*, **21**, 18–19.
- Findlay, S., Howe, K. & Austin, H. K. (1990). Comparison of detritus dynamics in two tidal freshwater wetlands. *Ecology*, **71**, 288–95.

- Findlay, S. E. G., Dye, S. & Kuehn, K. A. (2002a). Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. *Wetlands*, **22**, 616–25.
- Findlay, S., Tank, J., Dye, S. *et al.* (2002b). A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology*, **43**, 55–66.
- Fisher, S. G. & Likens, G. E. (1972). Stream ecosystem: organic energy budget. *Bioscience*, **22**, 33–5.
- Frost, P. C., Stelzer, R. S., Lamberti, G. A. & Elser, J. J. (2002). Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C:N:P ratios in lentic and lotic habitats. *Journal of the North American Benthological Society*, **21**, 515–28.
- Fuss, C. L. & Smock, L. A. (1996). Spatial and temporal variation of microbial respiration rates in a blackwater stream. *Freshwater Biology*, **36**, 339–49.
- Gessner, M. O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early decay in a standing position. *Aquatic Botany*, **69**, 325–39.
- Gessner, M. O. & Chauvet, E. (1994). Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, **75**, 1807–17.
- (1997). Growth and production of aquatic hyphomycetes in decomposing leaf litter. *Limnology and Oceanography*, **42**, 496–595.
- Gessner, M. O. & Newell, S. Y. (2002). Biomass, growth rate, and production of filamentous fungi in plant litter. In *Manual of Environmental Microbiology*, 2nd edn, ed. C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney & L. D. Stetzenbach. Washington, DC: ASM Press, pp. 390–408.
- Gessner, M. O. & van Ryckegem, G. (2002). Water fungi as decomposers in freshwater ecosystems. In *Encyclopedia of Environmental Microbiology*, ed. G. Bitton. New York: Wiley & Sons, pp. 3353–64.
- Gessner, M. O., Suberkropp, K. & Chauvet, E. (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. In *The Mycota*, Vol. IV. *Environmental and Microbial Relationships*, ed. D. T. Wicklow & B. E. Söderström. Berlin: Springer-Verlag, pp. 303–22.
- Graça, M. A. S., Maltby, L. & Calow, P. (1993). Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus* II. Effects on growth, reproduction and physiology. *Oecologia*, **96**, 304–9.
- Graça, M. A., Newell, S. Y. & Kneib, R. T. (2000). Grazing rates of organic matter and living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh invertebrates. *Marine Biology*, **136**, 281–9.
- Grattan, R. M. & Suberkropp, K. (2001). Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of the North American Benthological Society*, **20**, 33–43.
- Guest, R. K. & Smith, D. W. (2002). A potential new role for fungi in wastewater MBR biological nitrogen reduction system. *Journal of Environmental Engineering and Science*, **1**, 433–7.
- Gulis, V. & Suberkropp, K. (2003a). Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology*, **45**, 11–19.
- (2003b). Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquatic Microbial Ecology*, **30**, 149–57.
- (2003c). Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, **48**, 123–34.
- (2004). Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. *Mycologia*, **96**, 57–65.

- Gulis, V., Rosemond, A. D., Suberkropp, K., Weyers, H. S. & Benstead, J. P. (2004). Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. *Freshwater Biology*, **49**, 1437–47.
- Gunasekera, S. A., Webster, J. & Legg, C. J. (1983). Effect of nitrate and phosphate on weight losses of pine and oak wood caused by aquatic and aero-aquatic hyphomycetes. *Transactions of the British Mycological Society*, **80**, 507–14.
- Hamilton, S. K., Tank, J. L., Raikow, D. F. *et al.* (2001). Nitrogen uptake and transformation in a midwestern US stream: a stable isotope enrichment study. *Biogeochemistry*, **54**, 297–340.
- Hieber, M. & Gessner, M. O. (2002). Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026–38.
- Högberg, M. N. & Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, **154**, 791–5.
- Huryn, A. D., Butz Huryn, V. M., Arbuckle, C. J. & Tsomides, L. (2002). Catchment land-use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshwater Biology*, **47**, 401–15.
- Hynes, H. B. N. & Kaushik, N. K. (1969). The relationship between dissolved nutrient salts and protein production in submerged autumnal leaves. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **17**, 95–103.
- Iqbal, S. H. & Webster, J. (1973). Aquatic hyphomycete spora of the River Exe and its tributaries. *Transactions of the British Mycological Society*, **61**, 331–46.
- Kaushik, N. K. & Hynes, H. B. N. (1971). The fate of dead leaves that fall into streams. *Archiv für Hydrobiologie*, **68**, 465–515.
- Komínková, D., Kuehn, K. A., Büsing, N., Steiner, D. & Gessner, M. O. (2000). Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. *Aquatic Microbial Ecology*, **22**, 271–82.
- Kreeger, D. A. & Newell, R. I. E. (2000). Trophic complexity between producers and invertebrate consumers in salt marshes. In *Concepts and Controversies in Tidal Marsh Ecology*, ed. P. Weinstein & D. A. Kreeger. Dordrecht: Kluwer Academic Publishers, pp. 187–220.
- Kuehn, K. A. & Suberkropp, K. (1998a). Decomposition of standing litter of the freshwater macrophyte *Juncus effusus* L. *Freshwater Biology*, **40**, 717–27.
- (1998b). Diel fluctuations in microbial activity associated with standing-dead litter of the freshwater emergent macrophyte *Juncus effusus*. *Aquatic Microbial Ecology*, **14**, 171–82.
- Kuehn, K. A., Churchill, P. F. & Suberkropp, K. (1998). Osmoregulatory strategies of fungal populations inhabiting standing dead litter of the emergent macrophyte *Juncus effusus*. *Applied and Environmental Microbiology*, **64**, 607–12.
- Kuehn, K. A., Gessner, M. O., Wetzel, R. G. & Suberkropp, K. (1999). Standing litter decomposition of the emergent macrophyte *Erianthus giganteus*. *Microbial Ecology*, **38**, 50–7.
- Kuehn, K. A., Lemke, M. J., Suberkropp, K. & Wetzel, R. G. (2000). Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*. *Limnology and Oceanography*, **45**, 862–70.
- Kuehn, K. A., Steiner, D. & Gessner, M. O. (2004). Diel mineralization patterns of standing-dead plant litter: implications for CO<sub>2</sub> flux from wetlands. *Ecology*, **85**, 2504–18.
- Larmola, T., Alm, J., Juutinen, S., Martikainen, P. J. & Silvola, J. (2003). Ecosystem CO<sub>2</sub> exchange and plant biomass in the littoral zone of a boreal eutrophic lake. *Freshwater Biology*, **48**, 1295–310.

- Laughlin, R. J. & Stevens, R. J. (2002). Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. *Soil Science Society of America Journal*, **66**, 1540–8.
- Mann, K. H. (1988). Production and use of detritus in various freshwater, estuarine and coastal marine ecosystems. *Limnology and Oceanography*, **33**, 910–30.
- Melillo, J. M., Aber, J. D. & Muratore, J. F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, **63**, 621–6.
- Melillo, J. M., Naiman, R. J., Aber, J. D. & Eshleman, K. N. (1983). The influence of substrate quality and stream size on wood decomposition dynamics. *Oecologia*, **58**, 281–5.
- Melillo, J. M., Naiman, R. J., Aber, J. D. & Linkins, A. E. (1984). Factors controlling mass-loss and nitrogen dynamics of plant litter decaying in northern streams. *Bulletin of Marine Science*, **35**, 341–56.
- Methvin, B. R. & Suberkropp, K. (2003). Annual production of leaf-decaying fungi in 2 streams. *Journal of the North American Benthological Society*, **22**, 554–64.
- Mille-Lindblom, C., von Wachenfeldt, E. & Tranvik, L. J. (2004). Ergostrol as a measure of living fungal biomass: persistence in environmental samples after fungal death. *Journal of Microbiological Methods*, **59**, 253–62.
- Mitsch, W. J. & Gosselink, J. G. (2000). *Wetlands*, 3rd edn. New York: Wiley & Sons.
- Moliner, J., Pozo, J. & Gonzalez, E. (1996). Litter breakdown in streams of the Agüera catchment: Influence of dissolved nutrients and land use. *Freshwater Biology*, **36**, 745–56.
- Moran, M. A., Legovic, T., Benner, R. & Hodson, R. E. (1988). Carbon flow from lignocellulose: a simulation analysis of a detritus-based ecosystem. *Ecology*, **69**, 1525–36.
- Moran, M. A., Benner, R. & Hodson, R. E. (1989). Kinetics of microbial degradation of vascular plant material in two wetland ecosystems. *Oecologia*, **79**, 158–67.
- Mulholland, P. J., Tank, J. L., Sanzone, D. M. *et al.* (2000). Nitrogen cycling in a forest stream determined by a N-15 tracer addition. *Ecological Monographs*, **70**, 471–93.
- Newbold, J. D., Elwood, J. W., O'Neill, R. V. & van Winkle, W. (1981). Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 860–3.
- Newell, S. Y. (1993). Decomposition of shoots of a saltmarsh grass: methodology and dynamics of microbial assemblages. *Advances in Microbial Ecology*, **13**, 301–26.
- (2003). Fungal content and activities in standing-decaying leaf blades of plants of the Georgia Coastal Ecosystem research area. *Aquatic Microbial Ecology*, **32**, 95–103.
- Newell, S. Y. & Bärlocher, F. (1993). Removal of fungal and total organic matter from decaying cordgrass leaves by shredder snails. *Journal of Experimental Marine Biology and Ecology*, **171**, 39–49.
- Newell, S. Y. & Fallon, R. D. (1991). Toward a method for measuring instantaneous fungal growth rates in field samples. *Ecology*, **72**, 1547–59.
- Newell, S. Y. & Porter, D. (2000). Microbial secondary production from salt marsh-grass shoots, and its known potential fates. In *Concepts and Controversies in Tidal Marsh Ecology*, ed. M. P. Weinstein & D. A. Kreeger. Dordrecht: Kluwer Academic Publishers, pp. 159–86.
- Newell, S. Y. & Statzell-Tallman, A. (1982). Factors for conversion of fungal biovolume values to biomass, carbon and nitrogen: variation with mycelial ages, growth conditions, and strains of fungi from a salt marsh. *Oikos*, **39**, 261–8.
- Newell, S. Y., Moran, M. A., Wicks, R. & Hodson, R. E. (1995). Productivities of microbial decomposers during early stages of decomposition of leaves of a freshwater sedge. *Freshwater Biology*, **34**, 135–48.
- Niyogi, D. K., Simon, K. S. & Townsend, C. R. (2003). Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand. *Freshwater Biology*, **48**, 1698–708.

- Ostrofsky, M. L. (1997). Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society*, **16**, 750–9.
- Paul, E. A. & Clark, F. E. (1989). *Soil Microbiology and Biochemistry*. San Diego: Academic Press.
- Qualls, R. G. (1984). The role of leaf litter nitrogen immobilization in the nitrogen budget of a swamp stream. *Journal of Environmental Quality*, **13**, 640–4.
- Quinn, J. M., Burrell, G. P. & Parkyn, S. M. (2000). Influences of leaf toughness and nitrogen content on in-stream processing and nutrient uptake by litter in a Waikato, New Zealand, pasture stream and streamside channels. *New Zealand Journal of Marine and Freshwater Research*, **34**, 253–71.
- Ramírez, A., Pringle, C. M. & Molina, L. (2003). Effects of stream phosphorus levels on microbial respiration. *Freshwater Biology*, **48**, 88–97.
- Rier, S. T., Tuchman, N. C., Wetzel, R. G. & Teeri, J. A. (2002). Elevated-CO<sub>2</sub>-induced changes in the chemistry of quaking aspen (*Populus tremuloides* Michaux) leaf litter: subsequent mass loss and microbial response in a stream ecosystem. *Journal of the North American Benthological Society*, **21**, 16–27.
- Roden, E. E. & Wetzel, R. G. (1996). Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. *Limnology and Oceanography*, **41**, 1733–48.
- Rodewald-Rudescu, L. (1974). Das Schilfrohr. (Die Binnengewässer, vol. 27). Stuttgart: Schweizerbart.
- Sanzone, D. M., Tank, J. L., Meyer, J. L., Mulholland, P. J. & Findlay, S. E. G. (2001). Microbial incorporation of nitrogen in stream detritus. *Hydrobiologia*, **464**, 27–35.
- Scanlon, D. & Moore, T. (2000). Carbon dioxide production from peatland soil profiles: the influence of temperature, oxic/anoxic conditions and substrate. *Soil Science*, **165**, 153–60.
- Shearer, C. A. (1992). The role of woody debris. In *The Ecology of Aquatic Hyphomycetes*, ed. F. Bärlocher. Berlin: Springer-Verlag, pp. 77–98.
- Shoun, H., Kim, D. H., Uchiyama, H. & Sugiyama, J. (1992). Denitrification by fungi. *FEMS Microbiology Letters*, **94**, 277–81.
- Silliman, B. R. & Newell, S. Y. (2003). Fungal farming in a snail. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 15643–8.
- Simon, K. S. & Benfield, E. F. (2001). Leaf and wood breakdown in cave streams. *Journal of the North American Benthological Society*, **20**, 550–63.
- Sinsabaugh, R. L. & Findlay, S. (1995). Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. *Microbial Ecology*, **30**, 127–41.
- Sinsabaugh, R. L., Antibus, R. K., Linkins, A. E. *et al.* (1993). Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology*, **74**, 1586–93.
- Sridhar, K. R. & Bärlocher, F. (2000). Initial colonization, nutrient supply, and fungal activity on leaves decaying in streams. *Applied and Environmental Microbiology*, **66**, 1114–19.
- Stelzer, R. S., Heffernan, J. & Likens, G. E. (2003). The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology*, **48**, 1925–37.
- Suberkropp, K. (1991). Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. *Mycological Research*, **95**, 843–50.
- (1992). Interactions with invertebrates. In *The Ecology of Aquatic Hyphomycetes*, ed. F. Bärlocher. Berlin: Springer-Verlag, pp. 118–34.

- (1995). The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. *Canadian Journal of Botany*, **73** (Suppl. 1), S1361–9.
- (1997). Annual production of leaf-decaying fungi in a woodland stream. *Freshwater Biology*, **38**, 169–78.
- (1998a). Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. *Mycological Research*, **102**, 998–1002.
- (1998b). Micro-organisms and organic matter processing. In *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*, eds. R. J. Naiman, & R. E. Bilby. New York: Springer-Verlag, pp. 120–43.
- Suberkropp, K. & Chauvet, E. (1995). Regulation of leaf breakdown by fungi in streams: Influences of water chemistry. *Ecology*, **76**, 1433–45.
- Suberkropp, K. & Klug, M. J. (1980). The maceration of deciduous leaf litter by aquatic hyphomycetes. *Canadian Journal of Botany*, **58**, 1025–31.
- Suberkropp, K. & Weyers, H. (1996). Application of fungal and bacterial production methodologies to decomposing leaves in streams. *Applied and Environmental Microbiology*, **62**, 1610–15.
- Suberkropp, K., Godshalk, G. L. & Klug, M. J. (1976). Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology*, **57**, 720–77.
- Suberkropp, K., Arsuffi, T. L. & Anderson, J. P. (1983). Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. *Applied and Environmental Microbiology*, **46**, 237–44.
- Tank, J. L. & Dodds, W. K. (2003). Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology*, **48**, 1031–49.
- Tank, J. L., Webster, J. R. & Benfield, E. F. (1993). Microbial respiration on decaying leaves and sticks in a southern Appalachian stream. *Journal of the North American Benthological Society*, **12**, 394–405.
- Tank, J. L., Meyer, J. L., Sanzone, D. M. *et al.* (2000). Analysis of nitrogen cycling in a forest stream during autumn using a N-15-tracer addition. *Limnology and Oceanography*, **45**, 1013–29.
- Thormann, M. N., Bayley, S. E. & Currah, R. S. (2001). Comparison of decomposition of belowground and aboveground plant litters in peatlands of boreal Alberta, Canada. *Canadian Journal of Botany*, **79**, 9–22.
- Thornton, D. R. (1963). The physiology and nutrition of some aquatic hyphomycetes. *Journal of General Microbiology*, **33**, 23–31.
- (1965). Amino acid analysis of fresh leaf litter and the nitrogen nutrition of some aquatic hyphomycetes. *Canadian Journal of Microbiology*, **11**, 657–62.
- Triska, F. J. & Sedell, J. R. (1976). Decomposition of four species of leaf litter in response to nitrate manipulation. *Ecology*, **57**, 783–92.
- Updegraff, K., Bridgham, S. D., Pastor, J., Weishampel, P. & Harth, C. (2001). Response of CO<sub>2</sub> and CH<sub>4</sub> emissions from peatlands to warming and water table manipulation. *Ecological Applications*, **11**, 311–26.
- Wallander, H., Nilsson, L. O., Hagerberg, D. & Rosengren, U. (2003). Direct estimates of C:N ratios of ectomycorrhizal mycelia collected from Norway spruce forest soils. *Soil Biology & Biochemistry*, **35**, 997–9.
- Wallander, H., Goransson, H. & Rosengren, U. (2004). Production, standing biomass and natural abundance of N-15 and C-13 in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia*, **139**, 89–97.
- Webster, J. (1959). Experiments with spores of aquatic hyphomycetes I. Sedimentation, and impaction on smooth surfaces. *Annals of Botany New Series*, **23**, 595–611.
- Webster, J. R. & Benfield, E. F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567–94.

- Webster, J. & Davey, R. A. (1984). Sigmoid conidial shape in aquatic fungi. *Transactions of the British Mycological Society*, **83**, 43–52.
- Webster, J. R. & Meyer, J. L. (eds.) (1997). Stream organic matter budgets. *Journal of the North American Benthological Society*, **16**, 3–161.
- Welsch, M. & Yavitt, J. B. (2003). Early stages of decay of *Lythrum salicaria* L. and *Typha latifolia* L. in a standing-dead position. *Aquatic Botany*, **75**, 45–57.
- Wetzel, R. G. & Howe, M. J. (1999). High production in a herbaceous perennial plant achieved by continuous growth and synchronized population dynamics. *Aquatic Botany*, **64**, 111–29.
- Weyers, H. S. & Suberkropp, K. (1996). Fungal and bacterial production during the breakdown of yellow poplar leaves in two streams. *Journal of the North American Benthological Society*, **15**, 408–20.
- Yavitt, J. B. (1997). Methane and carbon dioxide dynamics in *Typha latifolia* (L.) wetlands in central New York State. *Wetlands*, **17**, 394–406.