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Short communication

Growth measurements of saprotrophic fungi and bacteria reveal differences between canopy and forest floor soils

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ABSTRACT

Canopy-held organic matter develops into a distinct soil system separate from the forest floor in wet temperate coniferous forests, creating a natural microcosm. We distinguished between fungal and bacterial components of the decomposer community in one site with Maple (*Acer macrophyllum*) and one site with Alder (*Alnus rubra*) by using direct measurements of growth; acetate incorporation into ergosterol, and leucine incorporation for fungi and bacteria, respectively. The higher organic matter content of the canopy soils correlated with higher fungal growth. The relative importance of fungi, indicated by fungal:bacterial growth ratio, was higher in the canopy soil of the Maple site, while there was no difference in the Alder site. The high C:N ratio of the Maple canopy soil likely contributed to this difference. These results demonstrate a divergence between canopy and forest floor that should be explored to gain insights in decomposer ecology using the natural microcosms that the canopy soils provide.

Understanding the processes of decomposition is critical to predict many aspects of forest ecosystem dynamics, including nutrient capture and circulation (Zheng et al., 2006), limits on primary productivity (Rothstein et al., 2004), and determinants of long-term soil fertility (Feller et al., 2003). Hundreds of studies have documented the fluxes of nutrients via the decomposition pathway, and ecosystem ecologists have established ranges for the rates of nutrient flow among most components (Dent et al., 2006). In a parallel way, microbiologists have ascertained the basic aspects of the organisms and processes that determine these rates and have established that the major decomposer groups in soils principally responsible for organic matter mineralisation are fungi and bacteria. However, assessing the relative importance of these two groups for decomposition has been hampered by insufficient methods of partitioning the contribution of specific microbial components.

New methodological developments have enabled specific, sensitive, and powerful ways to measure fungal and bacterial growth, which provide direct proxies for their relative contributions to decomposition. Acetate incorporation into the fungal specific lipid ergosterol (Ac-in-erg, Bååth, 2001; Rousk and Bååth, 2007a,b) has been used to measure fungal growth and leucine (Leu) and thymidine incorporation (Bååth et al., 2001) has been used to measure bacterial growth. The combination of these growth techniques to measure and separate between the decomposer groups has revealed details of decomposer ecology. For instance, substrate identity (Rousk and Bååth, 2007b) and pH (Rousk et al., 2009) strongly affect the relative importance of fungi and bacteria, and a strong competitive interaction exists between the two groups (Rousk et al., 2008).

Two forest types with complex decomposition regimes are wet temperate coniferous forests and tropical cloud forests. In both, organic matter is sequestered in a variety of forms; nitrogen-fixation is present; and the microenvironment is highly patchy due to the high stature of the trees and the presence of structural elements such as snags, fallen trees, and small-scale gaps (Matelson et al., 1995). Another structural element that is unique to these forest types is the presence of "canopy mats", live and dead components of canopy-held organic matter that is distinct from terrestrial soil (Nadkarni et al., 2002). This material is derived from shoots and roots of vascular and non-vascular epiphytic plants, intercepted leaves from host trees and from epiphytes, invertebrates, and microorganisms. This results in a soil system solely derived from dead organic matter that coexists with the mixture of dead organic

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matter and mineral constituents making up the forest floor soil in the same area. This distinctive system has been partially characterised previously, including assessments of soil chemistry, microbial ecology, and nutrient transformations, (Vance and Nadkarni, 1990; Nadkarni et al., 2002, 2004; Cardelús et al., 2009). Microbial biomass and activity are different in the canopy-held soil compared with the terrestrial forest floor soil, which suggests that dissimilar decomposer communities exist (Vance and Nadkarni, 1990).

In this study, we investigated the relative importance of the two major decomposer groups in canopy and forest floor soils of a North American temperate wet forest. We sampled canopy soil and the corresponding terrestrial forest floor immediately below in habitats dominated by two tree species: Bigleaf Maple (*Acer macrophyllum*; hereafter, Maple), and Red Alder (*Alnus rubra*; hereafter, Alder). We assessed the relative contributions of the fungal and bacterial soil components with direct measures of their growth.

The study site was a temperate rainforest in the Quinault River Valley in the Olympic National Park, Washington, USA (47° 29′ 46″ N; 123° 49′ 40″ W). Sampling was performed similarly to Nadkarni et al. (2002). In June 2007, six Maple trees were chosen ca. 500 m apart. For each tree, three canopy sites, with corresponding forest floor site directly beneath, were sampled ($n = 18$ for canopy, $n = 18$ for forest floor). In May 2008, another set of canopy soils was sampled from nine Alder trees, with two canopy sites per tree, and corresponding terrestrial forest floor pairs per tree ($n = 18$ for canopy, $n = 18$ for forest floor). Canopy material was collected from branches (about 0–5 cm depth). Accumulated standing litter was removed before sampling the forest floor, and samples were collected immediately below the O-horizon with a 10 cm corer (0–10 cm depth).

The bacterial growth rate was estimated using Leu incorporation (Kirchman et al., 1985), on bacteria extracted from soil using the homogenisation/centrifugation technique described by Bååth (1992, 1994) and Bååth et al. (2001). We used 2 μl radio-labelled Leu [^{3}H]Leu (37 MBq ml^{-1}, 5.74 TBq mmol^{-1}, Amersham) and non-labelled Leu to each tube, resulting in 275 nM Leu in the bacterial suspensions. We used the amount of Leu incorporated into extracted bacteria per h and g soil as a measure of bacterial growth.

Fungal growth was assessed using the Ac-in-erg method (Newell and Fallon, 1991) adapted for soil (Bååth, 2001) with modifications (Rousk and Bååth, 2007b) using an 8 h incubation at 22 °C without light. Ergosterol was recalculated to fungal biomass assuming 5 mg ergosterol g^{-1} fungal biomass (Joergensen, 2000; Ruzicka et al., 2000). We used the amount of Ac incorporated into ergosterol per h and g soil as a measure of fungal growth.

Water content was measured by drying soil samples overnight at 105 °C. Subsequent determination of organic-C was achieved by combusting the samples at 600 °C for 6 h. The loss-on-ignition was assumed to contain 45% C. pH was determined by extracting fresh soil with distilled water (1:10). Total N was determined as Kjeldahl-N.

Table 1
Organic matter-C and total N are given in percent of soil dry weight, and C:N is the unitless ratio between them.

	Maple			Alder		
	Canopy	Floor	Stat. sign.	Canopy	Floor	Stat. sign.
Organic matter-C (%)	40.0 (1.62)	10.6 (1.62)	***	42.3 (2.41)	28.1 (2.41)	**
Total N (%)	1.57 (0.16)	0.46 (0.16)	***	2.10 (0.18)	1.65 (0.18)	ns
C:N	30.7 (6.01)	22.9 (6.01)	ns	20.5 (1.26)	18.1 (1.26)	ns
Water content (%)	47.8 (4.46)	38.5 (4.46)	ns	62.9 (3.84)	63.9 (3.84)	ns
pH	4.65 (0.19)	4.19 (0.19)	ns	4.32 (0.11)	3.74 (0.11)	**

Values are given as means with standard errors from ANOVAs within brackets. Statistical significance is reported as ***$P < 0.001$, **$P < 0.01$, or ns, not significant.

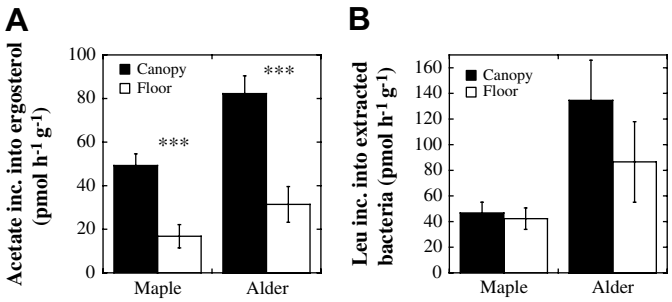

Fig. 1. The fungal growth measured using acetate incorporation into ergosterol (A) and bacterial growth measured using leucine incorporation into extracted bacteria (B) of the Maple and Alder sites. Closed bars designate canopy soils and open bars designate forest floor soils. The error bars designate standard errors from ANOVA (see description in text). ***$P < 0.001$.

All analyses were based on all collected samples ($n = 18$ for canopy, $n = 18$ for forest floor) for each species. Estimates of variation, given as standard error (SE), were based on analysis of variance (ANOVA), with tree as blocked factor to partition and isolate the canopy – forest floor variation from the inter-tree variation. For organic-C, total N, water content, and pH, we bulked within-tree replicates, resulting in $n = 6$ and $n = 9$ replicates for Maple and Alder, respectively. The fungal:bacterial (F:B) growth ratio was calculated for each replicate, i.e., fungal growth was related to bacterial growth in the same soil sample. To avoid any bias that the difference in sampling date between the Alder and Maple sites may have led to, the canopy and forest floor comparisons were always related within site (i.e. site-standardised), and never contrasted between sites.

In the Maple site, organic matter-C and total N were 3.8 ($P = 0.0002$) and 3.4 ($P = 0.004$) times higher in canopy compared with forest floor, respectively (Table 1). The C:N ratios, water content, and pH for canopy samples were overall slightly, but not significantly, higher than forest floor samples (Table 1).

The bacterial growth of canopy samples in the Maple site did not differ from those of the forest floor (47 and 42 pmol Leu h^{-1} g^{-1}, respectively). However, fungal growth was three times higher in the canopy (49 compared with 17 pmol Ac h^{-1} g^{-1}; $P < 0.0001$; Fig. 1). This resulted in a 4.5 times higher fungal-to-bacterial (F:B) growth ratio in the canopy compared with the forest floor ($P = 0.0004$, Fig. 2). The fungal biomass estimated with ergosterol was three times higher in the canopy (2.7 mg g^{-1}) compared with the forest floor (0.9 mg g^{-1}) ($P < 0.0001$, Fig. 3). When the microbial parameters were expressed per organic material in the Maple site, most differences between canopy and forest floor were eliminated, except for a low bacterial growth, which were more than three times lower than in forest floor, in the canopy (Table 2).

In the Alder site, organic-C and total N were both higher in the canopy (42% and 2.1% respectively), compared with the forest floor, at 28% and 1.7% respectively (Table 1). Only organic-C was significantly different ($P = 0.005$). Samples from canopy and forest floor were not significantly different for C:N ratios or water content (Table 1). However, the pH in the canopy was significantly higher ($P = 0.01$) in the canopy compared with the forest floor (mean = 4.3 and 3.7, respectively) (Table 1).

Bacterial growth was more variable in the Alder site. For canopy soils values were not significantly higher than the forest floor (135 vs. 87 pmol Leu h^{-1} g^{-1}; $P = 0.21$, Fig. 2). The fungal growth was almost three times higher ($P = 0.0002$) in the canopy (82 pmol Ac h^{-1} g^{-1}) than in the forest floor (31 pmol Ac h^{-1} g^{-1}; Fig. 1). When

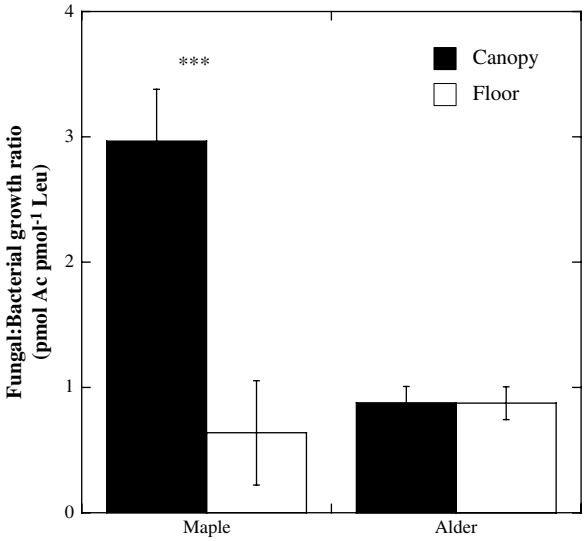

Fig. 2. The fungal:bacterial growth ratio of the Maple and Alder sites. Closed bars designate canopy soils and open bars designate forest floor soils. The error bars designate standard errors from ANOVA (see description in text). $^{***}P < 0.001$.

the fungal and bacterial growth were related in each soil sample, this resulted in similar F:B growth ratios (0.9 in both; Fig. 2). The fungal biomass was significantly higher ($P = 0.003$) (2.7 mg g^{-1}) in the canopy compared with 1.4 mg g^{-1} in the forest floor (Fig. 3). When expressing the microbial parameters per organic material in the Alder site, the fungal and bacterial growth measurements as well as the fungal biomass showed no differences between canopy and forest floor (Table 2).

In summary, there was a higher fungal growth in the canopy soils of both sites, whereas bacterial growth was higher only in the canopy soil of the Alder site, with no differences in the Maple site. A possible explanation for the differences between forest floor and canopy soils is the differences in organic carbon between the systems, which were emphasised when the growth measurements

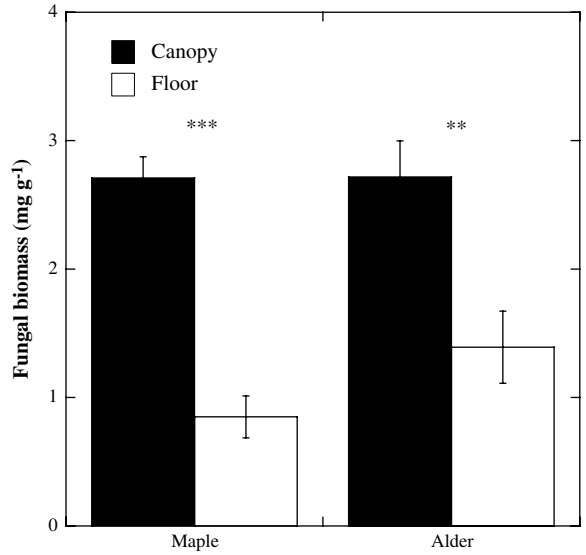

Fig. 3. The fungal biomass estimated using ergosterol of the Maple and Alder sites. Closed bars designate canopy soils and open bars designate forest floor soils. The error bars designate standard errors from ANOVA (see description in text). $^{***}P < 0.001$, $^{**}P < 0.01$.

Table 2
Fungal growth, bacterial growth, and fungal biomass related to the amount of organic matter in the soil.

	Maple		Alder	
	Canopy	Floor	Canopy	Floor
Fungal growth (Ac-in-erg, pmol h^{-1} g^{-1} OM)	55.4 (6.0)	71.6 (22.8)	87.5 (8.7)	50.3 (13.2)
Bacterial growth (Leu, pmol h^{-1} g^{-1} OM)	52.5 (10.0)	180 (38.8)	143 (33.4)	139 (50.4)
Fungal biomass (mg g^{-1} OM)	3.0 (0.18)	3.6 (0.68)	2.9 (0.28)	2.2 (0.43)

Values are given as means with standard error of the ratio between the measurements and the organic matter content.

were related to organic matter content (Table 2). Only one measurement still stood out – the very low bacterial growth in the canopy soil of the Maple site (Table 2). This resulted in a higher importance of the fungal decomposer group, as indicated by the F:B growth ratio, in the canopy of the Maple, but not in the Alder site.

In many soil studies, biomass assessments of fungi and bacteria have indicated that bacteria benefit relatively more from high pH, while low pH tends to favour fungi (Blagodatskaya and Anderson, 1998; Marstorp et al., 2000; Gong et al., 2001; Bååth and Anderson, 2003). This finding has been corroborated with growth-based measurements (Pennanen et al., 1998; Rousk et al., 2009). However, both the Maple and the Alder sites showed a tendency for slightly higher pH in the canopy compared with forest floor, which does not explain fungi being more important only in the Maple site.

It is more likely that the high C:N ratio found in the canopy soil of Maple, due to initially high C:N ratio of the litter compared to Alder (with nitrogen fixer symbionts), is of importance. The high C:N ratio of substrate seem to disfavour bacterial growth, and selective organic matter quality effects on the two decomposer groups has frequently been found (Bossuyt et al., 2001; De Vries et al., 2006, 2007; Rousk and Bååth, 2007b; Lauber et al., 2008). Consistent with our results, tree species have recently been shown to have strong effects on the C:N ratios of tropical canopy soils (Cardelús et al., 2009).

In conclusion, we have shown that the fungal contribution to decomposition was higher in the canopy compared with the forest floor in the Maple site, while no discernible differences between the relative importance of decomposer groups could be found in the Alder site. It is probable that the differences between canopy and forest floor soils were related to the organic matter quality. These results demonstrate an interesting divergence between canopy and forest floor soils that should be explored to gain insights in decomposer ecology using the natural microcosms that the canopy soils provide.

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