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Effects of fungal inocula on the decomposition of lignin and structural polysaccharides in *Pinus sylvestris* litter

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Abstract Litter bags containing sterile Scots pine (*Pinus sylvestris*) needles (19.8% lignin, 26.5% cellulose and 0.34% N) were inoculated with two species of fungi in the laboratory and then placed in the litter layer of a pine plantation. *Marasmius androsaceus*, which can degrade lignocellulose, was initially displaced by other fungal colonisers and was not detected in the litter after 2–3 months; but was re-isolated from the needles after 12 months. *Trichoderma viride*, which is a cellulolytic species and also antagonistic to other fungi, dominated the litter throughout the experiment. The control litter was naturally colonised by litter fungi. After 12 months, mass losses were similar at 52% for *M. androsaceus* and 48% for *T. viride*, compared with 36% for the control litter colonised by a more complex fungal community. Lignin concentrations increased with time in control litter and with *T. viride* because mass losses of carbohydrates were greater than those of lignin. Litter inoculated with *M. androsaceus* showed significant lignin decomposition throughout the experiment but cellulose concentrations showed a proportional increase in the first 6 months, suggesting that the fungus was preferentially exploiting hemicellulose and non-structural carbohydrates. Analysis of TFA-extractable sugars (mainly from hemicellulose) and CuO-derived phenylpropanoid moieties from lignin confirmed the differential patterns of resource decomposition which were not evident from total mass losses. During the initial stages of decomposition, *T. viride* was as effective in utilising structural polysaccharides as the complex fungal community in the control litter. Furthermore, *M. androsaceus* not only exhibited unexpectedly low cellulolytic activity but also facilitated lignin depolymerisation after the fungus was no longer detectable in the litter. The pre-inoculation of litter with these two fungal species therefore affected the overall dynamics of decomposition at a biochemical level. This study illustrates the importance of understanding the effects and interactions of specific fungi, rather than assumptions about the functional competence of diverse communities, on the processes of litter decomposition.

Keywords Fungal diversity · Litter decomposition · *Pinus sylvestris* · Cellulose · Lignin

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Introduction

The decomposition of conifer litter is largely carried out by fungi (Millar 1974) but the composition of the fungal communities exploiting the resource has rarely been considered as a variable determining patterns of mass losses in the field. Berg et al. (1993) showed that decomposition rates of a standard pine needle (*Pinus sylvestris*) litter in conifer forests along a latitudinal transect were largely explained by climatic variables and there was little residual variance which could be attributed to soil biotic effects. This suggests that the complement of functional groups of fungi required for litter decomposition have a ubiquitous distribution, although species composition of these consortia can vary within and between forest systems (Gourbière 1988).

On the other hand, there have been many detailed studies of microbial succession on conifer needles (Kendrick and Burges 1962; Millar 1974; Gourbière and Corman 1987) and other litter types (e.g. Kjøller and Struwe 1987; Robinson et al. 1994; Rosenbrock et al. 1995) which emphasised the roles of particular fungal species in the decomposition of different resource components. Decomposition is usually initiated by generalist primary colonisers involving a diverse community of fungi (and bacteria) which utilise simple sugars, oligosaccharides and other low molecular weight compounds. After this initial flush of microbial activity, specialist secondary colonisers, notably the Basidiomycetes, which are less competitive than the micro-fungi

in exploiting labile resources (Frankland 1992), effect the decomposition of more recalcitrant plant polymers such as ligno-cellulose complexes. Exceptions to this paradigm occur where the time course of fungal succession is attenuated by dominant lignolytic basidiomycetes, such as *Mycena gallopus* (Pers. Fr.) Kummer and *Marasmius androsaceus* (L: Fr), invading from established mycelial networks in the underlying litter (Newell 1984; Frankland 1992). In such cases, decomposition processes may reflect the attributes of particular fungal species (e.g. Frankland 1969; Gourbière and Corman 1987) rather than the net effects of functional groups comprising diverse species. Cox et al. (1997) investigated the survival of two common fungal species, *M. androsaceus* and *Trichoderma viride* Pers.: S.F. Fry, which were inoculated onto sterile pine needles in litter bags before they were placed in the field. The two species were chosen because they have contrasting ecological attributes that could influence the decomposition of different litter constituents; *M. androsaceus* is a strongly lignolytic and cellulolytic species, and one of the major decomposers of needle litter (Gourbière and Corman 1987; Holmer and Stenlid 1991), whilst *T. viride* can actively decompose cellulose (Bowen and Harper 1990; Mandels and Reese 1999) although Papavisas (1985) suggested that it mainly utilises oligosaccharides and other non-structural compounds. It is, however, well known for its antagonistic properties towards other fungi and has been used for the biological control of plant fungal pathogens, e.g. on conifer seedlings (Zhang et al. 1994; Asiegbu et al. 1999). The results presented here show the effects of these two fungal inocula, and those of natural fungal colonisers, on the decomposition of structural carbohydrates and lignin in pine needles.

Materials and methods

Scots pine needles were collected in 1995 from a forest at Jadrås, Sweden (60°49′N, 16°01′E) which was one of the sites used by Berg et al. (1993) for litter standards. The litter was air dried to 96.2% dry matter and 5 g aliquots weighed into nylon (1 mm mesh) litter bags. The bags were then autoclaved at 120 °C, inoculated with plugs of mycelium from laboratory cultures of *T. viride* and *M. androsaceus* and then incubated at 20 °C for 5 weeks until the needles appeared thoroughly colonised. The control litter was autoclaved and incubated without an inoculum. In January 1996, the litterbags were placed in random groups of the three treatments in the litter fermentation (F) layer of a *P. sylvestris* plantation at Haldon Forest near Exeter, UK (50°37′N, 3°40′W). Three bags were collected from each treatment at monthly intervals for the first 6 months and then a final collection was made after 12 months. Small fragments of needles (of negligible mass) were removed, plated on 2% malt extract agar and incubated for 5 weeks for identification of litter fungi. The remaining litter was oven-dried at 40 °C, to determine mass losses, and stored for chemical analysis. Further details of litter preparation and the identification of fungal colonisers are given in Cox et al. (1997).

Chemical analysis

Samples were ground to <800 μm using a hammer mill (Glen Creston, Stanmore) and subsamples were oven-dried at 105 °C for moisture content corrections. All chemical analyses were carried out in triplicate.

Total C was determined using a Leco HF10 gravimetric carbon analyser (Leco Instruments, Stockport) and total N by modified Kjeldahl digestion (Allen 1989) followed by ammonium determination using a Bemas auto-analyser (Burkhard, Uxbridge).

Proximate analysis for acid detergent fibre (ADF), cellulose and lignin was performed using the cetyltrimethylammonium bromide-sulphuric acid method of Rowland and Roberts (1994).

Phenylpropanoid derivatives of lignin (PPDs) were determined in samples (50 ± 5 mg) of ground litter using an alkaline CuO-oxidation technique (Hedges and Ertel 1982). Samples were mixed with CuO, 2 M NaOH and heated in a digestion block with magnetic stirring for 2.5 h at 170 °C under a headspace atmosphere of N_2. After oxidation, samples were centrifuged (2,598 RCF for 30 min) and the supernatant decanted. Solutions were acidified to pH 2 with 6 M HCl and, after centrifugation (2,598 RCF for 30 min) the supernatant was eluted through a C18 extraction column. Silyl derivatives of the PPDs extracted from these columns were determined on a Shimadzu GC14-A capillary GC with a BPX 25 m, 0.25 mm i.d. column (Sanger et al. 1996).

Component carbohydrates were determined in samples (25 ± 5 mg) of ground litter extracted by trifluoracetic acid (TFA) hydrolysis using a modified procedure of Guggenberger and Zech (1994). The sample was digested at 100 °C for 4 h using a 100 μl ribulose internal standard and 250 μl (4 M) TFA. The solution was then passed through DOWEX 50 exchange resin. A 200 μl aliquot was blown down with air to dryness and 100 μl of methoxyaminehydrochloride in pyridine (25 mg ml^{-1}) added and left to react for 30 min at 70 °C. After cooling to room temperature the sample was silylated for 1 h using 100 μl of *N*-trimethylsilylimadazole. Final sample clean-up involved shaking the silylated solution with 300 μl of hexane. Carbohydrate were determined in aliquots of the hexane phase using a Shimadzu GC14-A capillary GC with a PB5 25 m $\times$ 0.25 mm i.d. column (Sanger et al. 1998).

Statistics

Data are expressed as means $\pm$SE. Analysis of variance (using arcsin-transformed data for percentages) and principal component analysis (PCA) was carried out using Microsoft Exel 97 SR-2. PCA is a statistical technique for reducing multivariate data to a smaller number of explanatory factors. It works by calculating a set of hierarchical factors or axes (PC1, PC2, etc.) that explain most of the contributory variance (high eigenvalues) and assigning each set of measurements to values on these axes.

Results

Mass loss

Mass losses after pre-incubation of litter for 5 weeks with *Marasmius* were greater ($10.0 \pm 0.6\%$) than with *Trichoderma* ($8.0 \pm 0.7\%$) or the uninoculated control litter ($5.7 \pm 0.2\%$). These treatment differences were maintained for 4 months in the field after which mass losses from litter with *Trichoderma* increased relative to *Marasmius* and control litters (Fig. 1). Over the last 6 months, mass losses from litter with *Marasmius* increased sharply so that this treatment showed the highest final mass losses of $52.2 \pm 1.8\%$ after 12 months compared with $47.9 \pm 4.9\%$ from litter with *Trichoderma* and $45.7 \pm 2.3\%$ from the controls. There were significant fungal treatment ($F_{2,48} = 15.9$, $P < 0.001$) and treatment $\times$ time interactions ($F_{14,48} = 2.55$, $P < 0.05$).

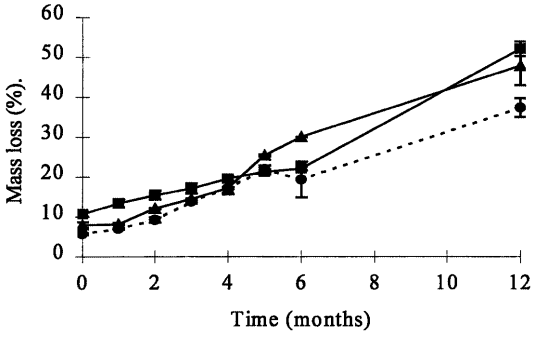

Fig. 1 Cumulative mass losses (mean % ±SE) from pine needles in litterbags inoculated with *Trichoderma viride* (▲) or *Marasmius androsaceus* (■) (without control –●–)

C:N ratios

Initial composition of the litter was 51.7±0.51% C and 0.34±0.01% N with a C:N ratio of 155:1. Following pre-incubation, the C:N ratio of litter with *Marasmius* was lower (130:1) than with *Trichoderma* (145:1). The C:N ratio of the sterile control litter was 154:1; possibly reflecting losses of soluble carbohydrates after autoclaving. For the first 4 months in the field, the C:N ratio decreased to between 90:1 and 100:1 for all three treatments. Thereafter C:N ratios in the *Trichoderma* and control treatments continued to decrease at about the same rate to about 50:1. The litter with *Marasmius*, however, had a significantly lower C:N ratio at 5 months (96:1), but then converged with the other treatments to a final value of 58:1 at 12 months. Again, fungal treatment ($F_{2,48}=3.47$, $P<0.05$), time ($F_{7,48}=13.66$, $P<0.001$) and treatment × time interaction ($F_{14,48}=2.83$, $P<0.01$) were significant. Orthogonal comparisons showed that main treatment effects were significant only between *Trichoderma* and *Marasmius* ($F_{1,32}=4.34$,$P<0.05$).

Lignin and cellulose

Mean lignin and cellulose concentrations in the sterilised litter prior to inoculation were 19.8±0.0% and 26.5±0.9% respectively. After pre-incubation, lignin concentrations increased to about 25% in both fungal treatments but was unchanged in the control litter (Fig. 2A). Litter with *Marasmius* showed a steady decrease in lignin concentrations to 18.9±0.7% after 6 months but was not significantly different from initial concentrations after 12 months (i.e. there was significant lignin decomposition relative to mass losses). In contrast, lignin concentrations increased with time in the controls and litter with *Trichoderma* to about 40% after 12 months, indicating mass losses exceeded lignin decomposition over this period. There were significant fungal treatment ($F_{2,48}=66.9$, $P<0.001$) and time × treatment interactions ($F_{14,48}=2.63$, $P<0.05$).

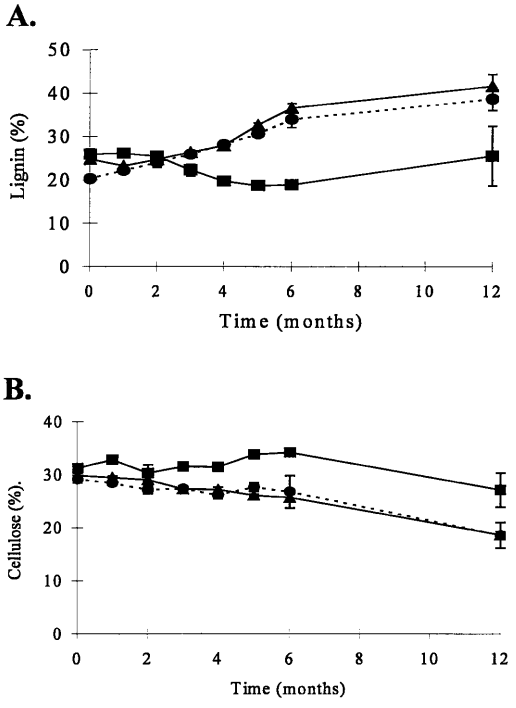

Fig. 2 Percentage concentrations (±SE) of lignin (**A**) and cellulose (**B**) in pine needles from litterbags inoculated with *Trichoderma viride* (▲) or *Marasmius androsaceus* (■) (without controls –●–)

The litter with *Trichoderma* and controls showed the same trends of decreasing cellulose concentrations from initial values of 27–29% to 17–18% after 12 months (Fig. 2B). The trends in litter with *Marasmius* were completely different with relative increases in percentage cellulose from 31.4±0.6% to 34.2±0.6% over the first 6 months. Final concentrations decreased to 27.1±3.2% at 12 months but were still significantly higher than in the other two treatments. There was a significant effect of fungal treatment on cellulose concentrations ($F_{2,48}=14.91$, $P<0.001$) but no significant treatment × time interactions.

Phenylpropanoid derivatives of lignin (PPDs)

Results for PPD analyses were complex and so concentrations of different moieties are only shown for 0, 6 and 12 months in Table 1 rather than the full data set for time trends. In all three treatments, total PPD concentrations showed little change over the first 6 months and then increased fourfold in the control and *Marasmius* treatments and doubled in the litter with *Trichoderma.* The effect of treatment on total PPD levels was highly significant ($F_{2,48}=65.94$, $P<0.001$); although time was not significant as a main effect, the time × treatment interaction was highly significant ($F_{14,48}=26.2$, $P<0.001$), indicating that temporal differences between treatments were significant and not solely due to random variation.

Table 1 Mean concentrations (mg g^{-1}) of CuO-extracted phenylpropanoid moieties in pine needles incubated in the field without (*control*) or with inoculation of *Marasmius androsaceus* or *Trichoderma viride*. Data for vanillyl, syringyl and *p*-hydroxyl are the sum of moiety species. Standard errors of means are shown *in parentheses* ($n=3$)

	Time (months)	Vanillyl	Syringyl	*p*-Hydroxyl	Coumaric acid	Ferulic acid	Vanillyl: syringyl	Vanillyl acid: aldehyde	Total moeities
	Initial	70.0 (2.1)	2.6 (0.3)	3.0 (0.0)	4.6 (0.3)	2.1 (0.1)	27.9	0.10	82.4 (2.7)
Control	0	62.0 (3.4)	4.0 (0.2)	2.7 (0.1)	5.7 (0.2)	2.5 (0.1)	15.4	0.10	76.8 (4.1)
	6	55.3 (1.9)	3.7 (0.8)	2.4 (0.1)	4.7 (0.5)	2.0 (0.2)	16.4	0.11	68.1 (3.5)
	12	61.2 (11.0)	4.7 (0.7)	2.2 (0.4)	6.0 (0.5)	2.8 (0.3)	12.9	0.13	76.9 (12.8)
Marasmius	0	38.3 (3.3)	2.0 (0.2)	1.9 (0.1)	3.6 (0.1)	1.4 (0.1)	19.4	0.13	47.2 (3.2)
	6	28.8 (2.8)	2.3 (0.1)	1.8 (0.2)	2.7 (0.4)	1.1 (0.1)	12.4	0.25	36.8 (3.3)
	12	36.0 (11.2)	3.2 (0.3)	1.9 (0.2)	3.1 (0.8)	1.5 (0.3)	12.1	0.28	45.5 (12.3)
Trichoderma	0	58.1 (4.1)	4.6 (0.7)	2.5 (0.4)	6.2 (0.6)	2.8 (0.4)	22.9	0.11	74.2 (6.0)
	6	77.9 (8.9)	5.6 (0.6)	2.8 (0.2)	8.5 (1.5)	3.5 (0.7)	14.6	0.10	36.8 (3.3)
	12	68.1 (10.2)	4.4 (0.4)	2.6 (0.3)	5.4 (1.1)	2.69 (0.3)	15.7	0.14	83.1 (11.3)

Concentrations of specific PPD moieties also showed treatment effects using PCA on the PPDs as percentages of total concentrations. The PCA resulted in three principal components with eigenvalues greater than unity that collectively accounted for 78.2% of the total variation. Pre-inoculation with *Marasmius* resulted in significantly more negative PC1 scores than with *Trichoderma* or control litters ($P<0.001$). This indicated significantly lower levels of vanillin and higher levels of vanillic acid, acetovanillone, *p*-hydroxybenzoic acid and acetosyringone in litter with *Marasmius*. PC1 scores from litter inoculated with *Trichoderma* did not differ significantly from those of uninoculated controls. PC2 scores were also significantly more negative with *Marasmius* than *Trichoderma* or in controls ($P<0.001$), indicating lower levels of coumaric and ferulic acids in the former case. PC2 scores from litter with *Trichoderma* were greater than those of the controls ($P<0.01$) indicating higher levels of coumaric and ferulic acids. Time did not significantly affect scores on either PC axis and there were no significant interactions. Litter with *Marasmius* showed a significant, time-dependent increase in the acid:aldehyde ratio of vanillyl residues (a shift reflecting the oxidative degradation of PPDs) from 0.13 to 0.28 after 12 months (Table 1). No increase in PPD acidity occurred with *Trichoderma* or in controls. For total PPDs, treatment ($F_{2,48}=66.94$, $P<0.001$), time ($F_{14,48}=12.66$, $P<0.001$) and time × treatment interactions ($F_{14,48}=2.63$, $P<0.05$) were all significant.

Carbohydrate derivatives

Concentrations of sugars hydrolysed by TFA from structural carbohydrates (which exclude crystalline cellulose) at 0, 6 and 12 months are shown in Table 2. Inoculation with *Marasmius* resulted in significantly lower ($P<0.001$) concentrations of total sugars after pre-incubation (from 249 ± 9.3 mg g^{-1} in the initial litter to 198 ± 6.7 mg g^{-1}) than with *Trichoderma* (245 ± 11.3 mg g^{-1}) or in the controls (252 ± 3.1 mg g^{-1}). In *Trichoderma*-inoculated litter and controls, concentrations of total TFA-extractable carbohydrates decreased steadily with time to 192 ± 20.0 mg g^{-1} and 179 ± 4.6 mg g^{-1}, respectively, at 12 months. In litter with *Marasmius*, however, concentrations showed little change until 12 months, when they had decreased slightly to 160 ± 11.6 mg g^{-1}. As with total PPDs, treatment ($F_{2,48}=14.9$, $P<0.001$), time ($F_{7,48}=13.7$, $P<0.001$) and interaction terms ($F_{14,48}=2.59$, $P<0.01$) were all significant for total TFA-extractable carbohydrates. In con-

Table 2 Mean concentrations (mg g^{-1}) of TFA-extracted sugars from pine needles incubated in the field without (*control*) or with inoculation of *Marasmius androsaceus*, or *Trichoderma viride*. Standard errors of means are shown *in parentheses* ($n=3$)

	Time (months)	Xylose	Arabinose	Rhamnose	Fucose	Mannose	Galactose	Glucose	Total
	Initial	21.5 (1.1)	43.2 (1.6)	7.9 (6.0)	2.4 (0.1)	57.3 (2.5)	44.3 (1.7)	72.0 (1.8)	249 (9.3)
Control	0	21.3 (2.0)	42.5 (0.7)	8.3 (0.7)	2.2 (0.2)	53.5 (0.0)	43.9 (2.0)	80.7 (1.3)	252 (3.1)
	6	21.7 (0.7)	21.5 (1.0)	6.9 (0.5)	2.7 (0.2)	48.6 (2.2)	36.4 (0.8)	64.5 (0.6)	202 (3.0)
	12	21.2 (0.4)	18.1 (0.6)	6.5 (0.1)	3.0 (0.2)	40.5 (2.4)	34.4 (1.4)	55.0 (2.1)	179 (4.7)
Marasmius	0	19.4 (0.6)	29.0 (1.0)	7.6 (0.5)	2.0 (0.1)	48.5 (2.8)	36.2 (1.2)	55.4 (2.8)	198 (6.7)
	6	23.2 (1.5)	20.3 (1.0)	9.2 (0.4)	2.3 (0.1)	55.9 (3.5)	37.2 (1.8)	64.8 (4.8)	213 (12.2)
	12	18.2 (1.6)	11.7 (2.5)	5.1 (1.2)	2.4 (0.3)	40.5 (1.5)	28.7 (2.9)	53.3 (1.5)	160 (11.6)
Trichoderma	0	23.0 (1.0)	38.1 (0.4)	8.0 (0.5)	2.3 (0.1)	64.4 (4.8)	45.9 (1.4)	63.7 (4.2)	245 (11.9)
	6	19.1 (0.4)	18.2 (0.4)	6.4 (0.5)	2.1 (0.1)	44.1 (1.1)	33.4 (1.4)	57.9 (2.6)	181 (5.6)
	12	24.0 (1.7)	19.8 (3.6)	6.7 (1.5)	4.4 (0.6)	40.4 (4.5)	34.0 (4.3)	62.6 (6.2)	192 (20.0)

trast to lignin transformations, PCA suggested that there were no effects of inoculation treatments, but there were significant changes in PC1 ($P<0.01$) and PC2 ($P<0.001$) scores with time. This reflected increases in xylose, fucose, galactose (PC1) and glucose (PC2) were accompanied by decreases in mannose (PC1) and arabinose (PC2).

Discussion

Cox et al. (1997) and Cox (1998) showed that no other species of fungi were isolated from litter inoculated with *T. viride* over 6 months in the field. At 12 months it was still the dominant isolate but *Mortierella* spp. and *Acremonium* spp. were also present. There was no *M. androsaceus* recorded in the inoculated litter after 2–3 months and no hyphae with clamp-connections were observed on needles in this treatment between 3 and 6 months in the field. However, the same karyotype was isolated from the final samples of the *Marasmius* litter at 12 months (Cox 1998), indicating its persistence in, or in the vicinity, of the experimental litter. After 2 months, *Trichoderma polysporium* was a prominent coloniser of both the *Marasmius* and control bags. In total, 26 species of common hyphomycetes and deuteromycetes were isolated from these two treatments. Most of these fungi are known to be cellulolytic but not lignolytic (Rai et al. 1988) while *T. polysporum* is also known to inhibit the growth of other fungi (Widden and Scattolin 1988). However, *T. polysporum* did not colonise the bags incoculated with *T. viride*.

After 12 months, mass losses from the *Marasmius* and *Trichoderma* treatments were similar and significantly higher than from the control litter which had been colonised by a more diverse fungal community. The patterns of lignin and structural polysaccharide decomposition were completely different for *Marasmius* and *Trichoderma*. Combining data for cellulose, lignin and mass losses indicated that the litter inoculated with *Marasmius* litter lost 17% of initial lignin over the first 6 months and 38% over the last 6 months. Cellulose decomposition was 15% at 6 months and 51% at 12 months. This means that hemicellulose and other labile compounds comprised 55% of the mass losses at 6 months and 47% at 12 months. In contrast, litter inoculated with *Trichoderma* showed no significant reduction in the initial mass of lignin over 12 months. However, cellulose decomposition was 33% and 26% over 6 months in the *Trichoderma* and control treatments respectively, and about 62% in both litters at 12 months. This means that mass losses of 38% and 31% at 6 months, and 40% and 43% at 12 months, were non-lignocellulose constituents in the *Trichoderma* and control treatments, respectively. The reduction in C:N ratios in all treatments with time reflected the conservation of N (in microbial biomass) relative to mass losses of C.

These patterns of litter decomposition revealed some interesting anomalies with respect to the initial assumptions about the ability of *Marasmius* and *Trichoderma* to decompose lignin and structural polysaccharides. Losses of cellulose in litter inoculated with *Marasmius* were small over the initial period when this fungus was present, while lignin decomposition was more extensive than in the other treatments. This suggests that *Marasmius* was degrading lignin to access hemicelluloses in preference to α-cellulose so that cellulose concentrations increased relative to mass losses. There was also a marked decrease in total TFA-extractable carbohydrates during pre-incubation of the litter inoculated with *Marasmius* followed by little change in this parameter during the next 6 months in the field. This also confirmed a rapid initial metabolism of labile carbohydrates rather than the utilisation of α-cellulose, which is not hydrolysed by TFA. Total TFA-extractable carbohydrates and cellulose in the control and *Trichoderma* treatments showed progressive losses with time, reflecting the decomposition of structural carbohydrates.

The higher amounts of lignin decomposition in litter inoculated with *Marasmius* was also reflected by changes in PPD concentrations. In particular, acid:aldehyde ratios of vanillyl residues increased during the first 6 months of incubation, indicating depolymerisation of core lignins (Goni et al. 1993). This shift in the acidity of PPDs was not observed in litter inoculated with *Trichoderma*, which is not considered to be lignolytic, or in the control litter, indicating that these litter bags were not colonised by actively lignolytic fungi during 12 months in the field.

In conclusion, the pre-inoculation of litter with these two fungal species affected the overall dynamics of decomposition at a biochemical level. The processes of decomposition in litter inoculated with *Trichoderma* were similar to those in litter that was naturally colonised by several species of fungi. This suggests that this single dominant species of fungus was as effective in the decomposition of structural carbohydrates in pine litter as more complex assemblages of fungal species. The absence of *Marasmius* from the inoculated litter after a few months is consistent with the poor ability of many basidiomycetes to compete with obligate 'sugar fungi' until simple C compounds are depleted (Garrett 1975; Frankland 1992). However, it was surprising to find that *Marasmius* preferentially exploited hemicellulose, and other labile C sources, while degrading lignin. The short period over which *Marasmius* was active in the litter, and the lack of lignolytic activity in the controls, which were naturally colonised by a succession of fungi, is not easily explained. Lignin decomposition in the *Marasmius* treatment occurred progressively throughout the experiment. This suggests that either *Marasmius* initially affected the biochemical structure of lignin so that it could be degraded by 'non-lignolytic' species or that a lignolytic fungus, which did not form clamp connections, replaced *Marasmius* but did not co-

lonise the control litter. The former explanation seems the more likely. The presence of *T. polysporum* in the *Marasmius* and control litters is of interest in this respect since *Trichoderma* spp. were therefore present in all treatments and have been shown to inhibit colonisation of wood by white-rot fungi (Filip and YangErve 1997). A final implication of this study is that the nature of the microbial inocula used in laboratory studies of decomposition, whether from specific cultures or by non-specific additions of litter homogenates, can predetermine the rates and processes of chemical transformations (Anderson 1993).

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