Soils in transition: dynamics and functioning of fungi

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Chapter 5

Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil type

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Submitted

Abstract

Fast-growing bacteria and fungi are expected to cause the initial stage of decomposition of woody fragments in and on soils, i.e. the respiration of sugars, organic acids, pectin and easily accessible cellulose and hemicellulose. However, little is known on the factors regulating initial wood decomposition. We examined the effect of wood fragment size, vertical position, nitrogen addition and soil type on initial wood decay and on the relative importance of fungi and bacteria therein. Two fractions of birch wood were used in microcosm experiments, namely wood blocks (dimensions: 3 x 0.5 x 0.5 cm) and sawdust (dimensions: 0.5-2 mm). The woody fragments were enclosed in nylon bags and placed on top of- or buried in an ex-arable soil and in a heathland soil. After 15, 25 and 40 weeks of incubation, fungal biomass was quantified (as ergosterol and chitin content) and bacterial numbers were determined. The results indicated that initial wood decay was mostly caused by fungi; bacteria were only contributing in sawdust in/on ex-arable soil. Fungal biomass and activity was stimulated by wood fragment size, burial of fragments and nitrogen addition. Both bacterial numbers and fungal biomass/activities were much lower in woody fragments incubated in/on heathland soil than in those incubated in/on ex-arable soil, indicating that soil origin is also an important factor determining initial wood decay.

Introduction

Wood is an important component of organic matter in ecosystems that are dominated by shrubs and trees, and is mainly composed of cellulose, hemicellulose and lignin (Schmidt, 2006). During the initial stages of wood decomposition, bacteria and fast growing opportunistic fungi (molds) can degrade simple soluble substrates, pectin and easily accessible cellulose and hemicellulose (De Boer et al., 2005; Van der Wal et al., 2006a). However, not much attention has been paid to factors regulating initial wood decomposition and the relative importance of bacteria and fungi therein. In later stages of decomposition, the lignocellulose matrix in wood is attacked by slow growing, saprotrophic, lignocellulolytic fungi (Boddy and Watkinson, 1995; De Boer et al., 2005).

Factors that influence the decomposing activity of fungi during wood decay are moisture content, oxygen concentration, acidity, temperature and nutrient concentrations, especially that of nitrogen (Nicholas and Crawford, 2003). However, these factors have mostly been studied for the more advanced stages of wood decay (Daniel, 2003; Schmidt, 2006). Little is known about the factors that regulate the first stages of decomposition.

The effect of nitrogen additions on litter decomposition has been studied extensively and it is generally concluded that initial decomposition of litter is nitrogen-limited as N addition often results in higher mass loss or respiration (Fog, 1988; Berg et al., 1998; Eiland et al., 2001; Moorhead and Sinsabaugh, 2006). This is attributed to the relief of N limitation for fast-growing fungi and bacteria (Fog, 1988; Carreiro et al., 2000). However, some studies found no effect of N addition on decomposition (Prescott, 1995; Hobbie and Vitoesek, 2000). Thus, results are not consistent and the mechanism of the effect of nitrogen on the initial decomposition rate is still not clarified. Differences in the relative importance of bacteria and fungi may contribute to the variation in decomposition response upon nitrogen addition. It is expected that bacteria will respond more to nitrogen addition than fungi, as the latter are thought to be more efficient in the use of nitrogen (re-allocation) (Griffin, 1985). Nitrogen addition may therefore result in a shift from fungal to bacterial dominance. In addition to possible shifts in microbial community structure, microbial growth efficiency is expected to be affected by high levels of nitrogen. Previous studies suggested that high levels of nitrogen increase the growth yield efficiency of micro-organisms, i.e. per unit of utilized substrate C a greater proportion of biomass is produced than under nitrogen limited conditions (Blagodatskiy et al., 1993; Agren et al., 2001; Thiet et al., 2006). Less energy is then required for obtaining N, resulting in a relatively lower respiration. Wood and litter have low levels of nitrogen, and decay fungi have developed strategies to deal with this sub-optimal nitrogen

concentration for growth. One of the strategies is the translocation of nitrogen via hyphae from soil to wood or litter (Frey et al., 2000).

Another potential factor affecting the rate of initial wood decomposition is the accessibility of the substrate. In a former study on constraints for fungal biomass development in abandoned arable fields, we showed that the biomass of opportunistic fungi was enhanced after mixing sawdust into ex-arable soils (Van der Wal et al., 2006a). However, the small size of the substrates and the location (mixing into the soil) may have had also a positive influenced on the growth of bacteria. Cellulose and hemi-cellulose are than more accessible and in close contact with soil nitrogen, providing a more favorable environment for bacterial growth relative to fungal growth (Holland and Coleman, 1987). Larger sizes of substrate and placement on top of the soil, e.g. resembling a litter layer with low nitrogen content, are expected to favor filamentous fungi over bacteria since the substrate may be far more difficult to reach for unicellular soil organisms like bacteria (Griffin, 1985; Frey et al., 2000; Klein and Paschke, 2004).

In this study we examined in microcosm experiments the effects of wood fragment size, vertical position of the woody fragments and nitrogen availability on dynamics of fungal biomass and – decomposition activities as well as growth yield efficiencies in birch wood. This was done for a recently abandoned ex-arable soil and a heathland soil. These types of soil were chosen to compare the fungal dynamics in a bacterial dominated soil (recently abandoned arable land) and a fungal dominated soil where birch wood is a generally occurring woody substrate (heathland). The effect of all treatments on development of bacterial numbers was taken into account to understand the fungal biomass dynamics.

Material and methods

Soil and sampling

One ex-arable field (abandoned since 2002), named Telefoonweg (about 4 ha), with a low fungal biomass and one heathland, named Mossel (about 4 ha), were selected from a previous study (Van der Wal et al., 2006b). The heathland soil is podzolic (Typic Haplorthod) developed in glacial sandy deposits and the ex-arable soil has a disturbed profile due to agricultural activities. The soil characteristics of both fields are listed in Table 1. We collected at least 80 kg of the upper 10 cm of mineral soil from a plot of 30 x 30 m. Since the upper 3 cm of the heathland soil consisted of organic matter, the 3-13 cm layer was collected. For each site, the soil was bulked, homogenized, sieved (4 mm mesh) and stored field moist at 4˚C for not more than 10 days until the start of the experiment.

Table 1: Soil characteristics of the mineral horizon; 0-10 cm for the ex-arable and 3-13 cm for the heathland site

Soil name	Soil type	pH	C g kg^{-1}	C:N ratio	Ergosterol content mg kg^{-1}	Soil texture
Telefoonweg	Arable field abandoned in 2002	5.4	22.5	26.2	0.16	Coarse sand
Mossel	Heathland	3.9	40.8	35.1	0.25	Coarse sand

Experimental set-up

In order to determine the effect of substrate accessibility on initial wood decay, two fragment sizes of birch wood were used in the experiments namely birch wood blocks (dimensions: 3 x 0.5 x 0.5 cm) and birch sawdust (dimensions: 0.5-2 mm). To create a similar thickness of woody layers (0.5 cm), twice as much weight (10.0 g dry weight) of wood blocks than of wood sawdust (5.0 g dry weight) was enclosed in nylon bags (7 x 7 cm, 1 mm mesh size), since the volume of sawdust was approximately twice as much as that of the blocks. Approximately 550 g of each soil was weighed into plastic boxes (10 x 10 x 5 cm). To examine the effects of burial on initial wood decay, the autoclaved wood-containing bags were either placed on top of the soil or buried in the soil (2 cm soil, wood-containing bag, 3 cm soil on top of the bag). Soil without wood addition served as controls. Finally, we included the effect of N enrichment on initial wood decay. A C: N ratio of 20 was created by autoclaving the wood-containing bag followed by dripping carefully a $(NH_4)_2(SO_4)$ solution in water on the wood. The wood absorbed all added $(NH_4)_2(SO_4)$ solution. These samples are hereafter referred to as samples with a low C: N ratio, whereas samples that did not receive the $(NH_4)_2(SO_4)$ solutions are referred to as samples with a high C: N ratio. Total moisture content of woodblocks was adjusted to 80% (w/w) which is a normal value for moisture content in hardwood. For sawdust fragments, having a higher surface/volume ratio, moisture content was adjusted to 150%, which is in the range of the moisture content of the litter layer in the heathland. Soils were incubated at their respective field moisture content (at the day of sampling) which was 11.8% (w/w) and 13.9% (w/w) for the ex-arable field and the heathland, respectively. Soils were incubated at 20° C and boxes were weighed during incubation to adjust for any water loss if needed. Four replicate samples per treatment were separately analyzed after 15, 25 and 40 weeks of incubation. After each incubation period, wood was collected from each bag and homogenized per sample and adjacent soil (0.5 cm) was collected to assess the colonization of soil by exploring hyphae from wood. The rest of the soil (hereafter referred to as 'bulk' soil) was mixed per sample. Wood blocks were homogenized and, after determining the dry weight, ground in a mill into sawdust. Wood and soil samples were stored at -20º C for not more than 1 week until analyses.

Mass loss and fungal biomass dynamics

Mass loss of wood in the nylon bags was calculated as the difference in dry weight between fresh and incubated woody fragments and is expressed as a percentage of the original mass. Ergosterol, a sterol only found in fungal cell membranes, was used as a fungal-specific biomarker and extraction from wood samples was based on extraction of soil ergosterol described by Bååth (2001). Briefly, 0.25 g of wood of each sample was added to 1 ml cyclohexane and 4 ml 10% KOH in methanol. After 15 min ultrasonic treatment, the samples were heated at 70° C for 90 min. Next, 1 ml distilled water and 2 ml cyclohexane was added to the tubes, which were then vortexed for 30 s and centrifuged. The top phase was removed and the remaining wood suspension was extracted again with 2 ml cyclohexane and the combined cyclohexane fractions were evaporated under N_2 at 40°C. The precipitates were dissolved in methanol and then filtered over a 0.2 μm filter. Samples were transferred to an auto-sampler for HPLC analysis. Ergosterol in soil samples was extracted according to Gong et al. (2001), since it has been shown to be as good as the more laborious Bååth method in samples with a low organic matter content (De Ridder-Duine et al., 2006). Briefly, 4 g of fresh soil was added to 6 ml methanol and 2 g 0.3-0.5 mm and 2 g 0.7-1 mm glass beads. After samples were shaken for 1 h, the supernatant was centrifuged for 10 min at 11000 rpm, filtrated over a 0.2 μm filter and samples were ready for HPLC analysis. Ergosterol was measured using a Dionex HPLC equipped with a C 18 reverse-phase column (De Ridder-Duine et al., 2006).

Chitin, an important structural component of the fungal cell wall, was measured by determining the hexosamine content of ground wood samples (500 mg) after hydrolysis with 5 ml 6 N HCl (24 h at 100°C) (Eikenes et al., 2005). Hexosamine was measured colorimetrically using the method of Johnson (1971).

Bacterial numbers

Bacteria were enumerated by plate counting. Wood samples (0.5 g) were transferred to polypropylene screw-cap tubes containing 10 ml of sterile phosphate buffer (0.25 g l^{-1} KH_2PO_4 in de-mineralized water; adjusted to pH 6.5). The tubes were shaken intensively on a bench top for 90 min at 20° C, sonicated at 47 kHz for 10 min, and shaken for an other 30 min. Ten-fold dilutions were made in phosphate buffer and 50 μl of appropriate dilutions were plated on 1/10 strength TSB agar (NaCl 5 g l^{-1}, KH_2PO_4 1g l^{-1}, 3 g l^{-1} Tryptic Soy Broth [Oxoid, Basingstoke, England] 1g l^{-1}, agar 20 g l^{-1}; pH 6.5) Media contained 50 mg l^{-1} of the fungal inhibitor natamycin [Delvocid, DSM, Delft, NL]. Plates were incubated in the dark at 20° C for 2 weeks. During this period, plates were checked regularly for colony formation.

Enzyme assays

All enzyme activities were assayed in the same extracts. Briefly, 4 ml of milliQ water was added to 1 g milled, fresh wood and shaken for 1 h at room temperature. Enzyme extracts were made by pressing the moistened, milled wood over an iron filter (pore size 2 mm dia). Filtrates were transferred to Eppendorf cups and after centrifuging for 10 min at 10000 rev min^{-1}, the supernatants were frozen (- 20 °C) until analysis of enzyme activity.

Laccase and manganese peroxidase activities were measured as indicators for lignin degradation (Leonowicz et al., 1999). Laccase activity was measured via oxidation of ABTS (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) according to Bourbonnais and Paice (1990). Manganese peroxidase activity was measured via the oxidative coupling of DMAB (3-dimethylaminobenzoic acid) and MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride) in the presence of Mn^{2+} and H_2O_2, as described by Daniel et al. (1994).

Activity of endo-1,4-ß-glucanase as an indicator of cellulase was estimated using carboxymethyl cellulose linked with Remazol brilliant blue R(Azo-CM-Cellulose, Megazyme, Bray, Ireland). The reaction mixture contained 200 µl of 2% Azo-CM-Cellulose in MQ and 200 µl enzyme extract. Samples were incubated at 40°C for 30 min and the reaction was stopped by adding 1 ml of precipitation solution (20% sodium acetate trihydrate and 3% zinc acetate in 100 ml MQ). The formation of blue dye was measured spectrophotometrically at 590 nm. The conversion to activity was based on a calibration curve of Remazol brilliant blue (RBB) and expressed as mmol RBB/24 hours/gram dry weight. The activity of endo-1,4-ß-xylanase (xylanase) as indicator of hemicellulase was estimated by a similar procedure using birchwood xylan linked with Remazol brilliant blue (Azo-xylan, Megazyme, Bray, Ireland) as a substrate.

Analysis of nitrogen availability

To determine the leaching of mineral N from the wood into the soil, mineral N of both portions of soil (adjacent soil and 'bulk' soil) was extracted by shaking 2 g soil (dry weight) in 10 ml 1 M KCl for 1 hour. Mineral N in the wood was determined by shaking 0.5 g wood (dry weight) in 5 ml 1 M KCl for 1 hour. Concentrations of NH_4^+-N and NO_3^--N in the KCl extract were determined colorimetrically using a Traacs 800 auto-analyzer. Total C and N of wood after the second and third incubation period were analyzed on a FlashEA 1112 Series NC soil analyzer.

Statistical analyses

All data were analyzed per field using univariate regression within the general linear model (GLM) procedure in SPSS for Windows (Release 12.0.1; standard version) with C:N ratio,

placement of the wood-containing bag, wood fragment size and incubation time as factors. The assumption of normality was tested with Shapiro-Wilk statistics and homogeneity of variance was assessed with Levene's test. Differences between groups were tested with Tukey's honestly significant difference test or, when variances were unequal, with Tamhane's T2 test at $P<0.05$.

To determine the amount of fungal biomass growing into the soil, we first compared the amount of ergosterol content in control soil (without wood addition) with that of wood-containing bulk soil. The difference in ergosterol content between the control soil and the soil that was > 0.5 cm separated from the wood-containing bags turned out to be less than 4% in all treatments, thus we assumed that the amount of fungal biomass did not increase in the bulk soil due to the presence of wood-containing bags. Then, we calculated the percentage of fungal biomass growing from wood into adjacent soil (< 0.5 cm) as (the amount of ergosterol in adjacent soil - the amount of ergosterol in 'bulk' soil)/ ergosterol content measured in the wood)* 100%.

The relation between fungal biomass (ergosterol or chitin content) and mass loss of wood was calculated by the coefficient of the linear equation at $P = 0.05$. We defined the growth yield efficiency of fungi as the amount of fungal biomass produced per unit of substrate and calculated this as (ergosterol content/ % wood mass loss).

Results

Mass loss

Over the whole incubation period, strong differences in mass loss were observed between different treatments. The lowest decay (about 5 % mass loss) had occurred in N-enriched sawdust that was buried in heathland soil, whereas the strongest decay (about 47% mass loss) had occurred in N-enriched wood blocks buried in ex-arable soil. In general, the mass loss of woody fragments was higher for the ex-arable soil than for the heathland soil ($P<0.05$, Fig. 1). For the ex-arable soil, mass loss of wood increased with incubation time for all treatments, except for wood blocks with a high C:N ratio placed on top of the soil ($P>0.84$, Fig. 1). For the heathland soil, mass loss only increased with time in treatments with a high C:N ratio, except for wood blocks placed on top of the soil ($P>0.22$, Fig. 1). For both soils, mass loss of wood was, in general, higher in blocks than in sawdust. Over the whole incubation period, nitrogen addition increased mass loss of both wood blocks and sawdust in the ex-arable field, however, for the heathland soil nitrogen addition did not have such an effect. Burial of the woody fragments had a significant positive effect on decay for the ex-arable soil ($P<0.05$, Fig.

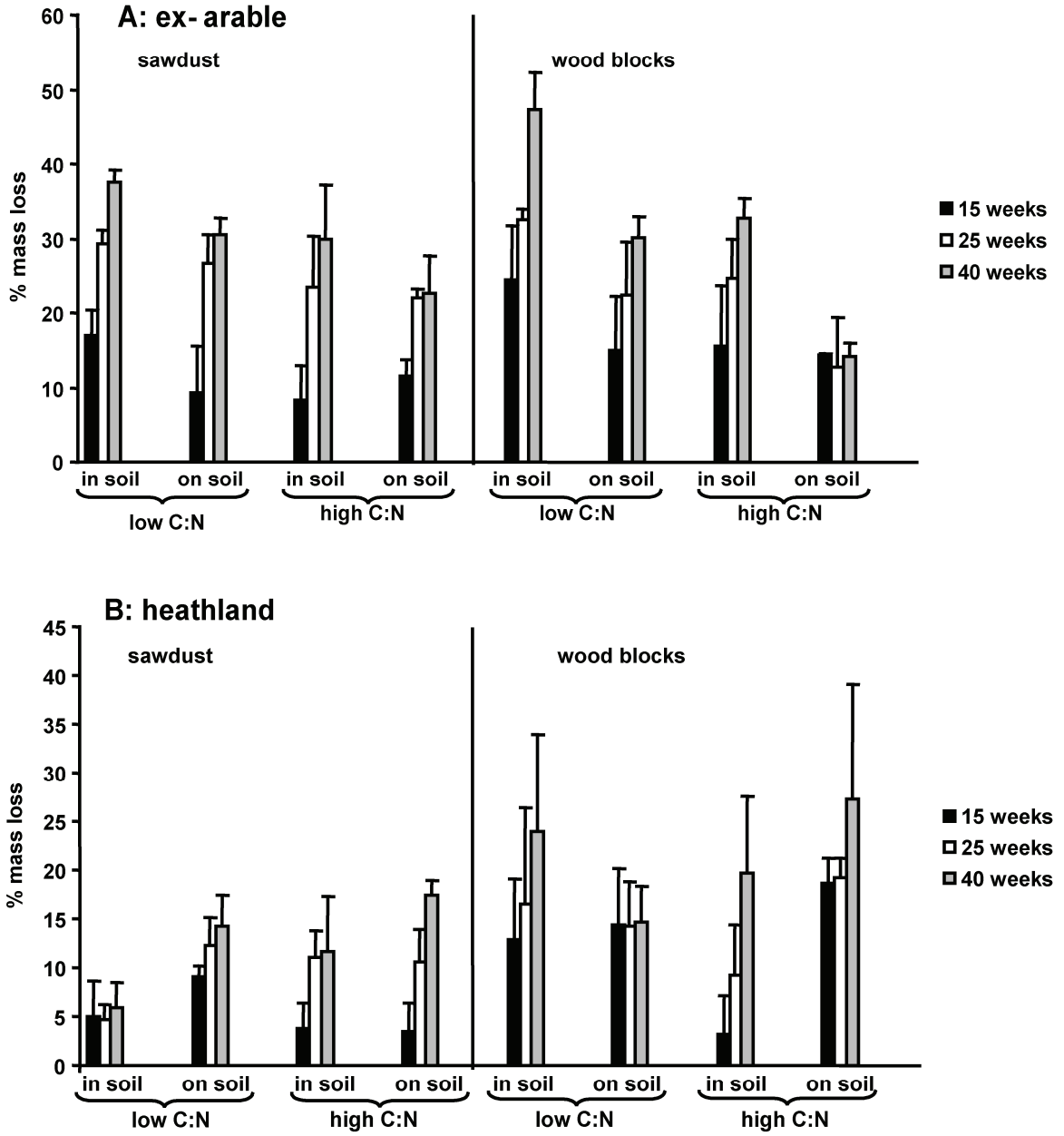

Figure 1: Effect of nitrogen addition (low C:N/ high C:N) and vertical position (in soil/on soil) on mass loss of sawdust and of wood blocks after 15, 25 and 40 weeks of incubation. A) wood incubated in/on ex-arable soil, B) wood incubated in/on heathland soil.

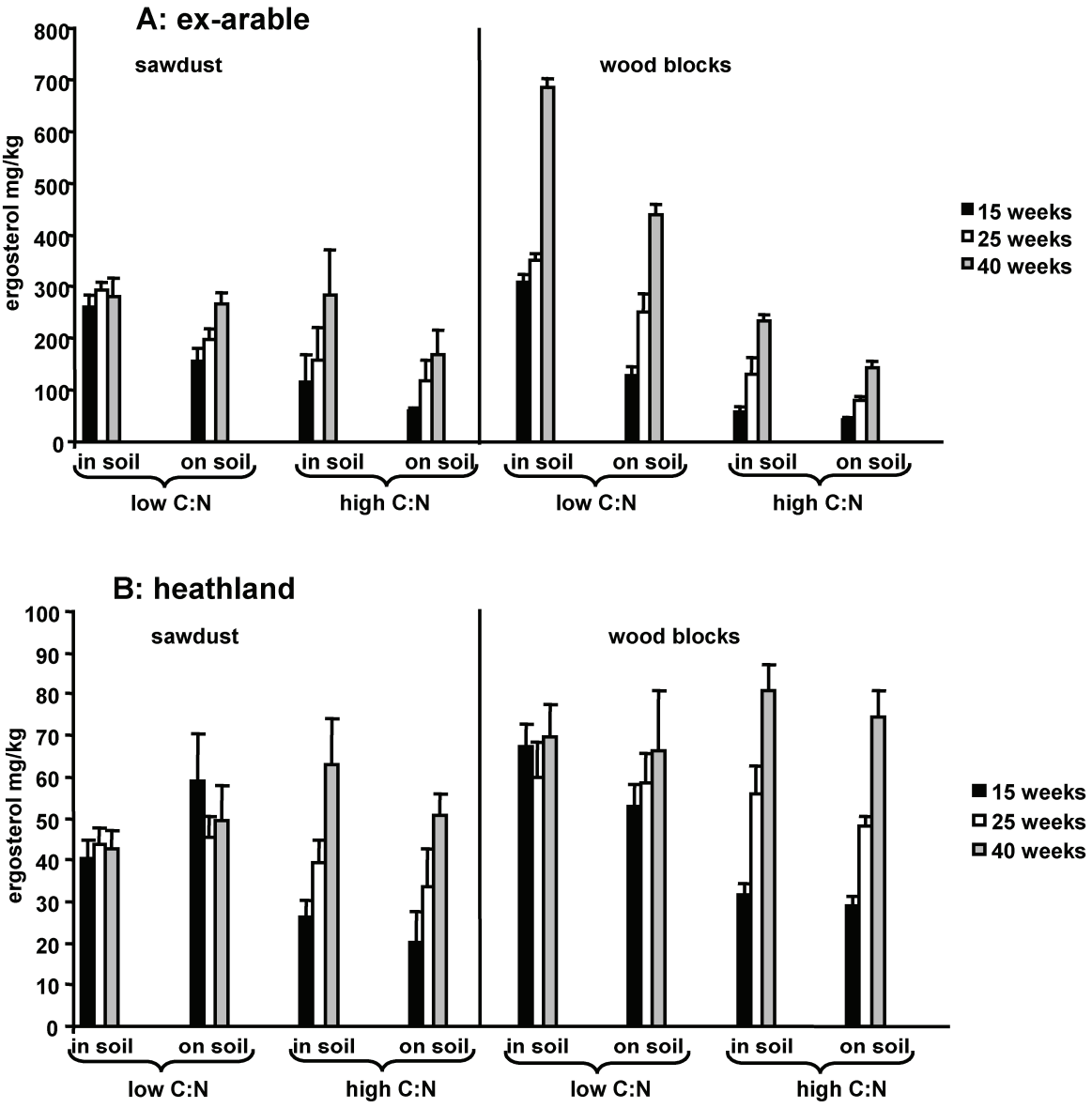

Figure 2: Effect of nitrogen addition (low C:N/ high C:N) and vertical position (in soil/on soil) on ergosterol content (mg/kg) in sawdust and in wood blocks after 15, 25 and 40 weeks of incubation. A) wood incubated in/on ex-arable soil, B) wood incubated in/on heathland soil.

1). The opposite was found for the heathland soil i.e. a higher decay of woody fragments that were incubated on top of the soil ($P<0.05$, Fig. 1).

For the ex-arable soil, fungal biomass (ergosterol) and mass loss correlated positively ($P<0.05$), except for wood blocks with a high C:N ratio placed on top of the soil and sawdust with a low C:N ratio buried in soil ($P> 0.08$ and 0.37 respectively). In the heathland, mass loss correlated significantly with ergosterol content in treatments with a high C:N ratio whereas this was not the case for treatments with a low C:N ratio ($P>0.05$).

Fungal biomass dynamics and bacterial numbers

Ergosterol and chitin, the two biomarkers that were used to follow fungal biomass dynamics during initial wood decay, were significantly correlated ($R^2= 0.93$, $P<0.05$). Therefore, the results of only one of the biomarkers, ergosterol, will be presented. For both soils, ergosterol was, in general, higher in woodblocks than in sawdust ($P<0.05$, Fig. 2). Nitrogen addition had a positive effect on the ergosterol content for all treatments during the first incubation period. However, at later sampling times the effect of nitrogen addition had disappeared, except for the woodblocks in/on ex-arable soil ($P<0.05$, Fig. 2). In contrast, all wood samples without N-enrichment showed an increase of ergosterol with incubation time. The vertical position of the woody fragments had also an effect on ergosterol content: buried fragments had, in general, a higher ergosterol content than those incubated on top of the soils ($P<0.05$, Fig. 2). There was a strong effect of soil origin on the amount of ergosterol: woody fragments incubated in/on heathland soil contained much less ergosterol than those incubated in/on ex-arable soil.

For both soils and all treatments, exploratory growth of fungi that colonized the wood was limited as ergosterol in soil adjacent to woody fragments was less than 0.3 % of that in wood.

Fragment size, soil origin and nitrogen addition had strong effects on bacterial numbers (Table 2a,b). For both soils, the numbers of bacteria in woodblocks were always lower than the detection limit ($8*10^4$ CFU g^{-1} dry wood) of the method used. For the heathland soil, bacterial numbers were below the detection limit in most of the sawdust samples. Detectable, but low, numbers of bacteria were only found to be present at the first harvest in sawdust without nitrogen enrichment (Table 2b). In contrast, high numbers of bacteria (up to $4*10^8$ CFU g^{-1} dry wood) were present in several sawdust samples that had been incubated in/on ex-arable soil. For this soil a strong inhibiting effect of nitrogen addition on bacterial CFU was observed during the first harvest ($P<0.05$, Table 2a). In fact, a similar effect was apparent for the heathland soil albeit that the numbers of bacteria in the non-nitrogen enriched sawdust samples were much lower than in the ex-arable soil ($P<0.05$, Table 2b). The negative effect of nitrogen addition on bacterial numbers in sawdust in/on ex-arable

Table 2a: Effect of nitrogen addition (low C:N/ high C:N) and vertical position (in soil/on soil) on bacterial numbers (g^{-1} dry weight wood) in sawdust and in wood blocks after 15 and 40 weeks (wks) of incubation in the ex-arable soil. Letters A and B denote differences at P<0.05 level between sawdust samples with a low and a high C:N ratio.

SAWDUST								WOODBLOCKS							
low C:N ratio				high C:N ratio				low C:N ratio				high C:N ratio			
in soil		on soil		in soil		on soil		in soil		on soil		in soil		on soil	
15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks
3.60E+0.6 A	2.00E+0.8	1.00E+0.6 A	1.34E+0.8	2.44E+0.8 B	2.80E+0.8	1.32E+0.8 B	4.26E+0.8	<8000	<8000	<8000	<8000	<8000	<8000	<8000	<8000

Table 2b: Effect of nitrogen addition (low C:N/ high C:N) and vertical position (in soil/on soil) on bacterial numbers (g^{-1} dry weight wood) in sawdust and in wood blocks after 15 and 40 weeks of incubation in the heathland soil. Letters A and B denote differences at P<0.05 level between sawdust samples with a low and a high C:N ratio.

SAWDUST								WOODBLOCKS							
low C:N ratio				high C:N ratio				low C:N ratio				high C:N ratio			
in soil		on soil		in soil		on soil		in soil		on soil		in soil		on soil	
15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks	15 wks	40 wks
<8000 A	<8000	<8000 A	<8000	<8000 A	<8000	1.20E+0.6 B	<8000	<8000	<8000	<8000	<8000	<8000	<8000	<8000	<8000

soil was still apparent at the last harvest. Yet, numbers of bacteria in the nitrogen treated samples were much higher than at the first harvest.

The presence of low numbers of bacteria in wood samples based on plate counts was confirmed by the inability to obtain PCR-amplified 16S RNA gene fragments from these samples (results not shown).

Enzyme activities

For all treatments and throughout the whole incubation period, both sawdust and woodblocks had very low activities of the enzymes laccase and manganese-peroxidase, indicating that degradation of lignin was insignificant (data not shown).

In contrast to the low lignolytic enzyme activities, both cellulase and hemi-cellulase activities were clearly detectable. Differences between treatments became already apparent after the first incubation period when both cellulase and hemi-cellulase activities peaked. The (hemi-) cellulase activities in the woody fragments were lower for the heathland soil than for the ex-arable soil ($P<0.05$, Fig. 3 & 4). Nitrogen addition had a strong positive effect on hemi-cellulase and cellulase activities in sawdust and woodblocks for both soils ($P<0.05$, Fig. 3 & 4). In addition to nitrogen-enrichment, fragment size had also an effect on the (hemi-) cellulase activities: in the ex-arable soil woodblocks had higher cellulase and hemi-cellulase activities than sawdust, whereas the reverse was observed for the heathland soil ($P<0.05$, Fig. 3 & 4). Burial of the woody fragments had a significant positive effect on hemi-cellulase and cellulase activities for both fields ($P<0.05$, Fig. 3 & 4).

Fungal growth yield efficiency

For the ex-arable soil, the fungal growth yield efficiency, the amount of fungal biomass produced per % mass loss (= indication of amount of utilized substrate), was higher in nitrogen-enriched wood than in untreated wood ($P<0.05$, 11.5 versus 7.3). Burial of woody fragments had also a positive effect on growth yield efficiency ($P<0.05$, 10.2 versus 8.5). However, the size of the wood fragments did not influence the efficiency ($P>0.88$). During incubation, the efficiency increased after the second harvest ($P<0.05$, from 9.9 to 10.2 to 9.9 again). For the heathland soil, the fungal growth yield efficiency showed no significant differences between treatments ($P>0.19$).

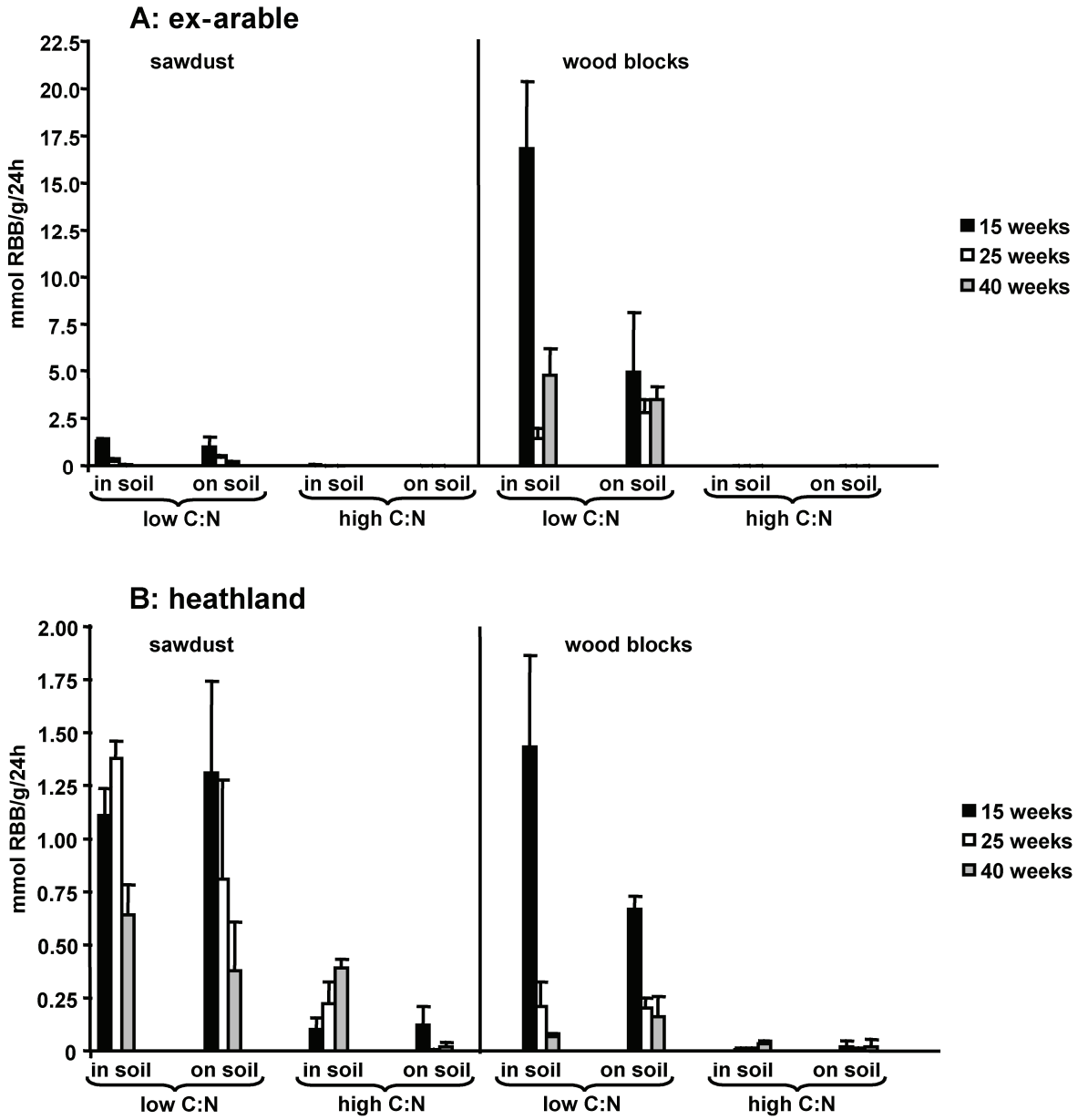

Figure 3: Effect of nitrogen addition (low C:N/ high C:N) and vertical position (in soil/on soil) on hemicellulase (mmol Remazol brilliant blue (RBB)/g/24h) activity in sawdust and in wood blocks after 15, 25 and 40 weeks of incubation. A) wood incubated in/on ex-arable soil, B) wood incubated in/on heathland soil.

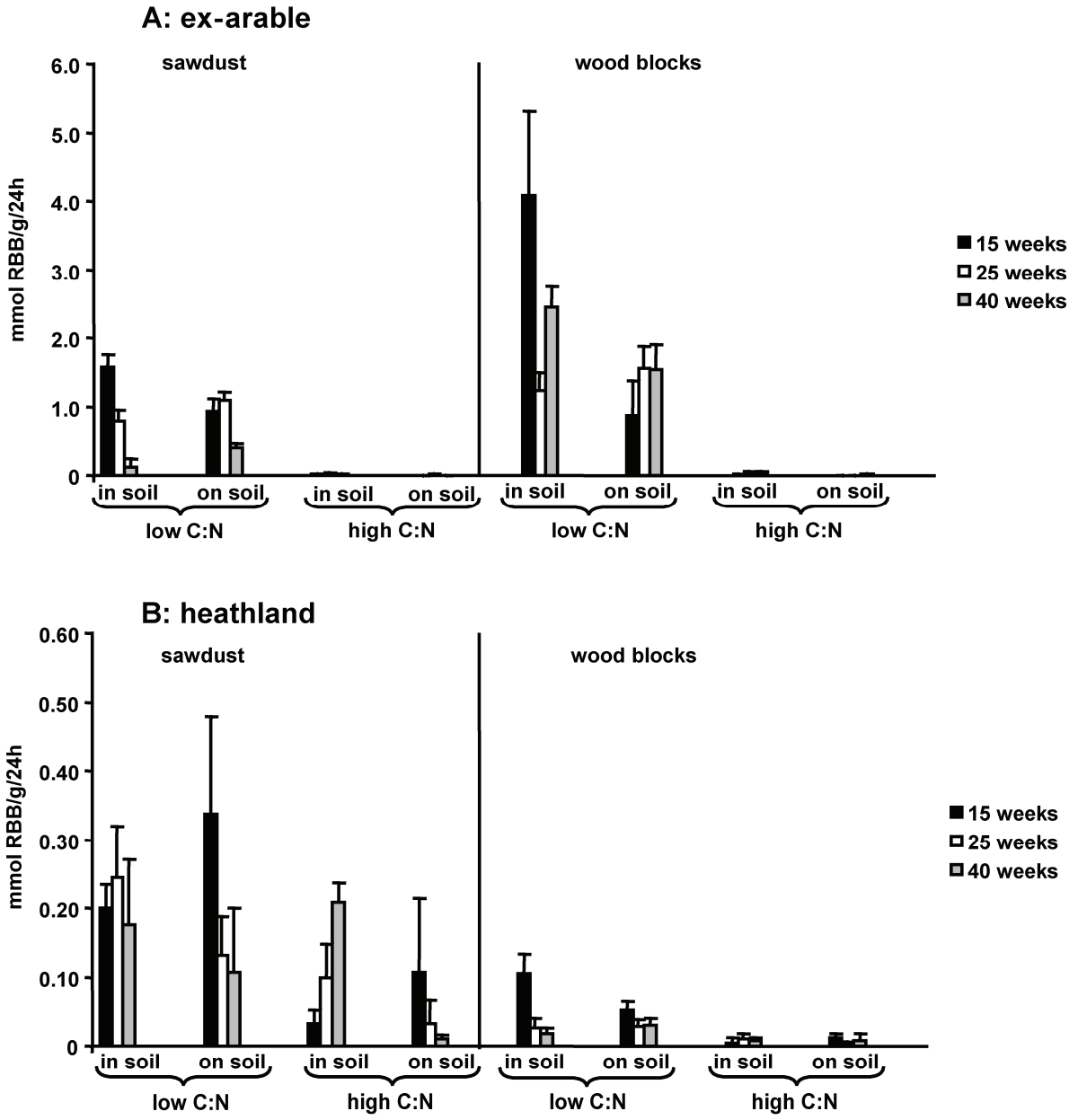

Figure 4: Effect of nitrogen addition (low C:N/ high C:N) and vertical position (in soil/on soil) on cellulase (mmol Remazol brilliant blue (RBB)/g/24h) activity in sawdust and in wood blocks after 15, 25 and 40 weeks of incubation. A) wood incubated in/on ex-arable soil, B) wood incubated in/on heathland soil.

Transfer of nitrogen between wood and soil

After the first incubation period, at least 90% of added mineral N had disappeared from the wood and about 90 % of this fraction could be recovered from the soil (mean of 2.2% in adjacent soil and 88.2% in 'bulk' soil).

The initial total amount of N in wood without nitrogen addition was 3.3 and 5.7 mg N for sawdust and woodblocks, respectively. This amount had increased after the second (mean of 18.3 mg N) and third incubation period (mean of 23.0 mg N). For the ex-arable soil, the increase in total N in wood between the second and third incubation period was 4.7 mg N in wood without nitrogen addition and 7.6 mg N in wood treated with nitrogen. The difference between these values is not significant ($P>0.10$), indicating that nitrogen addition did not influence the effect of nitrogen transfer to wood. For the heathland soil, nitrogen addition did not influence the effect of nitrogen transfer to the wood either ($P>0.10$).

Discussion

All factors that we have examined, i.e. soil origin, size of the woody fragments, their vertical position (in soil versus on top of soil) and nitrogen addition had an effect on fungal and bacterial dynamics and initial wood decomposition.

Soil origin

Wood decomposition (mass loss), cellulolytic enzyme activities, fungal biomass indicators (chitin and ergosterol) and bacterial numbers were much lower in woody fragments incubated in/on the heathland than in those incubated in/on the ex-arable soil. Therefore, the conclusion that soil origin is an important factor seems justified even though only 2 soils were studied. The lower density and activity of fungi in woody fragments in/on heathland may be due to the inhibiting activity of polyphenolic materials diffusing into the woody fragments. Organic matter composed of remainders of *Calluna vulgaris* (heather) is rich in polyphenolic compounds that have been shown to be inhibitory to microbial processes (Jalal and Read, 1983). Such inhibitory compounds may also explain the very low numbers of bacteria in woody fragments in/on the heathland soil. The lower pH of woody fragments in/on heathland soil as compared to that in/on ex-arable soil may also have affected growth and activity of wood-inhabiting microorganisms. However, this seems more likely for bacteria than for fungi as most fungi are well able to cope with low pH levels (Griffin, 1985; Mulder et al., 2005).

Size of the woody fragments

Both ergosterol and chitin determinations indicated that fungal biomass was higher in woodblocks than in sawdust. The decomposition rate (mass loss) of wood was also higher in woodblocks. For the ex-arable soil, bacterial numbers in woodblocks were much lower than in sawdust. Opportunistic bacteria may be constrained to colonize woodblocks as they do not form hyphae to penetrate solids (Griffin, 1985; Frey et al., 2000; Klein and Paschke, 2004). Hence, for the ex-arable soil a better fungal growth in woodblocks as compared to sawdust may be caused by a lower competitive pressure of bacteria. For the heathland soil, this explanation is not likely as numbers of bacteria were below the detection limit in most cases for both woodblocks and sawdust. Here, differences in fungal biomass and activity between sawdust and woodblocks may be caused by a different rate of absorbance of inhibitory heather-derived polyphenolics.

Our observations for the ex-arable soil appear to be in contrast with the findings of Griffith and Bardgett (2000) who found no differences in fungal activity in grass lamina fragment of different sizes. However, they used sterile pieces of grass and inoculated only isolated fungi. Therefore, in their study no other soil micro-organisms could negatively affect fungal colonization of smaller fragments. Thus, in a more natural environment the competitive pressure of bacteria against fungi in small-sized lignocellulose-rich materials may be substantial.

Vertical position of the woody fragments

For both soils, buried wood (sawdust and blocks) developed a higher fungal biomass and cellulase and hemi-cellulase activities than wood placed on top of the soil. These results, together with the undetectable or very low lignolytic enzyme activities, indicate that opportunistic, cellulolytic fungi were dominating the initial decomposition process and were favored by burial of the substrate. The positive effect of burial on fungal biomass and – activity was already apparent at the first harvest and appears not to be related to interactions with bacteria as the numbers of bacteria were not affected by burial at this stage. The positive effect of burial on fungal biomass and activity is in agreement with Osono et al. (2006) who found more fungal hyphae on needles placed beneath the litter layer than on needles on the litter surface. They ascribed this to the higher moisture content of the needles in the soil. In our study, the moisture content of woody fragments buried in soil was generally similar to those placed on top of soil. Therefore, a difference in moisture content between wood buried in soil and that on top of soil is not likely as an explanation for our results.

Nitrogen addition

In general, addition of nitrogen to wood resulted in an increase of decomposition and of fungal biomass formation. However, this was a short-tem effect since after prolonged incubation (40 weeks), differences between ergosterol content in wood with and without N addition diminished in most cases. Interestingly, colonization of sawdust by bacteria was reduced by addition of nitrogen, but also this effect disappeared with time. Hence, nitrogen addition appeared to be more advantageous for opportunistic fungi than for bacteria. This is surprising as bacteria are expected to be more affected by N limitation than fungi (Griffin, 1985). Degradation of purified cellulose is often stimulated by high levels of mineral N (Fog, 1988; Carreiro et al., 2000; Sjöberg et al., 2004; Waldrop et al., 2004). Wood is composed of cellulose, hemicellulose and lignin. Part of the cellulose and hemicellulose is easily accessible and, therefore, the first increase of decomposition activity after addition of nitrogen is probably due to the stimulation of degradation of this fraction of the wood. This is also confirmed by the N-induced increase of activity of (hemi-)cellulase after the first incubation period. Hence, it appears that the decay activities of the opportunistic fungi were nitrogen limited. The leveling-off of ergosterol concentrations and (hemi-)cellulase activity during subsequent incubation would than be explained by the complete utilization of all easily accessible (hemi-)cellulose, or by the immobilization of nitrogen into microbial biomass, resulting in renewed nitrogen limitation (Terziev and Nilsson, 1999). In addition, most of the added nitrogen (90%) appeared to have leached from the woody fragments to the soil during the first incubation period, making nitrogen less available for microbes during later incubation intervals.

The amount of exploratory hyphae was very low in all treatments. We expected that N translocation would be most pronounced in untreated wood to overcome nitrogen deficiency (Scheu and Schauermann, 1994). Although our results did not show differences in nitrogen translocation between N-enriched and non-enriched wood, there was an increase in nitrogen in almost all treatments. Therefore, it appears that a low amount of exploratory hyphae is sufficient for efficient transport of nitrogen. The fact that nitrogen addition to wood did not appear to have an effect on N translocation may be due to the aforementioned leaching of the added N from wood fragments.

In the ex-arable soil, the growth yield efficiency was higher after nitrogen addition which confirms previous studies (Blagodatskiy et al., 1993; Agren et al., 2001; Thiet et al., 2006). A possible explanation for this is that there is a shift in the fungal decomposer community towards fungi that are more efficient but have a greater nitrogen demand (Agren et al., 2001). However, in the heathland soil we could not detect such an increase in efficiency of fungi after nitrogen addition. This may point to a different fungal community composition

in wood exposed to heathland soil. The inhibiting compounds present in the heathland soil may be more important for selection of fungi than nitrogen addition.

In most treatments, loss of wood mass was positively correlated with ergosterol content confirming that fungi were responsible for the decomposition of wood. However, in nitrogen-enriched sawdust buried in ex-arable soil we observed an increase in mass loss without an increase in fungal biomass. In the nitrogen-enriched sawdust in/on ex-arable soil, bacterial numbers were relatively low at the first harvest and increased strongly during prolonged incubation. Hence, the lack of correlation between fungal biomass and mass loss may be due to an increase in bacterial decomposing activity during prolonged incubation.

The growth of rot fungi in wood was, even after 40 weeks of incubation, still very low as indicated by the low activity of lignin degrading enzymes. Therefore, the incubation time of this study appears to be too short for lignocellulolytic fungi to colonize the substrate or to produce lignin degrading enzymes. However, it is also possible that antagonistic, opportunistic fungi were hampering the colonization of rot fungi (Greaves, 1972).

In this study, initial wood decomposition by bacteria appeared to be mainly restricted to sawdust incubated in/on ex-arable soil. The use of bacterial colony forming units as indicators for bacteria biomass development may be questioned as many bacteria are not cultivable. However, PCR-amplification of 16S rDNA fragments of bacteria confirmed that bacteria were present in very low numbers in all treatments except sawdust in/on ex-arable soil.

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