Biol Fertil Soils (2002) 35:41–48
DOI 10.1007/s00374-001-0438-0

ORIGINAL PAPER

Trond M. Henriksen · Tor A. Breland

Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil

Received: 17 October 2001 / Published online: 25 January 2002

Abstract The degree of contact between crop residues and the soil matrix, as determined by the method of residue incorporation, affects decomposition dynamics both under natural and experimental conditions. In microcosm experiments we tested the hypothesis that poor residue-soil contact reduces the decomposition of structural plant constituents through delayed colonization by microorganisms degrading cellulose and hemicellulose. Barley straw or red clover foliage was either confined in buried mesh bags or homogeneously mixed into a loamy topsoil or a silty subsoil to create poor or intimate residue-soil contact in microbiologically rich and less rich environments, respectively. Soil type had no effect on decomposition of the easily degradable clover residues, but cumulative mineralization of barley straw C after 52 days at 15°C was less in the subsoil than in the topsoil by 12% of initial C. For clover material, poor soil contact increased cumulative C mineralization by 5% of initial C in the loamy topsoil but had no effect in the silty subsoil. For the more slowly degradable, cellulose- and hemicellulose-rich straw, on the other hand, poor soil contact reduced C mineralization by 6% of initial C. The results from the loamy topsoil were confirmed in a second experiment in a sandy topsoil. The reduced decomposition of straw with poor soil contact could not be explained by less favourable abiotic conditions, N deficiency nor exclusion of larger animals by mesh bags. Reduced straw-soil contact delayed measured increases in fungal ergosterol concentration, ratio of fungal to bacterial substrate-induced respiration, number of cellulase-producing, colony-forming bacterial units and activity of cellulases and hemicellulase on the residues. Thus, the results supported our hypothesis and underscore the importance of ensuring representative conditions for the soil microflora when decomposition dynamics are studied in microcosms experiments designed to mimic field conditions.

Keywords Crop residue · Soil contact · Decomposition · Fungi · Bacteria

T.M. Henriksen (✉) · T.A. Breland
Agricultural University of Norway,
Department of Chemistry and Biotechnology,
P.O. Box 5040, 1432 Ås, Norway
e-mail: trond.henriksen@planteforsk.no
Tel.: +47-61-166911, Fax: +47-61-166901

Present addresses:
T.M. Henriksen, The Norwegian Crop Research Institute,
Apelsvoll Research Centre, 2849 Kapp, Norway

T.A. Breland, Agricultural University of Norway,
Department of Horticulture and Crop Sciences, P.O. Box 5022,
1432 Ås, Norway

Introduction

Crop residues left in the field after harvest are the raw materials for humus formation and may represent a significant supply of nutrients to subsequent crops. Knowledge about residue decomposition dynamics is, therefore, essential for management of agroecosystems. Our present understanding is based on results from numerous decomposition studies performed under various field or laboratory conditions. These include different methods of application, e.g. residues placed on the soil surface, more or less evenly mixed into the soil or confined in mesh bags within the soil. Surface placement or heterogeneous distribution reduces the residue-soil contact as compared with a homogeneous distribution. This may affect the decomposition dynamics (Parr and Reuszer 1959; Beare et al. 1993; Breland 1994; Ladd et al. 1996). Knowledge of such effects is important when results from different studies are being compared and is essential when developing and calibrating decomposition models (Henriksen and Breland 1999a). It is also important when assessing effects of tillage practices resulting in different degrees of residue-soil contact, e.g. no-till, ploughing and rotovating.

The influence of reduced residue-soil contact on decomposition seems to be negligible or slightly positive for easily degradable materials such as alfalfa (McCalla and Duley 1943) and white clover (Breland 1994). The

positive effect of reduced soil contact may be explained by a reduced volume of detritusphere soil, the 4–5 mm surrounding zone that, for readily degradable material in particular, retains a significant part of decomposing residue C and N (Ladd et al. 1996; V. Gaillard, unpublished PhD thesis, ENGREF, Paris, 2001). Decomposition of straw, however, is reduced when it is placed on or above the soil surface as compared with burial in soil (Douglas et al. 1980; Christensen 1986). The density of fungal hyphae is also reduced by surface placement and the fungal species composition is different (Beare et al. 1993). Differences in decomposition have mainly been explained by differences in abiotic factors such as moisture and temperature (Christensen 1986; Douglas and Rickman 1992) or N deficiency when N-poor residues are spatially separated from soil (Ames et al. 1984; Holland and Coleman 1987). However, compared with a homogeneous distribution, decomposition is also reduced if straw is heterogeneously distributed within the soil matrix (Parr and Reuszer 1959). It seems, therefore, that close contact with the soil microflora is a key factor for the degradation of straw. This hypothesis is supported by observation of an increase in the decomposition rate when soil is added to straw residues confined in mesh bags (Malkomes 1980).

Straw residues are, in contrast with herbaceous material such as clover residues, poor in easily utilizable sugars and proteins but rich in cellulose and hemicelluloses. Consequently, straw decomposition depends on an appropriate colonization and growth of microorganisms producing extracellular cellulases and hemicellulases, an activity in which fungi play a prominent role (Swift et al. 1979). Our working hypothesis was, therefore, that a delayed colonization by microorganisms degrading cellulose and hemicellulose contributes to the reduced straw decomposition rate normally observed when residue-soil contact is poor.

Our objective was to determine effects of reduced crop residue-soil contact on degradation of herbaceous (red clover) and structural materials (cereal straw) at the process level and then to investigate our hypothesis that poor residue-soil contact delays colonization by holocellulose-degrading microorganisms in particular.

Materials and methods

Plant and soil material

Stems and leaves of flowering red clover (*Trifolium pratense* L.) and mature wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) straw were dried at 50°C, cut into 2-cm pieces and stored air dry. Selected chemical properties of the plant materials are given in Table 1. Three different soils were taken from sites in southeastern Norway. A loam topsoil was sampled from a spring barley field (Apelsvoll Research Centre, Div. Kise, at 60°47′N, 10°48′E) in October and moderately dried at room temperature until sieving (4-mm mesh) was feasible. A silty subsoil was sampled from a spruce forest (Hvam, at 60°6′N, 11°22′E) in October, dried at 30°C and sieved (1-mm mesh). A sandy topsoil was sampled from a ley near Elverum (at 60°54′N, 10°42′E) in May, moderately air dried and sieved through a 1-mm mesh. Selected soil properties are given in Table 2.

Table 1 Selected chemical properties of the plant materials used in the experiments

	Red clover foliage	Barley straw	Wheat straw
	(% of dry matter)		
Hemicellulose[a]	10.3	33.6	40.5
Cellulose[a]	20.3	47.5	41.5
Lignin[a]	3.4	6.1	5.0
Ash[b]	7.3	4.3	5.8
C[c]	46.2	47.3	46.6
N[c]	2.4	0.6	0.4
C/N ratio	19.4	80.2	116.5

[a] Proximate analysis (Goering and Van Soest 1970)
[b] Oven digestion (550°C)
[c] C/N analysis (Leco CHN-1000)

Experimental treatments

In Experiment 1, we studied C mineralization dynamics for red clover and barley straw as affected by the extent of residue contact with the loamy topsoil and the silty subsoil. Samples equivalent to 2.0 g ash-free dry matter of red clover foliage or barley straw were moistened with 5.0 ml water before placement in soil. The plant materials were either confined in circular nylon mesh bags (5.5 cm×2 cm, 1 mm mesh) and buried (2 cm depth) or homogeneously mixed into soil cores (150 g soil dry matter) contained in PVC pots (height 70 mm, inner diameter 68 mm) that were closed at the lower end by a 1-mm nylon mesh and placed on Petri dishes. Soil containing barley, but not red clover, was fertilized with $(NH_4)_2SO_4$ to a level of 870 mg inorganic N kg^{-1} dry soil to avoid N-limited straw decomposition (Henriksen and Breland 1999b). Control cores of the two soils without added plant materials were also prepared, and all cores were incubated at 15°C and –30 kPa water potential (adjusted weekly by addition of water to the target weight).

In Experiment 2, the objective was to study whether distribution effects on C mineralization from herbaceous and structural material, as observed in Experiment 1 (see Results section), were repeatable and, if so, to investigate further whether results for the holocellulose-rich straw could be explained by our hypothesis of a delayed colonization by holocellulose-degrading microorganisms. Samples of red clover foliage (5 g dry matter) or wheat straw (3 g dry matter) were pre-moistened with water (2.5 ml g^{-1} dry matter). The plant materials were either confined in nylon mesh bags or homogeneously mixed as described for Experiment 1 into cores with sandy topsoil. Soil containing wheat, but not red clover, was fertilized with $(NH_4)_2SO_4$ to a level of 150 mg inorganic N kg^{-1} dry soil. To investigate whether the N added to soil was fully available to organisms decomposing straw inside mesh bags, a supplementary treatment was applied where the same amount of N was added directly to wheat straw before confinement in mesh bags and burial in soil. Controls with fertilized and unfertilized soil only were also included. All samples were incubated in moist chambers in the dark at 15°C. The soil water potential was kept at –10 kPa by weekly addition of water to the target weight.

Measurements

In both experiments, C mineralization was measured at increasing time intervals. This was achieved by incubating three randomly chosen pots of each treatment in 2-l sealed glass jars containing alkali traps (3 M NaOH) for CO_2. Trapped CO_2-C was determined by injection of diluted alkali subsamples into 0.5 M H_2SO_4 contained in a volume of CO_2-free air circulating through an infrared gas analyser (ADC, Series 225, Hoddeson, England). Mineralization of residue C was calculated by the difference method (evolution of CO_2-C in samples with residues minus that from the respective control soil).

Table 2 Selected physical, chemical and biological soil properties

Properties	Units	Silty subsoil	Sandy topsoil	Loamy topsoil
Sand	%	51	91	44
Silt		47	7	35
Clay		2	2	21
Acidity	pH?H_2O)	5.0	6.5	6.8
C[a]	% of dry soil	0.2	1.8	2.6
N[a]		0.03	0.09	0.26
P	mg kg^{-1} dry soil	0.03	0.07	0.11
K		0.01	0.06	0.13
Soil inorganic N		1.1	5.9	1.7
Total SIR[b]	mg CO_2 -C kg^{-1} dry soil h^{-1}	1.4	3.0	4.3
Fungal SIR:bacterial SIR[c]	Ratio	1.8	1.6	3.4
Cellulase-producing CFU[d]	10^9 kg^{-1} dry soil	0.008	1.15	2.3

[a] C/N analysis (Leco CHN-1000)
[b] Glucose-induced respiration [substrate-induced respiration (SIR); Beare et al. 1990]
[c] SIR with selective inhibitors (Alphei et al. 1995)
[d] Plate dilution on carboxy-methyl-cellulose agar
CFU Colony-forming units

In Experiment 2, effects of wheat straw-soil contact on the microbial community and its activity were assessed on days 3, 9, 22 and 70. On each occasion, five microcosms of each treatment were destructively sampled, the content was very lightly dried (25°C), and soil and remaining straw were separated. For homogeneously distributed straw, this was done by sieving (1 mm mesh). The experimental error at the level of individual microcosms appeared to be very small as judged from the SEs for CO_2 evolution (Figs. 1B, 2B), whereas the subsampling error for microbiological and enzymatic analyses usually tends to be large owing to microscale heterogeneity. We gave priority to decreasing the subsampling error. Therefore, in order to have enough plant material for representative subsampling for all microbiological and enzymatic analyses, soil and remaining straw were pooled separately and treatment-wise. Three parallel subsamples (1 g plant material or 50 g soil) were dried at 105°C for dry matter determination and ashed at 550°C. The remaining wheat straw was then cut to approximately 5 mm length before further analysis.

Fungal ergosterol was extracted from remaining straw (single subsample only) by the method described by Seitz et al. (1977) and slightly modified by Henriksen and Breland (1999b). Briefly, 60 ml CH_3OH, 5 g KOH and 0.5 g pyrogallol were added to 1–2 g remaining (ash-free dry matter equivalents) wheat straw in a 250-ml glass bottle. Bottles were boiled in an autoclave (101 kPa, 10 min) and ergosterol extracted with petroleum ether. The concentration of ergosterol was determined quantitatively by HPLC (Henriksen and Breland 1999b). Fungal ergosterol concentration varies with composition of the fungal community and its physiological age (Stahl and Parkin 1996). Therefore, we did not calculate fungal biomass from ergosterol data.

Numbers of cellulase-producing colony-forming bacteria were determined by plate dilution. Three parallel subsamples (1.0 g) of remaining wheat straw were crusted in a ceramic mortar and washed into 20-ml sterile glass flasks with 19 ml sterile 0.15% NaCl solution. The subsamples were further homogenized with an ILADO X 10/20 homogenizer (Int Labor, Ballrechten-Dottingen, Germany) at 25,000 r.p.m. (3×1 min, intermittent cooling on ice) and diluted with sterile 0.15% NaCl solution. Appropriate dilutions were plated (5×0.1 ml) on carboxy-methyl-cellulose (CMC) agar as described by Henriksen and Breland (1999b). Staining for 15 min with Congo red (0.1% solution), followed by fixation with 1 M NaCl for 15 min, was used for visualization of zones of hydrolized CMC by endocellulase around bacterial colonies (Teather and Wood 1982). The number of clearing zones larger than 5 mm diameter were counted on dilutions giving <20 clearing zones per plate.

The total number and cell sizes of bacteria on wheat straw were determined by epifluorescence microscopy of the homogenates described above (Hobbie et al. 1977). Preparation of slides was done as described by Bakken (1985). To avoid a subjective bias, specimens were coded before size determination and counting. Average bacterial cell size and amount of bacterial biomass C were calculated from numbers and average cell volume within each of four determined volume groups, assuming a density of 1.1 g cm^{-3}, 30% dry weight and 44% C in bacterial dry matter (Bakken 1985).

Fungal and bacterial substrate-induced respiration (SIR; modified after Beare et al. 1990) were measured in both straw treatments. In each of three parallel measurements, four subsamples of remaining straw (0.5 g dry matter equivalents) or the surrounding soil (2.0 g dry matter equivalents) were added separately to 120-ml serum vials. Fungal and bacterial inhibitors (cycloheximide and streptomycin, respectively) were added to the plant materials

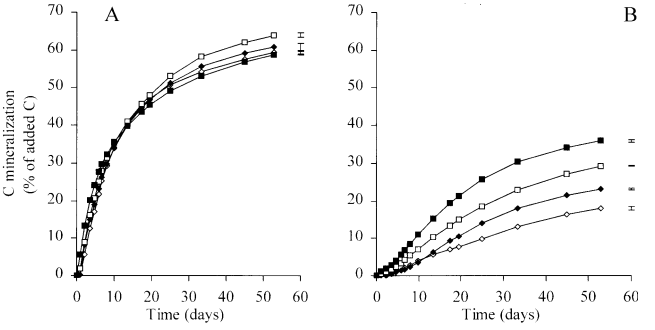

Fig. 1 C mineralization (difference method) from red clover foliage (**A)** and barley straw (**B)** homogeneously (filled symbols) or heterogeneously (open symbols) distributed in a loam topsoil (squares) or a silty subsoil (diamonds) in Experiment 1. Error bars represent ±SE (n=3)

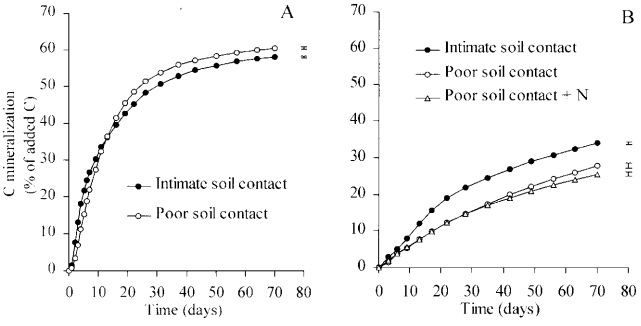

Fig. 2 C mineralization (difference method) from red clover foliage (**A)** and wheat straw (**B)** homogeneously (Filled symbols) or heterogeneously distributed (opensymbols) in a sandy topsoil. Results from a supplementary treatment, where N was applied directly on heterogeneously distributed wheat straw are also shown (triangles). *Error bars* represent ±SE (n=3)

as described by Neely et al. (1991) and to the soil as described by Alphei et al. (1995) before incubation for 12 h at 4°C. Before addition of glucose, the serum vials were brought to 25°C, flushed for 30 s with air (360 µl l^{-1} CO_2) and sealed. Respiration was measured 2.5 h after glucose addition by injection of headspace gas in an infrared gas analyser (ADC, Series 225, Hoddeson, England).

Exocellulase, endocellulase and hemicellulase activities were measured in two parallel extracts of remaining wheat straw. Extracts were prepared by adding 30 ml of 1 M NaCl to 3.0 g fresh weight of remaining straw in 100-ml glass bottles, shaking for 30 min (120 r.p.m.) and filtering (Whatman GFF) before addition of NaN_3 to 0.04% final concentration. The synthetic substrate *p*-nitrophenyl β-D-glucopyranoside (Sigma N-7006) was used to assay β-glucosidase activity (Van Tilbeurgh et al. 1982). Details are described by Henriksen and Breland (1999b). The activity was calculated as nmols nitrophenol released s^{-1} ml^{-1} extract (nkatals) based on a curve for *p*-nitrophenol standard solution (Sigma N-104–1).

Blue substrates (Loewe Biochemica nos. 04100, 04103) were used for the determination of *endo*-acting cellulase and hemicellulase activities. Details are described by Henriksen and Breland (1999b). The results were calculated as enzyme units h^{-1} g^{-1} ash-free dry matter on the basis of standard curves obtained by dilution of extracts with the highest measured activities of endocellulase and xylanase, respectively.

The potential activities of soil enzymes, as assayed under controlled conditions, are in general not easily related to their actual activities in soil (Ladd et al. 1996). A factor of uncertainty, specific to our experiment, is a possible difference in enzyme extraction efficiency owing to a different degree of contact between straw and soil particles. For example, enzymes may adsorb to organic and inorganic soil particles (Burns 1982; Ladd et al. 1996) and may be dissolved in water adhering to soil particles that were separated from the straw in the sampling procedure. Consequently, we focused on temporal patterns instead of absolute values by expressing enzyme activity data as percentage of maximal activity for the respective treatments. Absolute peak values are given in the text.

Differences in cumulative C mineralization at the end of the experiments caused by residue *distribution* were tested statistically by one-way ANOVA applied separately on data from the clover and straw treatments. Main effects of *distribution* and *time* and their interaction on C mineralization rates were tested for each plant material separately in a split-plot model. *Time* was tested against *time*×*replicate* as the error term and *distribution* and *distribution*×*time* against the residual. For microbial parameters measured in straw-amended soil in Experiment 2 (bacterial number and biovolume, cellulase-producing colony-forming bacteria, SIR and enzyme activities), true replicates of distribution treatments did not exist since five parallel microcosms were pooled in order to prioritize representative subsampling and analysis (see above). Therefore, main effects of *distribution* and *time* were tested against their interaction term, which could not be statistically tested then owing to the absence of true replicates.

Results

C mineralization

C mineralization from red clover material progressed rapidly with small but significant effects of the degree of residue-soil contact (Figs. 1A, 2A). Poor contact resulted in a slight delay in C mineralization initially (days 0–3; $P<0.001$). From day 3 onwards, C mineralization from clover material in mesh bags exceeded that of material homogeneously mixed into soil. In the topsoils but not in the subsoil, this resulted in a 5% and 3% increase with respect to initial C mineralized at the end of Experiments 1 and 2, respectively ($P<0.01$). At the end of Experiment 1, there was no significant difference between the microbiologically poor silty subsoil and the richer loamy topsoil (Table 2) with respect to cumulative C mineralization (Fig. 1A), and mineralization in the sandy topsoil was also comparable (Fig. 2A).

In contrast with the results for clover material, mineralization of straw was highly affected by degree of residue-soil contact (Figs. 1B, 2B, $P<0.001$) and soil type (Fig. 1B, $P<0.001$). At the end of the experiments, poor residue-soil contact had reduced cumulative CO_2-C evolution by 6.7%, 5.1% and 6.3% with respect to initial C in the loamy topsoil, the silty subsoil, and the sandy topsoil, respectively, as compared to mineralization when straw was homogeneously distributed. In the loamy topsoil, 32.7% of added barley straw C had evolved as CO_2-C as compared to 20.6% in the silty subsoil (mean of distribution treatments). The corresponding figure for wheat straw in the sandy topsoil was 30.8%.

The addition of mineral N directly to wheat straw inside mesh bags tended to reduce cumulative C mineralization by day 70 as compared to where N was added to the surrounding soil (Fig. 2B). However, the difference (2.2% of initial C) was not statistically significant.

Microbial numbers and biomass

Number of colony-forming, cellulase-producing bacteria showed different temporal patterns for the two treatments throughout the experiments (Fig. 3A). Intimate soil contact resulted in a higher number of colony-forming units than poor contact on the first 2 sampling days, whereas the relationship was opposite on days 22 and 70.

Fungal ergosterol increased more rapidly on wheat straw intimately associated with soil than on straw in poor contact with soil (Fig. 3B). On day 70, however, there was more ergosterol on confined straw. Although the validity of the ergosterol results may be questioned because each data point represents a single measurement only, the temporal pattern agrees with those of C mineralization (Figs. 1B, 2B) and cellulase-producing, colony-forming bacteria (Fig. 3A).

Throughout the experiment, the total counts of bacteria were higher on straw in intimate association with soil than on straw spatially separated from soil ($P<0.01$; Fig. 3C). This was counterbalanced by a significantly smaller bacterial cell size ($P<0.05$; Fig. 3D), resulting in essentially equal amounts of bacterial biomass in the two treatments (results not shown).

Microbial activities

Poor soil contact resulted in higher total SIR rates on remaining wheat straw than did intimate contact ($P<0.01$; Fig. 4A). For the surrounding soil it was the other way around ($P<0.05$; Fig. 4B). The SIR response was much stronger on plant material than in the surrounding soil.

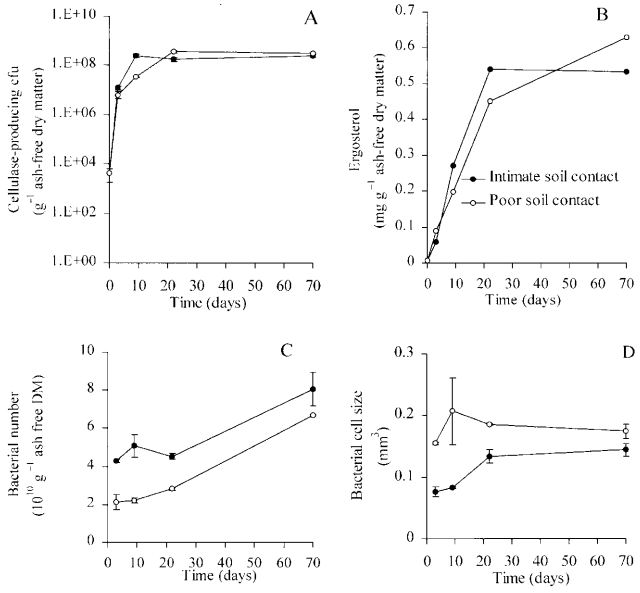

Fig. 3 Number of cellulase-producing, colony-forming bacterial units (*cfu*) on carboxy-methyl-cellulose agar (**A**), ergosterol concentration (**B**), total bacterial number (**C**) and mean bacterial cell volume (**D**) on remaining wheat straw with poor or intimate soil contact. *Error bars* in **A**, **C** and **D** represent ±SE (n=3, 2 and 2, respectively). Data points in **B** represent single values only. *DM* Dry matter

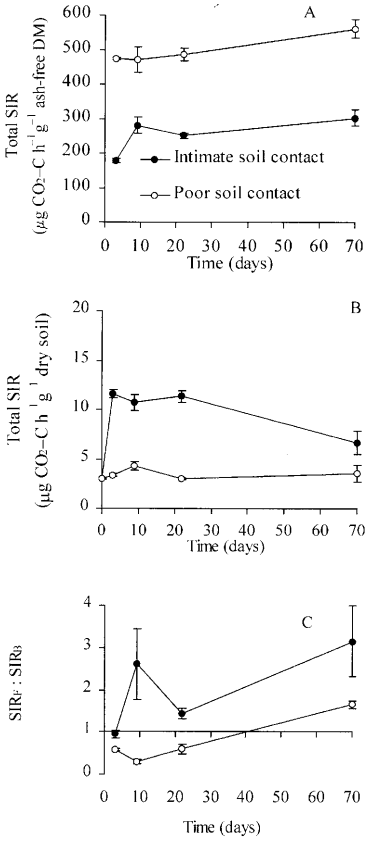

Fig. 4 Total substrate-induced respiration (*SIR*) on remaining wheat straw (**A**), in the surrounding soil (**B**) and ratio of fungal SIR (SIR_F) to bacterial SIR (SIR_B) (**C**) on straw residues with poor or intimate soil contact. *Note* differences in scales of *y*-axes between results of total SIR for residues and soil. *Error bars* represent ±SE (n=3)

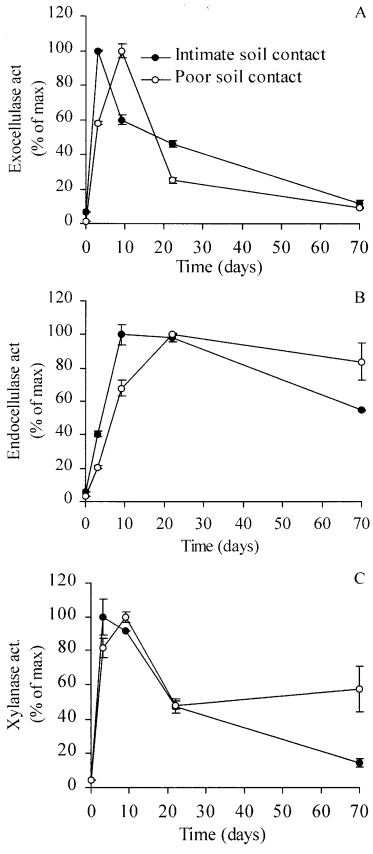

Fig. 5 Activity of exocellulase (**A**), endocellulase (**B**) and xylanase (**C**) in extracts prepared from decomposing wheat straw with poor or intimate soil contact. See Materials and methods for further explanation of units used and the text for absolute peak values measured. *Error bars* represent ±SE (n=2)

The fungal to bacterial SIR ratio (SIR_F:SIR_B) on wheat straw was below unity in the early phase of decomposition (day 3; P<0.01), and thus indicated a bacterial dominance. Later there was a shift towards a dominance of fungal activity. On day 70, the SIR_F:SIR_B ratio was above unity for both treatments (P<0.01; Fig. 4C). On every sampling day, SIR_F:SIR_B ratios were higher on straw with intimate soil contact than on straw with poor soil contact (P<0.05). In the soil, the initial SIR_F:SIR_B ratio was 1.6, and it remained above unity regardless of treatment throughout the study [mean value 2.0±0.1 (±SE); n=24]. No effect of treatment or time was detected (results not shown).

The activities of the measured enzymes showed different temporal patterns for the two treatments throughout the experiment and consistently peaked earlier where straw was mixed homogeneously with soil than where residue-soil contact was poor (Fig. 5A–C). However, the maximum activity of exocellulase and endocellulase, but not that of xylanase, was higher if residue-soil contact was poor. The exocellulase activity in extracts from homogeneously distributed wheat straw peaked at 0.10±0.0003 (n=2) nkatals g^{-1} organic dry matter (day 3) as compared with 0.74±0.03 nkatals g^{-1} (day 9) for het-

erogeneously distributed straw. The corresponding values for endocellulase were 55.7±3.4 (day 9) and 100±0.3 enzyme units h^{-1} g^{-1} ash-free dry matter (day 22). For xylanase, the peak values were 100±10.8 (day 3) and 92.4±2.9 enzyme units h^{-1} g^{-1} ash-free dry matter (day 9) for homogeneous mixing and mesh bags, respectively.

Discussion

Poor soil contact owing to the placement of residues in mesh bags reduced C mineralization from both red clover and wheat straw in the early phase of decomposition compared to residue distributed homogeneously in soil (Figs. 1, 2). For clover material, differences in C mineralization caused by the incorporation method were relatively small and transient. Apparently the microbial inoculum on heterogeneously distributed clover residues and in surrounding soil was biochemically adequate and was offered colonization and growth conditions comparable to those of homogeneously distributed material. This seemed to be the case even in the microbiologically poorer subsoil, where clover C mineralization differed little from mineralization in the loamy topsoil (Fig. 1A).

After the initial delay, limited soil contact in the topsoils had a slightly positive effect on C mineralization from clover (Figs. 1A, 2A). This agrees with similar observations made previously for readily degradable residues (McCalla and Duley 1943; Breland 1994; Henriksen and Breland, 1999a). The positive effect may be explained by a reduced volume of detritusphere soil, which by various mechanisms retains a significant fraction of decomposing C from herbaceous crop residues (Ladd et al. 1996; V. Gaillard, unpublished PhD thesis).

For straw, however, there was a negative effect of poor soil contact on C mineralization throughout the experiment (Figs. 1B, 2B). We anticipated this result, and our working hypothesis was that a delayed colonization or growth of microbes capable of producing extracellular cellulases and hemicellulases is a major reason for reduced straw decomposition under conditions where residue-soil contact is poor. However, there are also other possible explanations such as less favourable abiotic conditions, limited N availability and exclusion of macrofauna by the mesh bags.

In our experiment, less favourable abiotic conditions cannot explain the reduced C mineralization from straw with limited soil contact. Temperature was constant (15°C), and all straw residues were moistened before burial in soil. Moreover, when residues are placed within soil, their water potential eventually becomes equal to the soil water potential, regardless of incubation method (Parr and Papendick 1978).

We added N to soil in amounts sufficient to meet the microbial requirement (Recous et al. 1995; Henriksen and Breland 1999b). Even so, spatial segregation of C substrates from available N might possibly result in N-limited decomposition. However, the slightly reduced C mineralization in the supplementary treatment where N was applied directly to straw (Fig. 2B) excluded this possibility.

Mesh bags made of 1-mm mesh prevent larger animals from reaching the plant material. This may contribute to reduced decomposition (Jensen 1985). However, we did not observe large animals, e.g. earthworms, in any of the incubation pots, suggesting that the soil pretreatment (sieving and moderate air drying) prevented large animals from playing a significant role even in the decomposition of unconfined residues.

Fungi are generally regarded as the main decomposers of plant materials rich in holocellulose because of the widespread capability within this group of producing extracellular depolymerizing enzymes (Swift et al. 1979). In Experiment 2, the concentration of fungal ergosterol (Fig. 3B) and the SIR_F:SIR_B ratio (Fig. 4C) showed that fungal colonization was delayed when soil contact was poor. So also was the increases in cellulase and xylanase activity (Fig. 5A–C) and the growth of cellulase-producing colony-forming bacteria (Fig. 3A). Consequently, our results provided substantial support for our hypothesis that the functional group of holocellulose degraders may be a limiting factor for the degradation of straw heterogeneously distributed in soil.

Straw cellulose and hemicelluloses are partly embedded in lignin, which may provide some protection against attack by cellulases and hemicellulases. Bowen and Harper (1990) found that weight losses from straw in excess of 20% appeared to depend on the activity of lignin-modifying or -degrading microorganisms. It is possible, therefore, that impeded lignin degradation in the heterogeneously distributed straw also contributed to our results. This could only be a partial explanation, though. Straw decomposition was reduced from the start of the incubation (Figs. 1B, 2B), and our measurements of microbiological and enzymatic parameters strongly suggested that impeded colonization by holocellulose degraders was the major reason, at least during the early stages.

The physiological status of the microbial inoculum is very important for the potential of fungi to colonize and utilize a resource (Lockwood and Filonow 1981), and a supply of readily available energy influences cellulose decomposers positively if their activity is low (Alexander 1977). In our Experiment 2, microbial activity was low in soil surrounding mesh bags (Fig. 4B). This agrees with results of Rønn et al. (1996), who found that effects of residues on microbial activity and microfaunal populations were very local. Gaillard et al. (1999) also showed that the detritusphere soil is mainly limited to the 4- to 5-mm zone surrounding the decomposing residues and that this zone has a steep gradient in the concentration of residue-derived C and N and in microbial activity and growth. On this basis, we believe that the physiological status of the initial fungal community of our soil was insufficient for rapid ingrowth, proliferation and activity of cell wall-decomposing microorganisms when the distance between the inoculum and straw was

increased by placement in mesh bags. Strong competition with non-cellulolytic microorganisms for the small amount of readily available substrates in straw may have contributed further to this. In an experiment of Hu and van Bruggen (1997), fungal cellulose degradation started earlier when a bacterial inhibitor (streptomycin) was added. Our observations of a more bacteria-dominated microbial community (Fig. 4C), fewer but larger bacteria (Figs. 3C, 3D), and a stronger SIR response (Fig. 4A) on straw in mesh bags than on homogeneously distributed straw suggest that rapidly growing bacteria got hold of a relatively larger share of readily available resources from straw in mesh bags than from homogeneously distributed straw. On straw mixed into the soil, competition for simple substrates was less likely to limit the degradation of straw as the initial number of microbial propagules in intimate contact with straw residues was higher.

In another laboratory study (Henriksen and Breland 1999a), we incubated different plant residues in mesh bags in the sandy topsoil used in Experiment 2. In one treatment, we inoculated with 10% (w/w) of soil which had previously been amended with red clover material and was not dried or sieved before incubation. In the inoculated soil, we observed a prolific growth of fungal mycelium, and we found increased decomposition rates of some residues (barley straw, ryegrass foliage and potato haulm), while others (white clover foliage and cabbage leaves) were less affected. The increase in decomposition in inoculated soil was significantly correlated with the cellulose and hemicellulose concentration of the residues. This supports the above assumption that differences in the colonization potential of holocellulose decomposer may substantially influence straw decomposition.

It may be argued that observed differences in decomposition as affected by residue-soil contact (Experiment 2) or inoculation (Henriksen and Breland 1999a) could be due to soil treatment before incubation, for example sieving, which seems to damage fungi in particular (Petersen and Klug 1994). Thus, the differences might not be as distinct under natural field conditions with an undisturbed microflora. However, poor straw-soil contact consistently resulted in reduced straw decomposition in all three soils tested in our experiments, regardless of initial microbiological activity (Table 2) or the degree of soil macrostructure disruption before the start of the experiments (see the Materials and methods section). Mueller et al. (1997) estimated a substantially greater decay rate of structural plant material under field conditions in a soil with recent supplies of readily available energy than in a fallow soil. Moreover, Parr and Reuszer (1959) found that banding of wheat straw in a field soil caused a temporary initial reduction of decomposition. These results suggest that significant variations in the potential of the microflora to degrade holocellulose may occur even under field conditions and that our findings may be relevant to conditions where ploughing, for example, results in very heterogeneous straw distribution.

In conclusion, C mineralization from red clover material was unaffected or slightly stimulated when the residue-soil contact was poor, whereas that from barley and wheat straw was reduced significantly. Our results strongly suggested that the mechanism underlying the latter observation was an insufficient colonization and growth of holocellulose-degrading microorganisms. Straw decomposition also differed between soil types of different initial biological activity. This shows the necessity of ensuring realistic conditions for the decomposer microflora when studying effects of substrate quality on decomposition and when extrapolating results for holocellulose-rich residues to field conditions, e.g. by modelling.

Acknowledgements This work was funded through the Programme for Plants and Soil (Research Council of Norway), the Nordic Project on Nitrogen in Arable and Forest Soils (Nordic Council of Ministers and Research Council of Norway), and by The Norwegian Crop Research Institute. We thank Dr H. Riley for helpful comments on the manuscript.

References

Alexander M (1977) Introduction to soil microbiology, 2nd edn. Wiley, New York

Alphei J, Bonkowski M, Scheu S (1995) Application of the selective inhibition method to determine bacterial:fungal ratios in three beechwood soils rich in carbon-optimization of inhibitor concentrations. Biol Fertil Soils 19:173–176

Ames RN, Reid CCP, Porter LK, Cambardella C (1984) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytol 95:381–396

Bakken LR (1985) Separation and purification of bacteria from soil. Appl Environ Microbiol 49:1482–1487

Beare MH, Neely CL, Coleman DC, Hargrove WL (1990) A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. Soil Biol Biochem 5:585–594

Beare MH, Pohlad BR, Wright DH, Coleman DC (1993) Residue placement and fungicide effects on fungal communities in conventional and no-tillage soils. Soil Sci Soc Am J 57: 392–399

Bowen RM, Harper SHT (1990) Decomposition of wheat straw and related compounds by fungi isolated from straw in arable soils. Soil Biol Biochem 22:393–399

Breland TA (1994) Enhanced mineralization and denitrification as a result of heterogeneous distribution of clover residues in soil. Plant Soil 166:1–12

Burns RG (1982) Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol Biochem 14:423–427

Christensen BT (1986) Barley straw decomposition under field conditions: effect of placement and initial nitrogen content on weight loss and nitrogen dynamics. Soil Biol Biochem 18: 523–529

Douglas CL Jr, Rickman RW (1992) Estimating crop residue decomposition from air temperature, initial nitrogen content and residue placement. Soil Sci Soc Am J 56:272–278

Douglas CL Jr, Allmaras RR Jr, Rasmussen PE, Ramig RE, Roager NC Jr (1980) Wheat straw composition and placement effects on decomposition in dryland agriculture of the Pacific Northwest. Soil Sci Soc Am J 44:833–837

Gaillard V, Chenu C, Recous S, Richard G (1999) Carbon, nitrogen and microbial gradients induced by plant residues decomposing in soil. Eur J Soil Sci 50:567–578

Goering HK, Van Soest PJ (1970) Forage fibre analyses (apparatus, reagents, procedures, and some applications). USDA agriculture handbook no. 379. USDA, Washington, D.C. pp 1–19

Henriksen TM, Breland TA (1999a) Evaluation of criteria for describing crop residue degradability in a model of carbon and nitrogen turnover in soil. Soil Biol Biochem 31:1135–1149

Henriksen TM, Breland TA (1999b) Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil Biol Biochem 31:1121–1134

Hobbie JE, Daley RJ, Jasper S (1977) Use of Nucleopore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1229

Holland EA, Coleman DC (1987) Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology 68:425–433

Hu S, Van Bruggen AHC (1997) Microbial dynamics associated with multiphasic decomposition of ^{14}C-labeled cellulose in soil. Microb Ecol 33:134–143

Jensen MB (1985) Interactions between soil invertebrates and straw in arable soil. Pedobiologia 28:59–69

Ladd JN, Foster RC, Nannipieri P, Oades JM (1996) Soil structure and biological activity. In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 9. Dekker, New York, pp 23–78

Lockwood JL, Filonow AB (1981) Responses of fungi to nutrient-limiting conditions and to inhibitory substances in natural habitats. In: Alexander M (ed) Advances in Microbial Ecology, vol 5. Plenum, New York, pp 1–61

Malkomes HP (1980) Strohrotteversuche zur Erfassung von Herbizid-Nebenvirkungen auf den Strohumsatz im Boden. Pedobiologia 20:417–427

McCalla TM, Duley FL (1943) Disintegration of crop residues as influenced by sub-tillage and plowing. J Am Soc Agric 35: 306–315

Mueller T, Jensen LS, Magid J, Nielsen NE (1997) Temporal variation of C and N turnover in soil after oilseed rape straw incorporation in the field: simulations with the soil-plant-atmosphere model DAISY. Ecol Modell 99:247–262

Neely CL, Beare MH, Hargrove WL, Coleman DC (1991) Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. Soil Biol Biochem 23:947–954

Parr JF, Papendick RI (1978) Factors affecting the decomposition of crop residues by microorganisms. In: Oschwald WR (ed) Crop residue management systems. ASA, CSSA, SSSA, Madison, Wis. pp 101–129

Parr JF, Reuszer HW (1959) Organic matter decomposition as influenced by oxygen level and method of application to soil. Soil Sci Soc Am J 28:214–216

Petersen SO, Klug MJ (1994) Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Appl Environ Microbiol 60:2421–2430

Recous S, Robin D, Darwis D, Mary B (1995) Soil inorganic N availability: effect on maize residue decomposition. Soil Biol Biochem 27:1529–1538

Rønn R, Griffiths BS, Ekelund F, Christensen S (1996) Spatial distribution and successional pattern of microbial activity and micro-faunal populations on decomposing barley roots. J Appl Ecol 33:662–672

Seitz LM, Mohr HE, Burroughs R, Sauer DB (1977) Ergosterol as an indicator of fungal invasion in grains. Cereal Chem 54: 1207–1217

Stahl PD, Parkin TB (1996) Relationship of soil ergosterol concentration and fungal biomass. Soil Biol Biochem 28:847–855

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Blackwell, Oxford

Teather RM, Wood PJ (1982) Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43: 777–780

Van Tilbeurgh H, Claeyssens M, De Bruyne CK (1982) The use of 4-methylumbelliferyl and other chromophoric glycosides in the study of cellulolytic enzymes. FEBS Lett 149:152–156