

# Decomposition in soil and chemical changes of maize roots with genetic variations affecting cell wall quality

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## Summary

Roots of brown-midrib (F2bm1 and F292bm3) maize mutants and their normal isogenic counterparts (F2 and F292) were used to evaluate the changes in chemical cell wall features with regard to polysaccharides, lignin composition and interconnecting phenolic acids during root degradation in soil. To this end, the chemical variability of roots of brown-midrib mutants and their normal counterparts was compared and its subsequent impact on carbon (C) mineralization determined under controlled conditions. The bm1 mutation mainly caused an increase in lignin content and a decrease in polysaccharide content of maize roots whereas the bm3 mutation caused only a decrease in polysaccharide content. The lignin composition of bm roots differed from that of normal lines and the proportion of cell wall ester-linked hydroxycinnamic acids was also different. C mineralization kinetics differed markedly between the genotypes. Certain relevant factors concerning root decomposition in soil were studied from the relationships between the chemical characteristics of maize roots at different stages of decomposition and C mineralization rates. The Klason lignin-to-glucose ratio (KL/Glu), the Klason lignin-to-arabinoxylans ratio (KL/AX) and the arabinose-to-xylose ratio (A/X) were proposed as promising predictive indicators of C mineralization kinetics. Future estimations of soil residue decomposition could be improved by taking these initial chemical criteria into account on a wider range of residues.

## Décomposition dans le sol et évolution de la qualité chimique des racines de maïs présentant des modifications génétiques de la qualité des parois cellulaires'

### Résumé

Les racines des maïs mutants brown-midrib (F2bm1 et F292bm3) et celles de leurs lignées isogéniques normales (F2 et F292) ont été utilisées pour évaluer les modifications des caractéristiques chimiques des parois cellulaires, à travers la composition des polysaccharides, de la lignine et la nature des acides phénoliques, au cours de la dégradation des racines dans le sol. Pour cela, nous avons examiné, en conditions contrôlées, l'impact d'une variabilité de la qualité chimique des racines, en comparant les mutants bm et leurs lignées isogéniques normales, sur la minéralisation du C. La mutation bm1 engendre principalement une augmentation de la teneur en lignine et une diminution de la teneur en polysaccharides dans les racines de maïs alors que la mutation bm3 cause uniquement une diminution de la teneur en polysaccharides. Dans les racines des mutants bm, la composition de la lignine ainsi que les proportions en acides hydroxycinnamiques estérifiés des parois cellulaires diffèrent de celles des lignées non mutantes. Les cinétiques de minéralisation du C varient fortement entre les génotypes. Les relations entre les caractéristiques chimiques des racines de maïs à différents stades de décomposition et les taux de minéralisation du C ont permis d'étudier certains facteurs pertinents concernant la décomposition des racines dans le sol. Les rapports lignine Klason sur glucose (KL/Glu), lignine Klason sur arabinoxylanes (KL/AX) et arabinose sur xylose (A/X) ont été identifiés comme étant de bons indicateurs de prédiction des cinétiques de minéralisation du C. La prise en compte de ces critères de qualité chimique initiale sur une plus large gamme de résidus pourrait améliorer l'estimation de la décomposition des résidus dans le sol.

## Introduction

Crop residues are composed of a soluble fraction and an insoluble fraction corresponding essentially to the cell walls. The cell

wall composition of crop residues and the quality of their constitutive polymers have a great effect on their decomposition in soil and on the associated C and N fluxes (Bertrand *et al.*, 2006). The principal cell wall polymers are polysaccharides (such as cellulose and hemicelluloses) and lignin. In Gramineae, arabinoxylans represent the main type of hemicelluloses and consist of a backbone of  $\beta$ 1,4-linked xylose units mainly substituted by  $\alpha$ -linked arabinose. Hydroxycinnamic acids, namely ferulic acid (FA) and *p*-coumaric acid (PCA), are the principal inter-connecting agents between polymers in graminaceous cell walls (Kato & Nevis, 1985). FA is ester-linked to the O-5 position of the arabinose side chain of arabinoxylans, whereas PCA mostly esterifies the syringyl moieties of lignin. Phenolic acids thus play a key role in cross-linking arabinoxylans to lignin (Ralph & Helm, 1993). The chemical composition of cell wall polymers, as well as their interactions, influences the accessibility of these components to decomposers (Chesson, 1988). The fate of cell wall polymers, during the decomposition process, depends on their interactions within the cell walls. For this reason, a more thorough investigation of cell wall polymer quality should improve our understanding and quantification of the decomposition process.

In previous studies to determine the role of chemical quality of residues on their decomposition in soils, different types of crop residues (species, plant parts) were used. The chemical variations and differences in residue anatomy were found to be important factors influencing the decomposition process (Akin, 1989). Indeed, plant parts contain different types of tissues and cell wall quality can depend on the nature of these.

To our knowledge, few results have been published concerning the effect of cell wall polymer quality on root decomposition in soil (Herman *et al.*, 1977). Roots have now become the focus of particular interest, firstly because they often remain in the soil after harvest, and secondly because they may make a greater contribution to building up soil organic matter than aerial plant parts (Puget & Drinkwater, 2001; Rasse *et al.*, 2005). In the present study, we focused on roots of the natural maize mutant named brown-midrib (bm). This plant material was selected because: (i) it has a particularly high cell wall content compared with above-ground parts, (ii) it does not suffer from structural and chemical variations related to residue anatomy because the same tissue types are present, and (iii) the effects of the bm mutations in above-ground parts with respect to forage digestibility have been well reported (Barrière *et al.*, 2004), whereas no studies have as yet been performed on under-ground parts. The above-ground parts of bm1 and bm3 mutants are mainly recognized from their reduced lignin and phenolic acid contents compared with the normal lines (Barrière & Argillier, 1993). In contrast, little is known about the effects of bm mutations on root quality and their decomposition in soil. White *et al.* (2007) showed that total residue C mineralization in sorghum after 194 days of decomposition did not differ between bm mutants and normal lines. However, Hopkins *et al.* (2001) studied the mineralization of tobacco stems with genetic

modifications for lignin biosynthesis and found that transgenic plants mineralized more rapidly than the wild-type plants.

The aims of our study were therefore to assess the role of the chemical quality of cell wall polymers and their interactions in root decomposition in soils in order to determine new quality criteria. Identification of these criteria was based on the relationships between the chemical characteristics of maize roots and C mineralization rates determined under controlled conditions.

## Materials and methods

### Soil and maize roots

Soil was collected from a depth of 5–30 cm at the INRA Mons-en-Chaussée experimental station. The soil had a silty loam texture (17.8% clay, 77.3% silt, 3.8% sand), contained 0.95% organic C and had a pH (H<sub>2</sub>O) of 7.6. The soil was air-dried for 2 days to a moisture content of 120 mg g<sup>-1</sup> dry soil, and then immediately sieved to 2 mm. All visible organic residues were removed by hand after sieving. The soil was stored at 15°C for a week prior to incubation.

Two sets of maize (*Zea mays* L.) brown-midrib isogenic lines, within the genetic background of inbreds F2 (F2 and F2bm1) and F292 (F292 and F292bm3) were studied. All genotypes were cultivated in experimental fields at the INRA Lusignan experimental station and were harvested at physiological maturity. Only the roots were kept for experiments. These roots were washed with a 50 g l<sup>-1</sup> sodium metaphosphate solution for 24 hours, rinsed with deionised water to remove soil particles, and then dried for 1 week at 30°C. Roots with a diameter of 2–3 mm were selected for the study.

### Incubation experiment

Soil samples and maize roots (about 5 mm long) were mixed at a rate equivalent to 2 g C kg<sup>-1</sup> dry soil and incubated for 112 days at 15°C. Potassium nitrate (61 mg N kg<sup>-1</sup>) was added to the soil, which contained 9 mg NO<sub>3</sub><sup>-1</sup>-N kg<sup>-1</sup> (no ammonium was detected), to ensure that decomposition would not be limited by N (Recous *et al.*, 1995). The concentration of the added N solution was calculated such that the soil moisture was maintained at a potential of -80 kPa, corresponding to a water content of 190 g kg<sup>-1</sup>. Soil moisture was maintained throughout the incubation period by weighing at weekly intervals and readjusting with deionised water when necessary. A control incubation experiment was performed in the same way but without the addition of residues.

Carbon mineralization was measured in soil samples (equivalent to 100 g dry soil) incubated in 500 ml glass jars (four replicates per treatment) in the presence of a CO<sub>2</sub> trap (10 ml of 1 M NaOH). Mineral N was determined on separate soil samples equivalent to 30 g dry soil (three replicates per treatment)

placed in 2-litre glass jars containing 30 ml of 1 M NaOH as a CO<sub>2</sub> trap to ensure the same conditions as for the C mineralization measurements. Mineral N was extracted at 0, 14, 36, 57 and 112 days by adding 100 ml of a 1 M KCl solution to the soil. Carbon mineralization was measured at 3, 7, 10, 14, 21, 29, 36, 42, 51, 57, 70, 80, 87, 95 and 112 days after the beginning of incubation.

The chemical quality of the decomposing roots was determined after 14, 36, 57 and 112 days of incubation. At each date and for each genotype, ca. 5 g of root dry matter were removed manually from the soil, washed with deionised water and then with a sodium metaphosphate solution as described earlier, and dried for 1 week at 30°C prior to chemical analyses.

The concentrations of CO<sub>2</sub> trapped in the NaOH solutions were measured by continuous flow colorimetry (Chaussod *et al.*, 1986) using an auto-analyser (TRAACS 2000, Bran & Luebbe, Norderstedt, Germany). Mineral N in the soil and residue extracts was analysed by continuous flow colorimetry (TRAACS 2000, Bran & Luebbe). Concentrations of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> were determined using an adaptation of the method proposed by Kamphake *et al.* (1967). Ammonium ions were determined following the method described by Krom (1980).

#### Chemical analysis of maize roots

The chemical characteristics of non-decomposed and decomposed roots were determined on two replicates of each sample. The total C and N contents of non-decomposed and decomposed roots were measured by elemental analysis (NA 2000, Fisons Instruments, Milan, Italy).

Root residues were subjected to a cell wall preparation process, which consisted of the extraction of neutral detergent fiber (NDF) according to the method described by Goering & Van Soest (1970). Briefly, the soluble fraction was removed by boiling 1.5 g of roots (ca. 5 mm long/2 mm diameter) in deionised water at 100°C for 30 minutes and then extracting with a neutral detergent solution at 100°C for 60 minutes to remove cytoplasmic components and obtain the NDF fraction. This fraction was designated the cell wall residue. All residues from cell wall preparations were dried for 1 week at 30°C and ground to 80 µm.

The neutral sugar content of cell wall residues was determined according to the method described by Blakeney *et al.* (1983). Ten mg of sample were swollen in 125 µl 12 M H<sub>2</sub>SO<sub>4</sub> for 2 hours at 20°C followed by acid hydrolysis with 1 M H<sub>2</sub>SO<sub>4</sub> for 2 hours at 100°C. Monosaccharides released by the acid were separated by high performance anion-exchange chromatography (HPAEC) on a CarboPac PA-1 column (4 × 250 mm, Dionex, Sunnyvale, CA, USA) as described by Beaugrand *et al.* (2004). The composition of monosaccharides was analysed and quantified using 2-deoxy-D-ribose as internal standard and standard solutions of neutral carbohydrates (L-arabinose, D-glucose, D-xylose, D-galactose, D-rhamnose, D-mannose and L-fucose).

Klason lignin (KL) was determined as the acid-insoluble residue remaining after sulphuric acid hydrolysis of cell wall polysaccharides (Monties, 1984). Briefly, 200 mg of cell wall residues were suspended in 2 ml of 12 M H<sub>2</sub>SO<sub>4</sub> for 2 hours at room temperature. Suspensions were then diluted to 1 M with deionised water, heated at 100°C for 3 hours and filtered. The remaining residues were dried at 105°C and ash measurements made after 4 hours at 550°C.

The lignin monomer composition was determined by thioacidolysis. This reaction enables the specific disruption of labile-ether inter-monomer linkages, which represent the non-condensed lignin fraction. Ten mg of cell walls were heated in 10 ml dioxane:ethanethiol (9:1, v/v) containing 0.2 M boron trifluoride etherate for 4 hours at 100°C (Lapierre *et al.*, 1986). Monomer products were analysed as trimethylsilyl derivatives of guaiacyl and syringyl by capillary column gas chromatography using a J&W DB-1 column (30 m long, 0.3 mm i.d.; 0.25 µm film thickness; Folsom, California, USA) and a temperature gradient of 160–280°C at 2°C minute<sup>-1</sup>. The carrier gas was helium at 60 kPa, and detection was by flame ionization. The internal standard was tetracosane.

Ester-linked hydroxycinnamic acids (ferulic acid (FA) and *p*-coumaric acid (PCA)) were released by incubating 10 mg cell wall residue with 2 M NaOH (10 ml) for 2 hours at 35°C with constant stirring under a nitrogen atmosphere. After cooling for 10 minutes, the reaction was stopped by adjusting the pH to 1 with 6 M HCl, prior to the addition of 3,4,5-trimethoxy-*trans*-cinnamic acid as an internal standard. Hydroxycinnamic acids were then extracted three times with diethyl oxide (30 ml). The organic fractions were pooled and evaporated to dryness under reduced pressure. The dried extract was dissolved in 1.5 ml methanol:water (1:1, v/v) and filtered (0.45 µm) prior to injection on to a Spherisorb S50DS2 (Waters, RP-18, 250 × 2.6 mm) column. Hydroxycinnamic acids in the eluates were detected using a Waters photodiode array UV detector (Beaugrand *et al.*, 2004; PDA 996 Waters, Milford, MA, USA).

#### Data treatment and analysis

Carbon mineralization was calculated as the difference in the CO<sub>2</sub> released after each treatment between the amended residue and control soils, and was expressed as a percentage of the added residue C.

Chemical features related to cell wall residues were expressed in relation to the initial residue dry matter and were corrected by the extraction yield of the cell wall preparation step (i.e. the amount of material lost during NDF extraction). The chemical data for the decomposed roots were expressed as previously described, by taking into account: (i) the material lost during NDF extraction, and (ii) the loss of mass residue by decomposition, which was calculated from the cumulative amounts of mineralized C. In this way, the data obtained from NDF-extracted/soil-decomposed material were directly comparable with those from non-decomposed residues.

Differences in values between genotypes were evaluated by the least significant difference (LSD,  $P \leq 0.05$ ) derived from the analysis of variance (ANOVA) (Genstat 8.1).

## Results

### *Chemical characteristics of non-decomposed roots*

The cell wall contents of maize roots were particularly high as the NDS soluble fraction accounted for less than 20% dry matter, whatever the genotype (Table 1). The cell wall content rose as follows: F2bm1 < F292 = F2 < F292bm3.

The major constituents of the root cell walls were neutral sugars (Table 1). The total amount of neutral sugars was determined from the sum of glucose, xylose, arabinose, galactose, rhamnose, mannose and fucose. Before soil decomposition, the neutral sugars content differed significantly between genotypes and was ranked as follows: F292 > F292bm3 > F2 > F2bm1 (Table 1) ( $P \leq 0.05$ ). The three main cell wall monosaccharides were glucose, xylose and arabinose. The arabinose levels were low (< 3% of dry matter) compared with those of xylose and glucose in all genotypes. The relative proportions of these monosaccharides were similar in all genotypes. Glucose in the cell wall can be attributed to the cellulose fraction whereas the sum of arabinose and xylose gives a relatively good assessment of the hemicellulose fraction (arabinoxylans) (Brett & Waldron, 1996). The main cell wall polymer was cellulose, which was present at significantly smaller levels in F2bm1 (28.5% of dry matter) than in F2 (30.0%), F292bm3

(31.8%) and F292 (33.0%) (Table 1) ( $P \leq 0.05$ ). Hemicellulose was the second major polymer in root cell walls and the levels were significantly smaller in F2bm1 (18.2% of dry matter) than in F2 (19.0%), F292 (20.8%) and F292bm3 (20.9%) (Table 1) ( $P \leq 0.05$ ). The arabinose to xylose ratio (A/X) was equal in all four genotypes before soil decomposition, indicating a similar level of xylan substitution by arabinose.

The amount of Klason lignin (KL) accounted for less than 20% of root dry matter in all genotypes and its levels were significantly smaller in the F292 and F292bm3 genotypes than in F2 and F2bm1 (Table 1) ( $P \leq 0.05$ ). In F2bm1, the amount of KL exceeded that of the hemicellulose fraction (19.2% and 18.2% of dry matter, respectively). The lignin proportion in non-condensed structures, determined by the  $\beta$ -O-4 ether-linked syringyl (S) and guaiacyl (G) unit contents, varied considerably between genotypes: roots of bm mutants contained significantly less non-condensed lignin than their isogenic lines (F2 and F292) (Table 1) ( $P \leq 0.05$ ). In addition, the relative proportion of S units was smaller than that of G units in F292bm3, leading to an S to G ratio <1 which was only found in this genotype. Ester-linked PCA were more abundant than ester-linked FA and found at significantly greater levels in F2 and F292 than in F2bm1 and F292bm3 (Table 1) ( $P \leq 0.05$ ).

### *Carbon and nitrogen dynamics*

The cumulative amounts of mineralized C varied markedly between genotypes (Table 2). At the end of incubation

**Table 1** Total C, N and biochemical characteristics of maize root residues before soil decomposition (ND). The total amount of neutral sugars was represented by the sum of glucose, xylose, arabinose, galactose, rhamnose, mannose and fucose measured in non-decomposed cell wall residues (NDF-ND) and expressed as % of non-decomposed dry matter (%DM-ND). Means not sharing a common letter within individual genotypes differ in terms of their response to chemical characteristics ( $P \leq 0.05$ )

	F2	F2bm1	F292	F292bm3	LSD
Total C /% dry matter	47.8c	48.0c	46.6b	45.3a	0.40
Total N /% dry matter	1.1c	1.4d	0.8a	0.9b	0.06
C to N ratio	44b	34a	61d	53c	4.68
<i>Cell wall fraction</i>					
Cell wall /% dry matter	83.9b	80.6c	83.6b	86.0a	1.16
<i>Polysaccharides composition</i>					
Total neutral sugars /% dry matter	50.2b	48.2a	55.0d	53.9c	1.08
Glucose /% dry matter	30.0b	28.5a	33.0d	31.8c	0.96
Xylose /% dry matter	16.7b	15.9a	18.3c	18.4c	0.55
Arabinose/% dry matter	2.3a	2.3a	2.5c	2.5b	0.05
Arabinose to Xylose ratio	0.14a	0.14a	0.14a	0.13a	0.01
<i>Lignin fraction</i>					
Klason Lignin /% dry matter	17.6b	19.2c	15.6a	15.8a	0.37
Uncondensed lignin /% Klason lignin	17.8d	8.2a	15.5c	9.5b	1.03
S to G molar ratio	1.64d	1.30b	1.45c	0.66a	0.11
<i>Esterified phenolic acids</i>					
PCA / $\mu\text{mol g}^{-1}$ cell wall	162c	100a	165d	116b	1.48
FA / $\mu\text{mol g}^{-1}$ cell wall	37c	31a	36c	35b	0.69

**Table 2** Cumulative C mineralization and changes in cell wall, lignin quality and esterified phenolic acids as determined on roots decomposed for 112 days. Means not sharing a common letter within individual genotypes differ in terms of their response to chemical characteristics ( $P \leq 0.05$ )

	F2	F2bm1	F292	F292bm3	LSD
Cumulative mineralized C /% added C	37.2b	27.5a	45.8c	46.0c	1.12
Cell wall /% dry matter-ND	55.2c	63.5d	44.6b	41.9a	0.56
<i>Lignin quality</i>					
Uncondensed lignin /% KL-ND	11.8c	3.6a	8.0b	3.8a	0.60
S to G molar ratio	1.96d	1.34b	1.75c	0.56a	0.11
<i>Esterified phenolic acids</i>					
PCA / $\mu\text{mol g}^{-1}$ cell wall-ND	66.0d	43.0b	64.5c	34.9a	0.89
FA / $\mu\text{mol g}^{-1}$ cell wall-ND	9.7c	10.5d	7.9b	5.7a	0.37

(112 days), the total amounts of mineralized C were significantly smaller in F2bm1 (27.5% of added C) than in F2 (37.2%) and those in F2 were significantly smaller than in F292 (45.8%) and F292bm3 (46.0%) ( $P \leq 0.05$ ).

The observed differences in cumulative mineralized C for the entire incubation period resulted mainly from differences in the rates of C mineralization over the first 40 days (Figure 1a). Carbon mineralization rates with F292 and F292bm3 reached a maximum and similar value at day 14 whereas the rates with

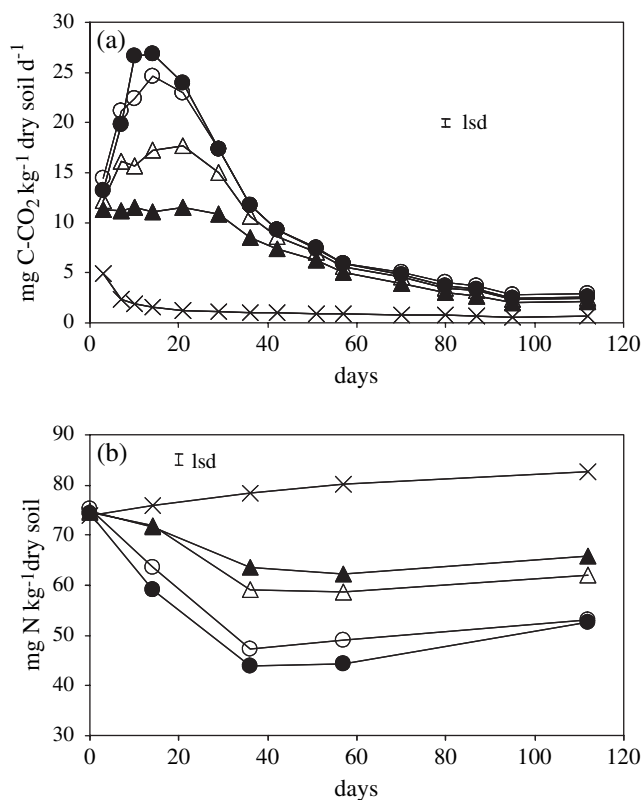
F2 and F2bm1 were much slower from the beginning, and peaked at day 21 with F2 only. At the end of incubation (95–112 day interval), C mineralization rates were significantly smaller in F2bm1 than in the other genotypes ( $P \leq 0.05$ ).

The average soil mineral N concentration at the beginning of incubation (day 0) was  $73.8 \text{ mg N kg}^{-1}$  dry soil (Figure 1b). Positive and almost linear net mineralization was observed in the control soil, resulting in  $+8.8 \text{ mg N kg}^{-1}$  dry soil after 112 days of incubation. During the first 36 days, F292 and F292bm3 exhibited a sharper decrease in soil mineral N (because of net immobilization) than F2 and F2bm1. The concentrations in soil mineral N then increased slightly under all treatments but remained below those of the control soil until the end of the incubation period.

#### Evolution of cell wall quality during decomposition

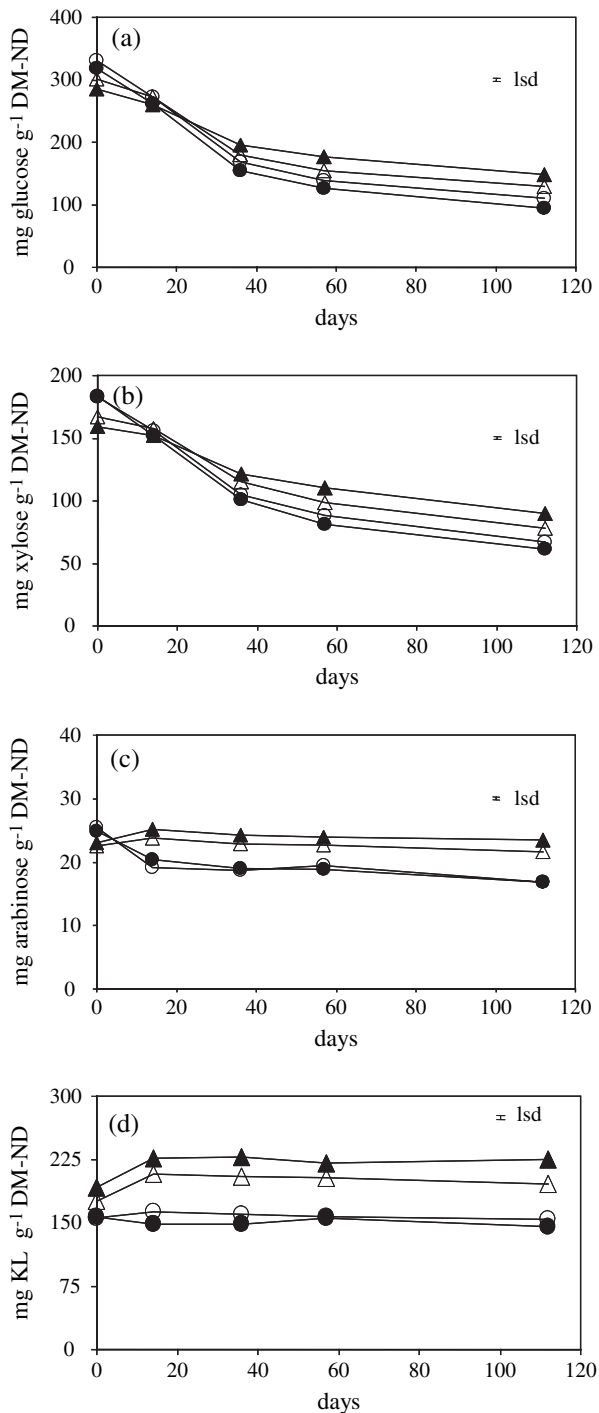
Root dry matter losses were calculated from the cumulative C mineralization values (expressed as a percentage of C added to soil) (Table 2) and represented the total amounts of C mineralized after 112 days. The F292 and F292bm3 genotypes lost more dry matter than F2 and F2bm1. Decomposition significantly affected the cell wall content of all genotypes after 112 days of incubation. A greater decrease was observed in F292 and F292bm3 (47% and 51% of the initial cell wall content, respectively) than in F2 (34%) and F2bm1 (21%) ( $P \leq 0.05$ ).

The evolution of cellulose (the main cell wall polymer degraded) was assessed from the changes in glucose content after 14, 36, 57 and 112 days of decomposition (Figure 2a). After 14 days of incubation, the glucose content decreased significantly in all genotypes, and after 112 days the extent of glucose depletion was significantly greater in F292 and F292bm3 (70% and 67% of the initial glucose content, respectively) than in F2 (57%) and F2bm1 (48%) ( $P \leq 0.05$ ). The evolution of hemicellulose was assessed from the changes in xylose and arabinose contents. Changes in the xylose content closely followed those of glucose (Figure 2b). After 112 days of incubation, the xylose loss was significantly greater in F292 and F292bm3 (63% and 67% of the initial xylose content, respectively), than in F2 (53%) and F2bm1 (44%) ( $P \leq 0.05$ ). The evolution of arabinose differed from that of glucose and xylose. After



**Figure 1** Carbon mineralization rates (a) and cumulative nitrogen mineralization kinetics (b) measured in soils without residues ( $\times$ , control) and after the addition of roots: ( $\Delta$ ) F2, ( $\blacktriangle$ ) F2bm1, ( $\circ$ ) F292 and ( $\bullet$ ) F292bm3. Data are means of four incubation replicates for C ( $n = 4$ , Figure 1a) and three incubation replicates for N ( $n = 3$ , Figure 1b).

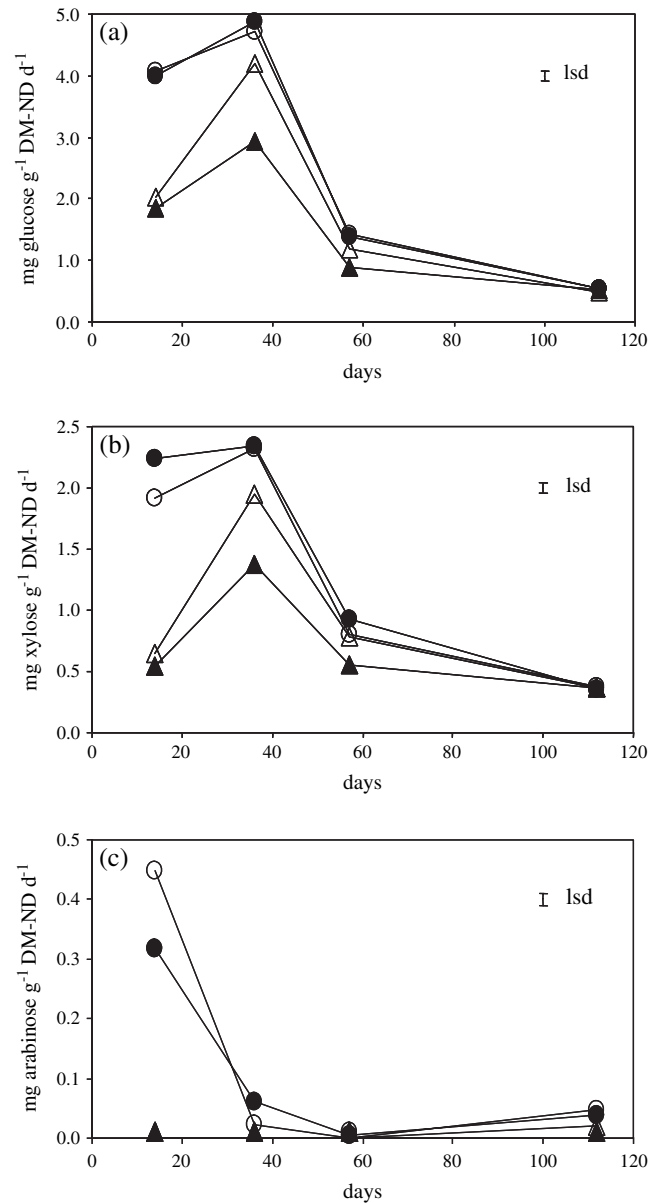
14 days, decomposition led to a significant decrease in arabinose content only in F292 and F292bm3 (Figure 2c)



**Figure 2** Changes in glucose (a), xylose (b), arabinose (c) and Klason lignin (d) levels determined on NDF-residues from roots decomposed for 112 days: ( $\Delta$ ) F2, ( $\blacktriangle$ ) F2bm1, ( $\circ$ ) F292 and ( $\bullet$ ) F292bm3. Values were corrected for dry matter loss during decomposition and are expressed as mg g<sup>-1</sup> dry matter of non-decomposed residues (DM-ND). Data are means ( $n = 2$ ).

( $P \leq 0.05$ ) whereas the roots of F2 and F2bm1 exhibited constant arabinose values throughout the incubation period (112 days).

On day 14 of incubation, the rates of glucose and xylose degradation were significantly greater in F292 and F292bm3 than in F2 and F2bm1 (Figure 3a,b) ( $P \leq 0.05$ ). Between 14 and 36 days of incubation, the glucose and xylose degradation rates increased for all genotypes, the slowest rates being observed



**Figure 3** Glucose (a), xylose (b) and arabinose (c) degradation rates determined on NDF-residues after 112 days of root decomposition: ( $\Delta$ ) F2, ( $\blacktriangle$ ) F2bm1, ( $\circ$ ) F292 and ( $\bullet$ ) F292bm3. Values were corrected for dry matter loss during decomposition and are expressed as mg g<sup>-1</sup> dry matter of non-decomposed (DM-ND) residues day<sup>-1</sup>. Data are means ( $n = 2$ ).

with the F2bm1 genotype. Thereafter, the glucose and xylose degradation rates decreased in all genotypes to reach similar values at day 112. In contrast, the arabinose degradation rate was significantly greater in F292 than in F292bm3 after 14 days ( $P \leq 0.05$ ) whereas no arabinose degradation in F2 and F2bm1 was noted during this period (Figure 3c). Between days 14 and 36, the arabinose degradation rates decreased significantly in F292 and F292bm3 ( $P \leq 0.05$ ) and were close to zero at the end of incubation, like those of F2 and F2bm1.

The Klason lignin (KL) levels remained almost stable in F292 and F292bm3 but showed a weak but significant increase in F2 and F2bm1 during decomposition (Figure 2d) ( $P \leq 0.05$ ). After 112 days, the ranking of KL content was similar to that observed before decomposition (Table 1), with F2bm1 presenting significantly greater KL values (22.5% of non-decomposed dry matter) than F2 (19.6%), F292 (15.4%) and F292bm3 (14.5%) ( $P \leq 0.05$ ). The amounts of non-condensed lignin decreased in all genotypes in line with decomposition, compared with the values found before soil decomposition (Tables 1, 2). The S to G ratio increased during decomposition in F2 and F292, which exhibited a preferential degradation of G units (Tables 1, 2). The S to G ratios for F2bm1 and F292bm3 remained stable during decomposition.

Residual FA and PCA were determined only after 112 days of decomposition. The levels of esterified FA and PCA were both affected, and a significant decrease was observed in all genotypes (Table 2) ( $P \leq 0.05$ ). The loss of ester-linked PCA was greater in F292bm3 than in F292, F2 and F2bm1. The same pattern was observed for the esterified FA content (Table 2).

Relationships between the cell wall chemical features and C mineralization rates were determined from the glucose, arabinoxylans and Klason lignin contents in roots decomposed for 14, 36, 57 and 112 days and the mean C mineralization rates at 0–14, 14–36, 36–57 and 57–112 days, for all four genotypes. Glucose and arabinoxylans contents exhibited positive relationships with C mineralization rates ( $R^2 = 0.47$  and  $0.46$ , respectively) (Figure 4a). In contrast, the Klason lignin content was not correlated with C mineralization rates ( $R^2 = 0.08$ ) (Figure 4a). The ratios of Klason lignin to glucose (KL/Glu) and of Klason lignin to arabinoxylans (KL/AX) exhibited negative relationships with C mineralization rates ( $R^2 = 0.81$  and  $R^2 = 0.85$ , respectively) (Figure 4b). In addition, the mean C mineralization rates at 0–14, 14–36, 36–57 and 57–112 days were negatively correlated with the A/X ratio ( $R^2 = 0.81$ ) (Figure 4b) whereas no correlation was shown between these rates and either the S/G ratio ( $R^2 = 0.04$ ) or the proportion of non-condensed lignin structure ( $R^2 = 0.30$ ) (data not shown).

## Discussion

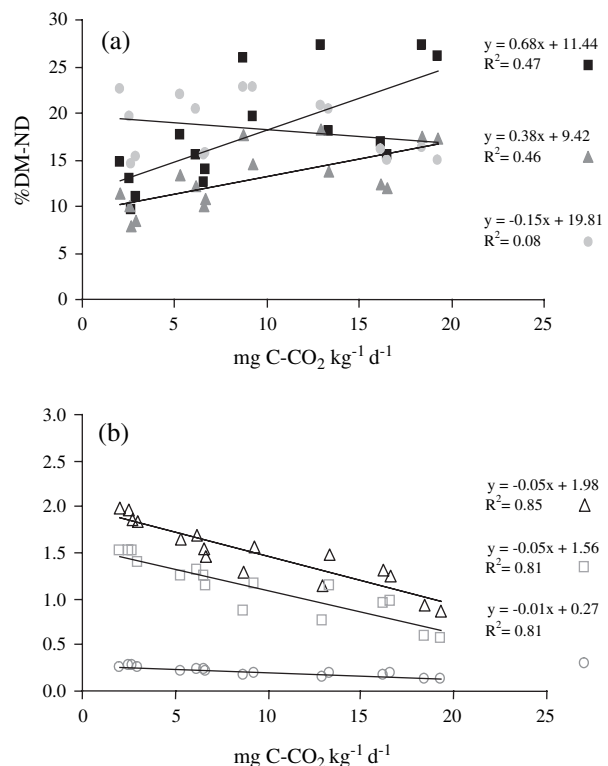
### Effects of brown-midrib mutations on root characteristics

Similarities exist between crop residue decomposition in soil and forage digestibility in the rumen (Chesson, 1997). Both require

specific enzymes to break down the plant structure. Studies of rumen digestibility have shown that the above-ground parts of brown-midrib maize mutants are more digestible than those from normal lines (Cherney *et al.*, 1991; Barrière & Argillier, 1993; Barrière *et al.*, 1994). The impacts of bm1 and bm3 mutations on the cell wall quality of maize stems have thus been characterized (Halpin *et al.*, 1998; Barrière *et al.*, 2004; Méchin *et al.*, 2005), whereas the present study was focused on bm roots and reports for the first time the cell wall quality of bm roots and their decomposition in soil.

The stems of bm1 and bm3 maize mutants are mainly characterized by lower levels of lignin and esterified PCA, and altered lignin composition compared with normal lines. However, the importance of these variations may depend on the genetic background of the plants (Barrière & Argillier, 1993; Barrière *et al.*, 2004). Accordingly, the cell wall chemical composition of roots of the normal F2 and F292 counterparts were also different.

Many of the cell-wall characteristics of brown-midrib maize stems were also found in the roots. Notably, smaller amounts of esterified PCA were found in mutant roots than in the normal lines. In addition, the differences in lignin composition (lower



**Figure 4** Relationships between mean C mineralization rates at 0–14, 14–36, 36–57 and 57–112 days of incubation and glucose (■), arabinoxylans ▲, Klason lignin contents ● expressed as mg g<sup>-1</sup> dry matter of non-decomposed (ND) residues (a), the Klason lignin to arabinoxylans ratio (▲), the Klason lignin to glucose ratio (□), and the arabinose to xylose ratio (○) (b).

S/G ratio in bm3, higher level of condensation) were similar to those of stems. However, the changes in lignin content induced by the mutation differed between the above-ground and underground plant materials. Indeed, our results showed that the bm1 mutation caused an increase in the lignin content of maize roots whereas the bm3 mutation had no such effect on lignin content. However, the brown-midrib mutations may impact stems and roots to different extents. Root cell walls are more lignified with higher condensed lignin and contain a lower proportion of ester-linked phenolic acids than stems. These variations can be partly explained by the differences in anatomy and tissues types between root and stems (Esau, 1977).

#### *Relationships between initial root quality and mineralization*

The C to N ratio of plant residues is often used as an indicator of their potential degradation (Nicolardot *et al.*, 2001), but this is mainly true if the N content of residues is an indirect consequence of plant type or plant maturity, or when N availability limits the activity of decomposers (Mary *et al.*, 1996). The overall N availability (soil + residue) was not limiting in our experiment and the C to N ratio (or root N content) of the four genotypes did not explain the observed ranking in C mineralization, although it satisfactorily explained the kinetics of soil mineral N. This is in agreement with work by Sall *et al.* (2007), which showed that the N content of residues did not influence the fate of added C when N availability was sufficient to meet overall microbial N requirements.

The genotypic variations in initial cell wall polysaccharides and initial Klason lignin contents followed the ranking of C mineralization rates. The lowest rates of C mineralization were found in the F2 and F2bm1 roots, which contained lower levels of cell wall polysaccharides and more Klason lignin than F292 and F292bm3 before decomposition.

Marked variations in cumulated C quantities were obtained even though the incubated residues were selected roots obtained from a single species (maize), and from plants grown under the same field conditions. The observed variations in total mineralized C were even greater than those measured during other studies using different plant parts from the same species or even different species (Gorissen & Cotrufo, 2000; Abiven *et al.*, 2005; Jensen *et al.*, 2005). For example, Abiven *et al.* (2005) showed that the differences in total amounts of mineralized C observed between wheat or sorghum roots after 100 days of soil incubation, were equivalent to only 5% of the added C, whereas a difference of 18.5% was measured between bm1 and bm3 maize roots incubated under the same conditions. Indeed, variations in chemical quality between wheat and sorghum roots were more marked than those found in the maize roots selected for this study, thus showing the lack of proportionality between variations in residue chemical quality and those of cumulated mineralized C. Crop residue quality in the previously mentioned studies was determined in terms of indepen-

dent fractions of different types (i.e. soluble, cellulose, hemicellulose and lignin). It should therefore be possible to determine relationships with C mineralization by analysing the quality of the cell wall polymers in greater detail. These relationships can be established by monitoring the fate of the cell wall polymers during decomposition.

#### *Dynamics of cell wall components during decomposition*

Polysaccharides were the cell wall components most affected by decomposition. Although polysaccharide decomposition in residue-amended soils mainly originates from the residues, microbial sugars can, to a lesser extent, also participate in C mineralization (Derrien *et al.*, 2007). In the present study, and assuming that only those polysaccharides originating from residues were degraded, the losses represented 60% of the total C mineralized after 112 days of decomposition (data not shown). The remaining glucose content measured during decomposition indicated that cellulose was the most markedly degraded cell wall polysaccharide, a finding in agreement with previous studies (Cheshire *et al.*, 1973). Cellulose and arabinoxylans were more degraded in the roots of F292 and F292bm3 (which exhibited greater levels of mineralized C at the end of the experiment) than in roots of F2 and F2bm1, which presented lower cumulated C kinetics.

The level of arabinoxylans substitution is represented by the arabinose to xylose ratio (A/X). Studies of the digestibility of cereal straws have shown that highly substituted arabinoxylans are less digestible (Chesson *et al.*, 1983). These studies also demonstrated that the A/X ratio increased with digestion time, showing a preferential degradation of the least substituted arabinoxylans. Our results also showed that the A/X ratio, which was similar in non-decomposed roots, increased with decomposition time in all four genotypes. However, the changes in A/X ratio during decomposition were not the same in all genotypes. After 14 days of incubation, the A/X ratio increased only in the roots of F2 and F2bm1, in which the lowest rates of arabinoxylans degradation were observed. This indicated that the degradation of arabinose-decorated xylans would be a limiting factor of root decomposition at an earlier stage in F2 and F2bm1 than in F292 and F292bm3. At the end of incubation (112 days), the A/X ratios were greater and similar for all genotypes when compared with the non-decomposed residues. Therefore, the remaining arabinoxylans showed relatively large substitution. Interactions between arabinoxylans and other cell wall polymers, as well as the substitution levels, may also hamper degradation (Chesson *et al.*, 1983). In this respect, phenolic acids, which play a key role in cross-linking arabinoxylans and lignin, are potential limiting factors in cell wall decomposition. The highest loss in phenolic acids after 112 days of decomposition was observed for F292bm3 roots in which the highest cumulative C mineralization occurred. A complete investigation of changes in PCA and FA at



different incubation times would be required to get a more comprehensive view of their impact on the soil decomposition process.

According to the Klason determinations, the lignin component was not degraded during the incubation period applied in this study. A slight increase in lignin levels was even observed in roots from F2 and F2bm1, as had previously been reported in wheat roots (Bertrand *et al.*, 2006). A preferential loss of non-lignified root material might have occurred when the residues were washed after removal from the soil.

Non-condensed lignins have been reported to be preferentially degraded during decomposition in soil compared with condensed lignins (Shimada, 1980; Bertrand *et al.*, 2006). This is in agreement with our results, which showed that the levels of non-condensed lignins decreased during decomposition in all four genotypes. However, this reduction was not detectable from the Klason lignin (acid-insoluble lignin) measurements. Indeed, non-condensed lignins, which accounted for less than 20% of the total lignin, may be partly acid-soluble (Yasuda *et al.*, 2001). After 112 days of decomposition, the degradation of non-condensed lignins represented 3% to 5% of the total C mineralized in all four genotypes (data not shown).

#### *Role of cell-wall chemical factors in the rates of C mineralization*

The Klason lignin to glucose ratio (KL/Glu) or the Klason lignin to arabinoxylans ratio (KL/AX) improved the relationships with the mineralized C rates compared with those based solely on glucose, arabinoxylans and Klason lignin. These ratios seemed more appropriate to describe the mineralization process because: (i) they were not influenced by dry matter losses, and (ii) they more clearly reflected the intimate association of lignin and cell wall polysaccharides in a cohesive network, which has been shown to influence biodegradation rates (Chesson, 1988). Therefore, the KL/Glu and KL/AX ratios determined during decomposition apparently constituted good chemical criteria to explain the kinetics of C mineralization. Another explanatory factor, the arabinose to xylose ratio (A/X), may also be relevant as it reflects the quality of one of the major cell wall components degraded during root decomposition. However, these ratios were deduced from dynamic experiments during which the C mineralization rates and changes in polymers were measured at different times during decomposition. Predictive criteria for decomposition are required as such experiments are time-consuming and expensive. If the above ratios (KL/Glu, KL/AX, A/X) are to be predictive of decomposition, as suggested by this study, it will be necessary to take into account the initial chemical characteristics of residues and to use a wider range of maize root residues. In addition, further investigations will be necessary to determine the role of cross-linking between cell wall polymers in the decomposition process.

## Conclusions

Maize roots presented marked variations in cumulated mineralized C, which were not proportional to those of their initial chemical quality. By monitoring cell wall quality evolution during decomposition, chemical criteria such as the KL/Glu, KL/AX and A/X ratios can be identified, which explain the kinetics of C mineralization. These ratios are better able to discriminate between initial residue qualities than the cell wall polymers considered separately. This is probably because they reflect interactions between polysaccharides and lignin within the cell walls that are suggested to affect soil decomposition.

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