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Plant residue chemistry impacts soil processes and microbial community structure: A study with *Arabidopsis thaliana* cell wall mutants

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ABSTRACT

The C:N ratio and concentrations of resistant compounds such as lignin in plant residues influences their biodegradation by microbial communities in soil. Soil texture is expected to modulate biodegradation rates through physical protection of residues and feedback on microbial communities. The model plant *Arabidopsis thaliana* can be modified genetically to produce residues with variable C:N ratio and acid unhydrolyzable fraction (AUF) concentrations for controlled biodegradation studies. This study assessed the C:N ratio and AUF concentration of stem and root residues of *A. thaliana* wild ecotypes and single gene knockout mutants of cinnamoyl-CoA reductase 1 (*CCR1*) and production of anthocyanin pigment 1 (*PAP1*/*MYB75*). Ground stem and root residues from *A. thaliana* lines were then mixed separately with soil (clay loam or sandy loam) and incubated for 63 d at 25 °C to evaluate the soil carbon dioxide (CO_2–C) production, mineral nitrogen (N) concentration, microbial biomass carbon (MBC) and microbial community structure by phospholipid fatty acid (PLFA) profiling. The *CCR1* mutant had 29% lower C:N ratio and 38% less AUF in stems than the wild ecotype, while *MYB75* mutant had ~2-fold higher C:N ratio in stems than the wild ecotype. The AUF concentration of roots did not differ among mutant lines and their wild ecotypes, but roots had ~2-fold higher AUF concentration than stems. Cumulative CO_2–C production was higher from soils (both texture types) amended with stem residues of *CCR1* and was lower from sandy loam soil amended with stem residues of *MYB75*, compared to their wild ecotypes. There was more CO_2–C production from soils amended with stem than root residues. Mineral N concentration was greater in soils (both texture types) amended with stem residue of the *CCR1* mutant line than its wild ecotype. There was more MBC in stem-amended than root-amended soils. PLFA profiling revealed lower fungal abundance in stem-amended than root-amended soils. In conclusion, *A. thaliana* stem residues with altered C:N ratio and AUF concentration affected the CO_2–C production, mineral N concentration, MBC and fungal:bacterial ratio of soil. Residue chemistry had a stronger influence on soil processes and microbial community structure than soil texture.

1. Introduction

Plant residue chemistry influences decomposition, nutrient mineralization, microbial biomass (Diallo et al., 2006; Zak and Kling, 2006; Bruijn and Butterbach-Bahl, 2010) and microbial community structure (Nicolardot et al., 2007; Potthast et al., 2010). Residues with lower C:N ratio and lower concentrations of resistant compounds such as lignin, condensed tannins, and insoluble waxes tend to decompose faster (Cortez et al., 1996; Flores et al., 2005; Fang et al., 2007; Yanni et al., 2011), increase N mineralization (Sun et al., 2009; Galicia and Felipe, 2011; Puttaso et al., 2011; Yanni et al., 2011) and increase microbial biomass (Saggar et al., 1999; Sun et al., 2009; Hoyle and Murphy, 2011). In contrast, plant residues with higher C:N ratio and higher concentrations of resistant compounds tend to decompose more slowly and release less mineral N (Potthast et al., 2010) and also cause an increase in relative abundance of microbial groups that are adapted to nutrient poor environments, e.g., fungi (Cerli et al., 2006; Fioretto et al., 2007; Hogberg et al., 2007; Paterson et al., 2008; Eskelinen et al., 2009; see also Arenz and Blanchette, 2011). Many studies characterize the concentrations of resistant compounds in plant tissues from the acid unhydrolyzable fraction (AUF) left after proximate chemical analysis, and this fraction has been inappropriately termed "lignin" as it has been shown to be composed of a mixture of compounds including condensed tannins, waxes, phenolics and as well as true lignins (Preston et al., 2009) the proportions of which can

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vary among species (Preston et al., 1997). Thus, in this paper we will use the terms "resistant compound" or "AUF" when referring to this fraction.

These experimental findings are summarized in a conceptual model (Fig. 1a) that contrasts the hypothesized CO_2 production, mineral N concentration, MBC and fungal:bacterial ratios in soil amended with two residue types, one having high C:N ratio and high AUF concentration, and the other with low C:N ratio and AUF concentration. The biodegradation rate is approximated from the CO_2 production rate during a short-term incubation (63 d corresponds to the length of an incubation experiment in this study), while changes in the mineral N (min. N, $NH_4 + NO_3$) concentration represent net N mineralization and nitrification in the residue–soil mixture. The "engine" for biodegradation is the microbial biomass, which increases in size when there are readily decomposable C substrates. A greater proportion of recalcitrant C (e.g., lignin and other resistant compounds) is expected to induce a shift toward more fungal-dominated than bacteria-dominated food web (i.e., greater F:B ratio).

It is not possible to predict plant residue biodegradation solely from its chemistry because other factors, like soil texture, also exert control on C and N mineralization and microbial community structure. Generally soils with higher clay contents better protect organic C, organic N (Chivenge et al., 2011), support larger microbial biomass (Six et al., 2006), and retain more nutrients (see Knops and Tilman, 2000) than soils with higher sand fraction. The interaction between chemical compounds in plant residue and soil minerals, particularly clays, can influence residue biodegradation. The N concentration of residue affects its binding affinity with mineral particles (Kleber et al., 2007) and immobilization in autochthonous organic matter (Sylvia et al., 2005). Therefore, plant residue is protected against biodegradation by its interactions with minerals and organo-mineral particles, in addition to chemical protection (resistant C compounds). A conceptual model illustrating the hypothesized effects of soil texture on CO_2 production, net mineral N concentration, MBC and fungal:bacterial ratios in sandy versus clayey soils are shown in Fig. 1b. Those diagrams can also be compared to the one on the left-hand side of Fig. 1a, which represents biodegradation of similar residue (high C:N ratio, high AUF concentration) in soil with an intermediate texture (between sandy and clayey). With increasing clay content, we predict more CO_2 production, no change in mineral N concentration, greater MBC and more proliferation of fungi, leading to a high F:B ratio. This assumes greater MBC in clayey soils, which stimulates biodegradation and CO_2 production. Short-term degradation of residue with a high C:N ratio and high AUF concentration should result in mineral N immobilization in microbial biomass, resulting in low mineral N concentration in all soil types. AUF-rich residue should support fungal proliferation, hence the medium to high F:B ratio in all soil types (Fig. 1a and b).

To test the interactive effects of residue chemistry and soil texture on biodegradation, we selected *Arabidopsis thaliana* because it can be genetically modified to yield residues with contrasting chemistry for controlled studies. Cell wall mutants of *A. thaliana* have down-regulation of single genes implicated in plant cell wall functions, which can alter the lignin concentration in their tissues (Li, 2009; Bhargava et al., 2010; Douglas, 2011). The cinnamoyl-CoA reductase 1 (*CCR1*) coding gene is involved in lignin biosynthesis in stems and its down-regulation is reported to cause marked reduction (25–50%) in lignin concentration in stems (Goujon et al., 2003; Leple et al., 2007; Ruel et al., 2009). Down-regulation of *MYB75* coding gene resulted in increased lignin deposition in inflorescence stems and higher guaiacyl (G) to syringyl (S) ratio of lignin monomers in *A. thaliana* under controlled environmental conditions (23 °C, 16/8-h light/dark photoperiod at 120 μmol m^{-2} s^{-1}, Bhargava et al., 2010). The expression of these genes is tissue specific with little (*MYB75*, Bhargava et al., 2010) or no expression (*CCR1*, Ma, 2007) detected in roots. Therefore, we can use *A. thaliana* cell wall mutants to obtain tissues that have diverse residue chemistry in plants that are genetically similar.

The present study investigated the biodegradation of stem and root residues from *A. thaliana* lines (two wild ecotypes and single gene knockout mutations of *CCR1* and *MYB75*) in soils with contrasting texture (clay loam and sandy loam). Cumulative CO_2–C processed, mineral N concentration, microbial biomass and microbial community structure were evaluated after 63 d incubation of plant residue–soil mixtures. The hypotheses underlying this study were described above and illustrated in Fig. 1.

2. Materials and methods

2.1. A. thaliana lines

Two wild ecotype accessions; Columbia-0 wt and Nossen wt and two mutant lines of *CCR1* and *MYB75* knockout genes were considered for this study. The *CCR1* knockout mutant line belongs to the Columbia-0 accession while *MYB75* knockout mutant line belongs to the Nossen accession. Seeds of *A. thaliana* lines were obtained from Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada. *CCR1* is the homozygous mutant of single gene mutation by T-DNA insertion in locus encoding *CCR1*, while *MYB75* line is the homozygous mutant of single gene mutation by *Ds* insertion in locus encoding *MYB75*. Further details about mutant lines used in this study have been reported in Derikvand et al. (2008) for *CCR1* and Bhargava et al. (2010) for *MYB75*.

2.2. Growth of A. thaliana lines

Plants were grown in a greenhouse at the Macdonald Campus of McGill University in October 2009 at about 27 °C temperature. Supplemental lighting was provided to ensure 12 h luminescence. Plants were grown in peat moss based growth medium (Jiffy pellets, Jiffy Products Ltd. Shippagan, New Brunswick, Canada) in plastic trays (53 cm × 27 cm). Each tray contained 50 Jiffy pellets with one plant grown per Jiffy pellet. Five trays were planted with each *A. thaliana* line. Plants were enriched with ^{13}C and ^{15}N according to the protocol of Bromand et al. (2001) at vegetative, bolting, and flowering stages. This involved the pulse labeling of plants with ^{13}C–CO_2 and applying $K^{15}NO_3$ after pulse labeling.

2.3. Biochemical analysis of plant tissues

A pooled sample of all plants grown in one tray was considered as one replication and hence 5 replications were made for each *A. thaliana* line. Plants were harvested at fruit ripening stage. Stems and roots were separated, fruits from inflorescence stems were removed, roots were washed, then stems and roots were dried at 50 °C for 48 h, crushed in a grinder and passed through 0.5 mm mesh sieve. Further, the C, N and C:N ratio analysis of stem and root residues from second cohort plants was done on two analytical subsamples from each replicated line. Organic C, total N and C:N ratio was analyzed using a ThermoFinnigan Flash EA 1112 CN Analyzer (Carlo Erba, Milan, Italy). Concentrations of resistant compounds in stems and roots were analyzed following the gravimetric method of Van Soest et al. (1991) using Ankom Fiber Analyzer (AnkomTechnology, Fairport, NY). This method involves the removal of cellular components and hemicellulose by washing the ground plant tissues with acid detergent, removal of cellulose with 72% concentrated H_2SO_4, and thereafter remaining AUF was weighed and values reported after subtracting the residue ash weight.

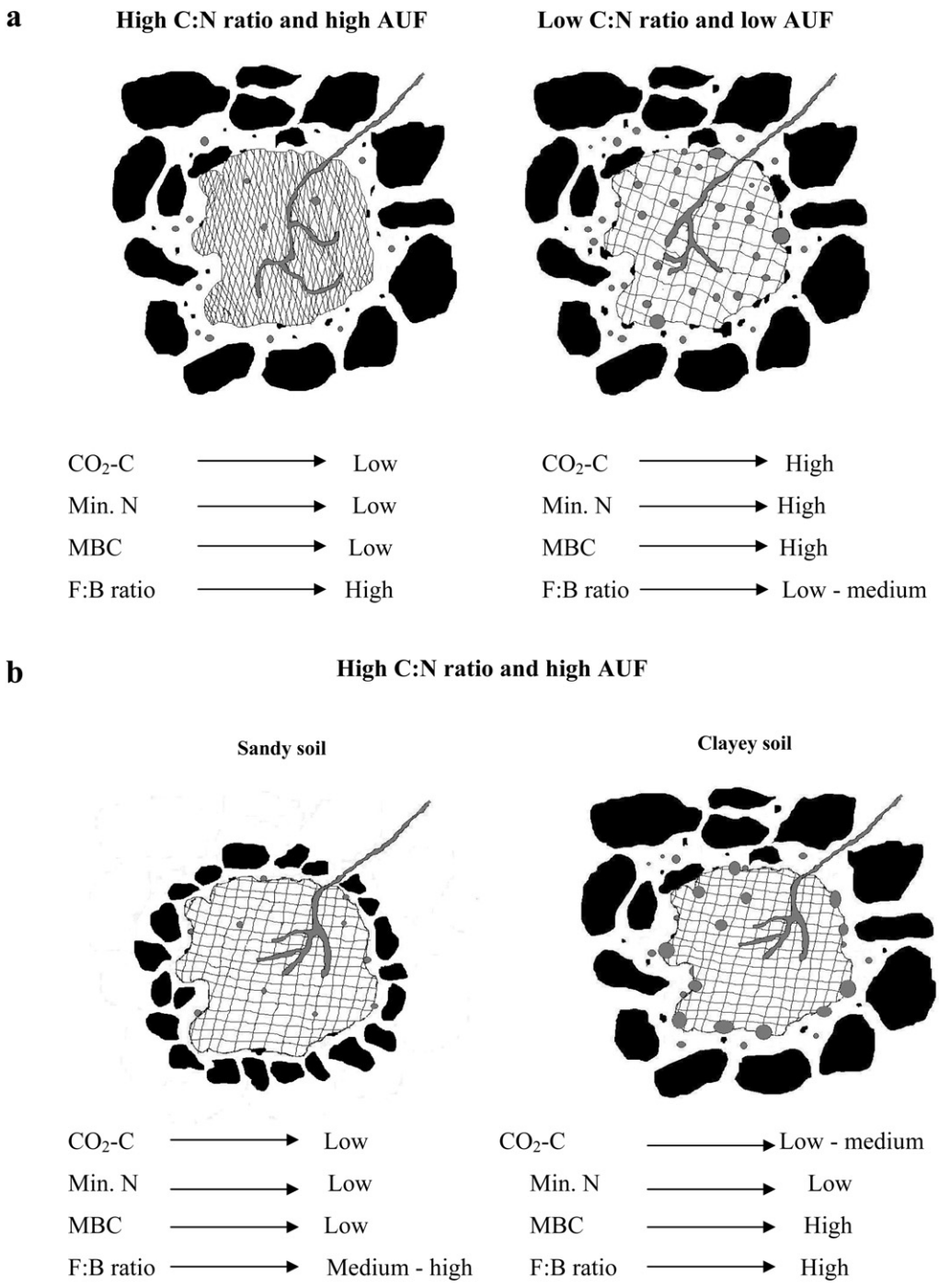

Fig. 1. Conceptual model illustrating (a) the influence of plant residue chemistry (C:N ratio and acid unhydrolyzable fraction (AUF) concentration) on CO_2–C emission, mineral N (min. N) concentration, microbial biomass C (MBC), and fungal:bacterial (F:B) ratio within a macro-aggregate (cross section shown) in a short-term (63 d) incubation, and (b) the interactive effect of plant residue chemistry and soil texture on CO_2–C emission, mineral N (min. N) concentration, microbial biomass C (MBC), and fungal:bacterial (F:B) ratio within macro-aggregates (cross section shown) in a short-term (63 d) incubation period. Soil, residue and microbial components are represented are as follows: microaggregate, ; fungal hypha, ; plant residue debris, ; small organo-mineral complex, ; microbial colony, .

Table 1
Soil physical and chemical characteristics.

Soil type	Fraction of mineral particles (g kg^{-1})[a]			pH	Organic C[b] (g kg^{-1} soil)	Total N[b] (g kg^{-1} soil)	C:N ratio
	Sand	Silt	Clay				
Clay loam	204	432	364	6.6	34.2	3.95	8.7
Sandy loam	815	89	96	6.0	18.8	2.01	9.3

[a] Soil texture was reported by Poirier et al. (2009) and Halpern et al. (2010).
[b] Organic C and total N was quantified with a CN analyzer (Carlo Erba, Italy).

2.4. Soil

Clay loam and sandy loam soils were collected for this experiment. The clay loam soil was obtained at the L'Acadie Experimental Farm of Agriculture and Agri-Food Canada (45°18′N, 73°21′W) from the top 20 cm of a 17-year-old no-till corn (*Zea mays* L.) plot. The sandy loam soil was obtained from the top 20 cm of an 18-year-old conventionally tilled plot under continuous corn silage production at the Macdonald Campus Farm, McGill University, Canada (45°30′N, 73°35′W). Soil physical and chemical characteristics are provided in Table 1.

2.5. Incubation trial

The incubation trial was designed as a factorial experiment with four *A. thaliana* lines, two residue types (stems and roots) and two soil types (sandy loam and clay loam), replicated five times for a total of 80 observations. The experimental unit was a 50 mL graduated translucent plastic bottle (Corning snap-seal container No. 1730, New York, USA) containing 20 g air-dried soil mixed completely with 1% (0.2 g) finely ground plant residue. Soil was moistened to 60% water-filled pore space, which is reported to maximize the activity of aerobic decomposer microorganisms (Linn and Doran, 1984). Plastic bottles were placed in 1 L Mason jars, 15 mL water was added to the bottom of the jar to maintain humidity, the jars were capped with lids equipped with air-tight rubber septa and placed in incubator at 25 °C. Residue–soil mixtures were incubated in closed jars for 63 d.

Gas sampling to determine CO_2–C production from residue–soil mixtures was carried out daily during first week of incubation, twice during second week and at 10 d intervals for the rest of the study. Gas sampling involved mixing headspace gas, removing a 20 mL aliquot and transferring it to a 12 mL exetainer (Labco, Wycombe, UK) using a gas-tight syringe. Then lids were removed and jars were aerated for 30–45 min before closing.

The CO_2 concentration was analyzed using a gas chromatograph (Hewlett-Packard 5890 Series II, Hewlett-Packard Company, Avondale, PA, USA) equipped with a Porapak Q column (ethylvinylbenzene and divinylbenzene copolymer beads; 80–100 mesh; length, 25 m; internal diameter, 0.20 mm; Supelco 20331). The carrier gas was helium (50 mL min^{-1}), oven temperature was set at 70 °C and CO_2 concentration was quantified with a thermal conductivity detector.

2.6. Soil CO_2–C production

The CO_2–C production from residue–soil mixtures was calculated in two steps. First, the gas concentration in headspace was converted from ppm to a mass per volume concentration (C_m in g CO_2–C L^{-1}) with the ideal gas equation (Holland et al., 1999):

$$C_m = \frac{C_v MP}{RT} \quad (1)$$

where C_v is the sample CO_2 concentration in ppm (L 10^6 L^{-1}) minus the CO_2 concentration of soil incubated without plant residue, M is the molecular weight of the trace species, e.g., CO_2 = 12 g C mol^{-1}, P is the atmospheric pressure (1 atm), T is the incubation temperature in K and R is the universal gas constant (0.0820575 L atm K^{-1} mol^{-1}). Thereafter, the CO_2–C production for each sampling event was calculated as:

$$f = V\left(\frac{C_m}{W}\right) \quad (2)$$

where f is the CO_2–C production in μg g^{-1} soil, V is the headspace of jar (0.95 L) and W is the dry mass of soil (20 g). Cumulative CO_2–C production (mg CO_2–C g^{-1} dry soil) was the sum of CO_2–C production from each sampling event during the 63 d incubation.

2.7. Assessment of soil mineral N and MBC concentrations

After 63 d incubation, the residue–soil mixture was extracted with 0.5 M K_2SO_4 (1:4 soil:solution ratio). Mineral N was the sum of ammonium (NH_4–N) and nitrate (NO_3–N) concentrations, analyzed colorimetrically using LachatQuikChem AE flow injection auto-analyzer (Lachat Instruments, Milwaukee, WI). Dissolved organic C (DOC) concentration was analyzed with a Shimadzu TOC-V analyzer (Shimadzu Corp., Kyoto, Japan). We used the chloroform fumigation direct extraction method to assess MBC, which was the difference in DOC concentration between fumigated and un-fumigated soils divided by an efficiency factor, k_{EC} = 0.45 (Joergensen, 1996).

2.8. PLFA assessment

Pooled samples from five experimental replicates of stem- and root-amended clay loam and sandy loam soils underwent PLFAs profiling using a modified Bligh and Dyer method (Frostegård et al., 1991). Soils were air dried for 24 h, crushed by hand with a mortar and pestle, then freeze dried and stored at −20 °C until they were sent to the Department of Forest Sciences, University of British Columbia, Vancouver, BC, Canada for PLFA analysis. Total lipids were extracted from 4 g of soil samples using citrate buffer, chloroform and methanol (1:1:2.5 v/v/v). Neutral lipids, glycolipids, and phospholipids were fractionated on silica bonded phase column by elution with chloroform, acetone, and methanol respectively. Phospholipids were transmethylated to fatty acid methyl esters (FAMEs) by alkaline methanolysis (Dowling et al., 1986). FAMEs were analyzed with Agilent 6890N GC connected to an Agilent 5975 Inert XL Mass Selective Detector (Agilent Technologies Inc., Santa Clara, CA), with a column 30-m J&W HP-5 with a film thickness of 0.25 μm and 0.32 mm ID. The column was kept at 76 kPa pressure. The samples were injected in a pulsed splitless mode. Methyl nonadecanoate (19:0) was the internal standard. The temperature program was as follows: initial temperature of 50 °C for 0.1 min, ramped to 150 °C at 15 °C min^{-1} and held for 2 min, ramped to 190 °C at 4 °C min^{-1}, then ramped to 200 °C at 10 °C min^{-1} and held for 5 min, ramped to 270 °C at 5 °C min^{-1} and kept for 10 min at 270 °C. The PLFAs i15:0, a15:0, i16:0, i17:0, a17:0 were designated as Gram-positive bacteria (Bååth et al., 1992; Zogg et al., 1997), i16:1ω7c, 16:1ω9c, 16:1ω7c, i17:1ω8c, cy17:0, 18:1ω7c, 18:1ω5c, cy19:0 as Gram-negative bacteria (Bååth et al., 1992; Zogg et al., 1997), 16:0 10Me, 10Me17:0, 17:0ω7 m/10Me18:0 as actinomycetes (McKinley et al., 2005; Zelles, 1997), 16:1ω5c, (McKinley et al., 2005), 18:2ω6, 9c, and 18:1ω9 as fungi (Zelles, 1999; Kaiser et al., 2010).

2.9. Statistical analysis

Data was assessed for normal distribution and normalized when necessary with log transformation. The data presented in tables and figures are untransformed means and ±standard deviations (SD). The effect of *A. thaliana* lines on residue chemistry (organic C, total N, C:N ratio, AUF concentration) was evaluated by one-way analysis of variance (ANOVA) using PROC GLM function of SAS (SAS Institute Inc., 2009). The main and interactive effects of *A. thaliana* line, residue type and soil texture on CO_2–C production, mineral N and MBC was also analyzed using the PROC GLM function. The correlation between cumulative CO_2–C production in stem-amended soils, plant residue chemistry (C:N ratio, AUF concentration) and soil mineral N was determined using the PROC CORR function of SAS (SAS Institute Inc., 2009). The difference in PLFA fungal:bacterial

ratio of stem-amended and root-amended soils was assessed with a Student's *t*-test.

3. Results

3.1. Organic C, total N, C:N ratio and AUF concentration of plant residues

Total N concentration of stem and root residues of the *CCR1* line were greater than its wild ecotype (Columbia-0 wt), while the N concentration was lower in stem residue from the *MYB75* line than its wild ecotype (Nossen wt) ($P \leq 0.05$, Table 2). *CCR1* line had a lower C:N ratio in stem and root residues than its wild ecotype while *MYB75* line had a higher C:N ratio in stem residue than its wild ecotype ($P \leq 0.05$, Table 2). The AUF concentration was lower in the stem residue of *CCR1* than its wild ecotype, but there was no difference in AUF concentration of stems from *MYB75* and its wild ecotype. Root residues had higher AUF concentration than stem residues ($P \leq 0.05$, Table 2).

3.2. Soil CO_2–C production

Cumulative CO_2–C production was higher when amended with stem residues of the *CCR1* (both soil texture classes) than the wild type ($P \leq 0.05$, Table 2). Sandy loam soil mixed with stem residues from the *MYB75* line produced lower CO_2–C than sandy loam soil amended with stem residues of its wild ecotype ($P \leq 0.05$, Table 2). There was no difference in CO_2–C production among root-amended soils, which produced less CO_2–C than stem-amended soils (($P \leq 0.05$, Table 2). The residue type × soil texture interaction and the *A. thaliana* line × soil texture interaction were not significant ($P > 0.05$) for stem-amended soil. Cumulative CO_2–C production from sandy loam and clay loam soils amended with stem residues was negatively correlated with the C:N ratio and AUF concentration in stem residues, and positively correlated with soil mineral N concentration (Table 3).

3.3. Soil mineral N and MBC concentrations

The mineral N concentration was higher in soil amended with stem residues of *CCR1* line than its wild ecotype in both soil texture classes, but there was no difference in the mineral N concentration of soils amended with stem residues of *MYB75* and its wild ecotype (Table 4). The MBC concentration was numerically higher in clay loam soil amended with stem residues from *CCR1* and *MYB75* lines than their wild ecotypes, and there was more MBC in stem-amended than root-amended soil ($P < 0.0001$, Table 4).

3.4. PLFA

The PLFA of soils amended with residues from *A. thaliana* lines showed greater fungal concentration in root-amended than stem-amended sandy loam soil ($P \leq 0.05$, Tables 5 and 6, Fig. 2a). The F:B ratio (summed fungal PLFA: summed bacterial PLFA) was higher for the root-amended than stem-amended sandy loam soil ($P \leq 0.05$, Fig. 2b).

4. Discussion

4.1. Plant residue chemistry and soil CO_2–C production

The *CCR1* and *MYB75* knockout mutations altered the C:N ratio and AUF concentration (in *CCR1* mutant line only) in stem residues. As expected, stem residues of *CCR1* that had 29% lower C:N ratio and 38% lower AUF concentration caused greater CO_2–C production from stem-amended soil than its wild ecotype. The higher C:N ratio in stems from *MYB75* caused lower CO_2 production in stem-amended sandy soil than its wild ecotype. Nitrogen is crucial for the growth and reproduction of aerobic decomposer microorganisms and the C:N ratio often controls plant residue biodegradation and CO_2 production in short-term (2–3 months) laboratory incubations(Cortez et al., 1996; Flores et al., 2005; Fang et al., 2007). There was a negative correlation between CO_2 production and stem residue chemistry (C:N ratio, AUF concentration) in both sandy loam and clay loam soils. Root residues that had ~2-fold higher AUF concentration than stems also resulted in lower CO_2–C emission from root-amended soils than soils. These findings are consistent with the hypotheses illustrated in Fig. 1a.

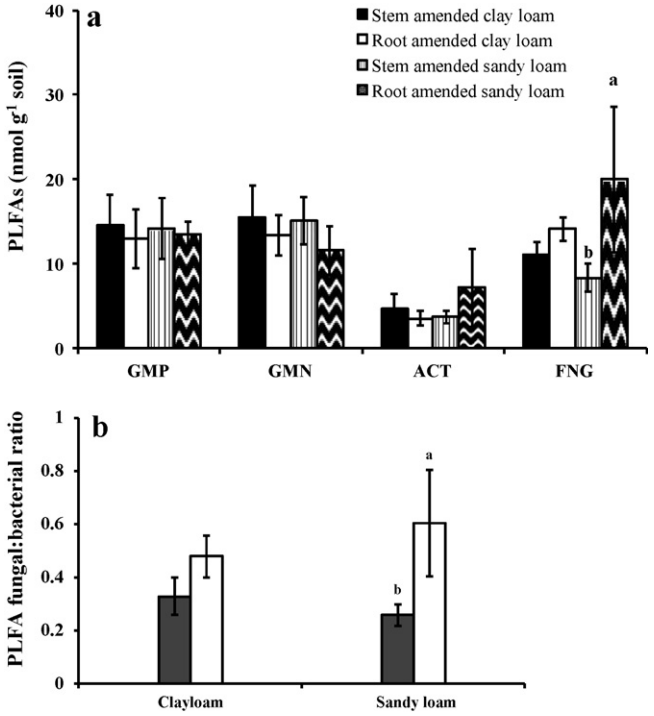

Fig. 2. (a) Average (±SD) of Gram-positive bacteria (GPB), Gram-negative bacteria (GNB), actinomycetes (ACT) and fungi (FNG) and (b) average abundance of fungal PLFA: bacterial PLFA in clay loam and sandy loam soils amended with stem (■) and root (□) residues of *A. thaliana* lines ($n = 4$). Bars with different letters represent significant ($P \leq 0.05$) differences between residue type in a given soil texture class.

Despite the higher C:N ratio, stem residues from the *MYB75* line did not reduce CO_2–C production from clay loam soil, although it did give lower CO_2–C production in sandy loam soil compared to its wild ecotype. Since clayey soils are generally rich in nutrients (see Knops and Tilman, 2000 and Table 1) and have higher microbial biomass (Six et al., 2006) than sandy soils, there was likely more biodegradation of *MYB75* stem residues (leading to more CO_2–C production) in clay loam than in sandy loam soil, which is consistent with the hypothesis illustrated in Fig. 1b.

4.2. Plant residue chemistry, mineral N concentration and microbial biomass

Consistent with the hypothesis in Fig. 1a, stem residues of the *CCR1* line (low C:N ratio and low AUF concentration, relative to other residues) caused significant increase in mineral N concentration in both soil texture classes, and tended to increase (numerically) the MBC in clay loam soil. Mineral N concentration was positively correlated with CO_2–C production, and negatively correlated with residue chemistry (C:N ratio, AUF concentration,

Table 2
Mean (±SD) of the organic C, total N and acid unhydrolyzable fraction (AUF) concentrations, and C:N ratio of stem and root tissues from *A. thaliana* lines and cumulative CO_2–C production of stem- and root-amended sandy loam and clay loam soils.

	A. thaliana line	Organic C ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	C:N ratio	AUF ($g\,kg^{-1}$)	Cumulative CO_2–C production[a] ($mg\,CO_2$–$C\,g^{-1}$ soil) (clay loam soil)	Cumulative CO_2–C production[a] ($mg\,CO_2$–$C\,g^{-1}$ soil) (sandy loam soil)
Stem tissue	Columbia-0 wt	448 ± 5[a]	8.8 ± 1.6[a]	52.5 ± 10.6[a]	104 ± 7.0[a]	2.89 ± 0.19[a]	2.58 ± 0.17[a]
	CCR1	443 ± 2[a]	11.9 ± 0.8[*a]	37.3 ± 2.5[*]	65.4 ± 5.6[*a]	3.17 ± 0.15[*]	3.02 ± 0.21[*a]
	Nossen wt	444 ± 5[a]	9.7 ± 1.5[a]	46.9 ± 10[a]	95.4 ± 8.5[a]	2.84 ± 0.12[a]	2.60 ± 0.15[a]
	MYB75	440 ± 3[a]	5.5 ± 0.8[*a]	81.4 ± 10[*a]	99.0 ± 8.9[a]	2.68 ± 0.08[a]	2.33 ± 0.09[*]
Root tissue	Columbia-0 wt	475 ± 9[b]	12 ± 1.0[b]	38.7 ± 2.9[b]	198 ± 10[b]	2.35 ± 0.05[b]	2.24 ± 0.12[b]
	CCR1	472 ± 2[b]	14 ± 0.6[*b]	34.7 ± 1.6	182 ± 1.9[b]	–[b]	2.32 ± 0.14[b]
	Nossen wt	477 ± 3[b]	14 ± 0.7[b]	34.6 ± 1.9[b]	207 ± 28[b]	2.56 ± 0.09[b]	2.35 ± 0.14[b]
	MYB75	476 ± 3[b]	13 ± 0.4[b]	37.0 ± 1.0[b]	195 ± 5.0[b]	2.42 ± 0.11[b]	2.43 ± 0.15

Within group (stem versus roots) difference probability (*P*)

Organic C	Total N	C:N ratio	AUF	CO_2–C production (clay loam)	CO_2–C production (sandy loam soil)
P = 0.0001	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001

Values within a column presented with * are significantly different at $P \leq 0.05$ from their wild ecotypes (wt) for a given tissue type (stem or root) and the values presented with letter within a column represent significant difference at $P \leq 0.05$ between residue type (stem versus root) of a given line.
[a] Corrected for CO_2 produced by soil incubated without plant residue.
[b] – Represents no data.

Table 3
Correlation (*r*) of cumulative CO_2–C production ($mg\,CO_2$–$C\,g^{-1}$ soil) of stem residue–soil mixtures after 63 d incubation with the initial C:N ratio and acid unhydrolyzable fraction (AUF) concentration in the stem residues, and the soil mineral N (min. N) concentration after 63 d incubation. Data was pooled across residue types and *A. thaliana* lines ($n = 40$ for clay loam soil, $n = 40$ for sandy loam soil). *P* is the probability level.

	Cumulative CO_2–C production (clay loam soil)		Cumulative CO_2–C production (sandy loam soil)	
	r	*P*	*r*	*P*
C:N ratio	−0.61	0.0052	−0.73	0.0004
AUF concentration	−0.60	0.0066	−0.73	0.0004
Min. N concentration	0.71	0.0004	0.78	0.0001

Table 4
Mineral N (NH_4–N + NO_3–N) concentration of stem-amended soils and microbial biomass carbon (MBC) in stem and root-amended clay loam soil after 63 d incubation. Values are the mean (±SD) of 5 replicates.

A. thaliana lines	Mineral N, stem-amended soils[a] ($mg\,N\,kg^{-1}$ soil)		MBC ($mg\,C\,kg^{-1}$ soil)	
	Clay loam soil	Sandy loam soil	Stem-amended soil	Root-amended soil
Columbia-0 wt	136.9 ± 2.5	110.0 ± 14.0	284.4 ± 161.3	104.19 ± 21.64
CCR1	159.3 ± 3.5[*]	132.1 ± 6.9[*]	321.4 ± 113.3	–[b]
Nossen wt	137.7 ± 3.2	105.2 ± 7.2	213.2 ± 118.9	60.15 ± 32.8
MYB75	135.4 ± 1.7	101.4 ± 8.3	397.1 ± 30.2	82.7 ± 33.2

[*] Within column, values were different from their wild ecotype at $P \leq 0.05$.
[a] Size of mineral N pool after 63 d, not corrected for the initial mineral N concentration or mineral N in soil incubated without plant residue.
[b] –, No data.

data not shown). Our results are in agreement with previous findings that C:N ratio and resistant C compounds in plant residues influence concentration of soil mineral N (Urquiaga et al., 1998; Nezomba et al., 2009; Sun et al., 2009) and microbial biomass (Saggar et al., 1999; Sun et al., 2009; Potthast et al., 2010; Hoyle and Murphy, 2011). Results from the *MYB75* stem-amended soils were inclusive, but this may due to the similarity in AUF concentration in stem residues from *MYB75* and the wild ecotypes, which

Table 5
Concentration of PLFAs ($nmol\,g^{-1}$ soil) and ratios of various group PLFAs in the clay loam and sandy loam soils amended with stem residues of *A. thaliana* lines after 63 d incubation.

PLFA	*A. thaliana* lines							
	Columbia-0 wt	*CCR1*	Nossen wt	*MYB75*	Columbia-0 wt	*CCR1*	Nossen wt	*MYB75*
	Clay loam				Sandy loam			
Gram-positive bacteria	12.3	18.8	10.8	16.3	15.2	12.7	18.7	10.3
Gram-negative bacteria	13.6	19.8	11.3	17.3	17.2	14.2	17.4	11.5
Actinobacteria	4.04	6.44	2.57	5.69	4.05	3.29	4.62	2.85
Fungi	9.42	12.9	10.8	11.4	10.2	8.63	8.21	6.22
Total bacteria	29.9	45.1	24.6	39.2	36.5	30.2	40.7	24.6
Gram-positive:Gram-negative	0.90	0.95	0.96	0.94	0.88	0.89	1.07	0.89
Actinobacteria:bacteria	0.16	0.17	0.12	0.17	0.12	0.12	0.13	0.13
Fungi:bacteria	0.31	0.29	0.44	0.29	0.28	0.29	0.20	0.25
Total PLFA	39.4	57.9	35.4	50.6	46.7	38.8	48.9	30.9

Table 6
Concentration of PLFA (nmol g^{-1} soil) and ratios of various group PLFAs in the clay loam and sandy loam soils amended with root residues of *A. thaliana* lines after 63 d incubation.

PLFA	*A. thaliana* lines							
	Columbia-0 wt	*CCR1*	Nossen wt	*MYB75*	Columbia-0 wt	*CCR1*	Nossen wt	*MYB75*
	Clay loam				Sandy loam			
Gram-positive bacteria	16.4	–[a]	9.47	13.1	11.5	14.0	14.6	14.2
Gram-negative bacteria	15.9	–	11.2	13.1	12.4	15.3	9.60	9.15
Actinobacteria	4.49	–	2.88	3.21	2.95	3.50	10.5	11.8
Fungi	14.3	–	12.6	15.4	11.0	14.3	27.3	27.4
Total bacteria	36.8	–	23.6	29.4	26.8	32.8	34.8	35.2
Gram-positive:Gram-negative bacteria	1.03	–	0.84	1.00	0.48	0.48	0.60	0.61
Actinobacteria:bacteria	0.14	–	0.14	0.12	0.12	0.12	0.43	0.51
Fungi:bacteria	0.39	–	0.54	0.53	0.41	0.44	0.78	0.78
Total PLFA	51.1	–	36.2	44.8	37.8	47.1	62.0	62.6

[a] –, No data

was important for the biodegradation, N mineralization and nitrification processes, and C accumulation in microbial biomass.

4.3. Plant residue chemistry and soil microbial community structure

Pooled samples of residue–soil mixtures were analyzed to examine general trends regarding the influence of residue type (stem versus root) and soil texture on microbial community structure. The abundance of Gram-positive bacteria, Gram-negative bacteria and actinomycetes was similar in clay loam and sandy loam soils. We noted greater fungal biomass and fungal:bacterial ratio in root-amended than stem-amended sandy loam soil, but not in the clay loam soil. Our findings suggest that root residues, which had a greater AUF concentration than stem residues, promoted fungal growth in the nutrient poor sandy loam soil, which is consistent with the hypotheses in Fig. 1a and b. However, there was no difference in the microbial community in the clay loam soil, which could indicate that the microbial community structure was less responsive to residue addition. Perhaps we would need to add residues with an even wider range of residue chemistry or increase the incubation period to see a significant shift in the fungal population and the F:B ratio in a clay loam soil, as hypothesized in Fig. 1b. These findings suggest that plant residue chemistry, especially the concentration of resistant C compounds, exerts a stronger influence on microbial community structure than soil texture.

4.4. Influence of soil texture on soil processes in response to residue chemistry

Soil processes such as CO_2–C production, mineral N concentration and microbial community structure gave similar responses in both soil texture classes when amended with the same residue type from a particular *A. thaliana* line. In addition, the residue type × soil texture interaction was not significant for CO_2–C production. This may be attributed to the fact that this short-term incubation relied upon finely ground plant residues, sieved soils and optimal conditions for microbial growth and biodegradation. Under such conditions, we conclude that soil texture exerts a minor influence on soil processes, with the impacts from plant residue chemistry having the dominant control on soil processes and microbial communities.

5. Conclusion

In conclusion, *CCR1* and *MYB75* knockout mutations altered the C:N ratio and AUF concentration in stem residues of *CCR1 A. thaliana* line. Plant residue chemistry (C:N ratio, AUF concentration) was negatively correlated with soil processes (CO_2–C production, soil mineral N concentration). Although similar levels of CO_2–C production were measured in sandy loam and clay loam soils, there was 20–34% higher mineral N concentration in the clay loam than sandy loam soil. Greater availability of soil mineral N in the clay loam soil could be linked to the stability of the microbial community in that soil, whereas a shift showing greater fungal biomass and higher F:B ratio was observed in the sandy loam soil when amended with root residues (about 2-fold greater AUF concentration) than stem residues. The influence of soil mineral N concentrations on microbial community structure in the presence of AUF-rich substrates merits further investigation. Stable isotope tracing with ^{13}C and $^{15}N$to monitor the dynamic transfers of these tracers from plant residues to soil microorganisms by PLFA-MS profiling would give greater insight into how soil microbial communities transform plant residue C and N compounds. Accumulation and depletion of ^{13}C and ^{15}N in aggregates and other organic fractions (e.g., particulate organic matter, fine organic matter) coupled with chemical analysis of bio-molecules with nuclear magnetic resonance and synchrotron-based techniques could also be helpful in this regard.

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