Soil biodegradation of aerial and underground litter of *Miscanthus*, a perennial energy crop

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Abstract
To predict the environmental benefits of energy crop production and use, the nature and fate of biomass residues in the soil need to be quantified. Our objective was to quantify *Miscanthus x giganteus* biomass recycling to soil (senescent leaves, roots and rhizomes) and to assess how harvesting time and N fertilization affect their characteristics and subsequent biodegradability. The quantification of aerial and belowground biomasses and their sampling were performed on 2- and 3-year-old Miscanthus stands, either fertilized with 120 kg N $ha^{-1}year^{-1}$ or not fertilized, in autumn (maximal biomass production) and winter (maturity). Plant biomass was chemically characterized and incubated in optimum decomposition conditions (15°C, -80kPa) for 263 days, for C and N mineralization. C mineralization kinetics was analyzed in relation to litter quality.

Key Words
Miscanthus, litter quality, soil biodegradation, carbon and nitrogen cycles

Introduction
The concerns of global fossil fuel depletion and environmental pollution from its combustion are driving the search for carbon-neutral, renewable energy sources. The use of ligno-cellulosic plant biomass as an energetic source is an alternative which is fully investigate nowadays. Substituting fossil fuels with crop biomass will require selection of the most suitable plant species and adequate management to meet the environmental constraints. Several species that produce high biomass from low inputs would be good candidates for energy production. Amongst these, the genus *Miscanthus*, which is a perennial rhizomatous grass, with a great adaptability to different environments and a high yielding potential (C_4-plant) appears as a good candidate (Heaton *et al*., 2004). New practices and/or the development of new energy crops will also modify the quantity and quality of crop residues entering the soil system and therefore affect the nutrient cycles (mainly Carbon (C) and Nitrogen (N)) (Bertrand *et al*., 2009; Amougou *et al*., 2010). The aim of our study was therefore to establish the relationships between *Miscanthu*s litter quality (aerial and underground parts) and their rate of decomposition in soil as a function of the agricultural practices (date of harvest and N fertilization rate).

Methods
Site and field experiment
Miscanthus sampling was realized at the INRA experimental station of Mons en Chaussée Northern France). The soil is a deep silt loam (Orthic Luvisol) with 19.9% clay, 2.0% silt, 7.8% sand, 0.3% $CaCO_3$ and pH of 7.8. The climate is oceanic temperate with annual precipitation and temperature means of about 713 ± 49 mm and 11 ± 1°C respectively since the establishment of the Miscanthus crop (spring 2006).
The field experiment design consisted of a randomized block design with (i) two Nitrogen rates (0 kg N ha^{-1} (0N) and 120 kg N ha^{-1}(120N)) added as urea ammonium nitrate solution applied each year at the beginning of growth (April), and (ii) two harvest dates, an early harvest in autumn (October) and a late harvest in winter (February-March). This gave four treatments: autumn/0N, autumn/120N, winter/0N, winter/120N. Each treatment had 3 replicates. The Miscanthus rhizomes were planted in April 2006 at a density of 15,625 plants ha^{-1}.

Sampling of aboveground and belowground biomass
The study concerned the 2007 and 2008 growing seasons, i.e. the plantation second and third years. At each harvest date (autumn 2007 and 2008, winter 2008 and 2009), 3 whole Miscanthus plants were destructively sampled from each 0N and 120N treatment plot. Each plant had 26 stems on average. Stems and leaves were weighed to obtain fresh weight and were then separated, subsampled and dried at 80°C for 48 hours for dry matter determination. The dry weight of the stems was then added to that of leaves to determine total aerial dry matter. The rhizomes were sampled to determine the below-ground biomass to a depth of 30 cm. The rhizomes + associated roots were cleaned of soil by manual washing on a sieve to avoid dry matter loss. The

roots were then separated from the rhizomes by hand. The rhizomes were manually cut into small pieces (5-10 cm). Senescent leaf fall was monitored through the autumn-winter 2007-2008 and autumn-winter 2008-2009 periods using a nylon net (mesh size 1 cm × 1 cm) in the 0N and 120N treatments. The yields of the different plant parts were then expressed in ton dry matter per hectare (t DM ha^{-1}). Subsamples of roots, rhizomes and leaves were kept for an incubation experiment and biochemical analysis; there were dried at 35°C for a week.

Chemical characteristics of the litters
Chemical characteristics were determined on leaf, rhizome and root samples from the first year of sampling only (2007). The total Carbon (C) and Nitrogen (N) concentrations of the plant parts were determined using an elemental analyzer. The total neutral sugar content of the plant samples was determined using the method described by Blakeney *et al*. (1983). The NDS-soluble fraction was determined using the method described by Goering and Van Soest (1970). The NDF fraction, designated as cell walls, was then dried for one week at 30°C and ground to 80 µm prior to Klason lignin determination. Klason lignin (KL) was determined as the acid-insoluble residue remaining after sulphuric acid hydrolysis of cell wall polysaccharides (Monties, 1984).

Incubation study
The soil from the field site was sampled from the top 5-10cm layer of one plot. It was sieved to 2 mm and stored at the incubation temperature (15°C) for a week prior to incubation. The rhizomes, roots, necrotic rhizomes and senescent leaves were hand cut into pieces 4-5 mm long and 5 mm wide prior to incubation. They were added at a rate equivalent to 2 g C kg^{-1} dry soil, mixed into the moist soil and incubated at 15°C, for 263 days for C mineralization and 114 days for N mineralization. Potassium nitrate was added to the soil to ensure that decomposition would not be N-limited (Recous *et al*., 1995). Soil moisture was maintained throughout the incubation period by weighing at weekly intervals and adding deionised water when necessary. A control treatment was performed in the same way but without the addition of residue. Carbon mineralization was measured from soil samples equivalent to 100 g dry soil, incubated in the presence of a CO_2 trap with four replicates per treatment. Mineral N was determined on separate soil samples with three replicates per treatment.

Results
The total aboveground biomass measured at autumn harvest was 20 to 22 t DM ha^{-1} for year 1 and 24 to 26 t DM ha^{-1} for year 2, declining to 14-15 and 19-20 t DM ha^{-1} at winter harvest, respectively. The aboveground biomass increased significantly between year 1 and year 2 except for the autumn/0N treatment, while N treatment had no significant effect (data not shown). The amount of senescent leaves collected over the winter was about 3 t DM ha^{-1} and did not vary between year 1 and year 2 or between N treatments. Belowground biomass was not significantly affected by harvest date or N treatment, and amounted 15 to 20 t DM ha^{-1} depending on the treatment.
Aboveground biomass sampled in winter accumulated significantly ($P \leq 0.05$) smaller amounts of N (22 to 41 kg N ha^{-1}) than the autumn sampled ones (90 to 118 kg N ha^{-1}), indicating that N lost from aboveground parts during winter amounted on average to 68 ± 7 kg N ha^{-1}. Application of fertilizer N had no effect on N accumulated in aboveground parts, except for autumn sampling in year 2, where the N content was significantly higher for the 120N treatment than for 0N. Total belowground parts sampled in winter accumulated higher amounts of N than those sampled in autumn, but the differences were not significant except for the winter/120N treatment in year 2, which exhibited higher N content than the winter/0N treatment.

Figure 1 presents cumulative C mineralization for rhizome, necrotic rhizome, root and senescent leaf obtained from the winter/0N treatment, incubated for 263 days. As expected, the cumulative CO_2 produced in the residue-amended soils was greater than in the control soil and ranked as follows: rhizome > necrotic rhizome = senescent leaf > root > control. At the end of incubation (263 days), the net mineralization of residue-C was significantly higher for rhizome (59% of added C) than for necrotic rhizome (51% of added C), leaf (53% of added C) and root (30% of added C) ($P \leq 0.05$). The observed differences at day 263 resulted mainly from differences in C mineralization during the first 30 days (Figures 1a). Over the 242-263 day interval, the rates of C mineralization were not significantly different ($P < 0.05$) between senescent leaf (1.6 mg C kg^{-1} day^{-1}), rhizome (1.4 mg C kg^{-1} day^{-1}) and necrotic rhizome (1.2 mg C kg^{-1} day^{-1}), but all these were significantly higher than for root (0.8 mg C kg^{-1} day^{-1}) and control (0.4 mg C kg^{-1} day^{-1}). These results

suggest that at the end of incubation the residues were still decomposing.

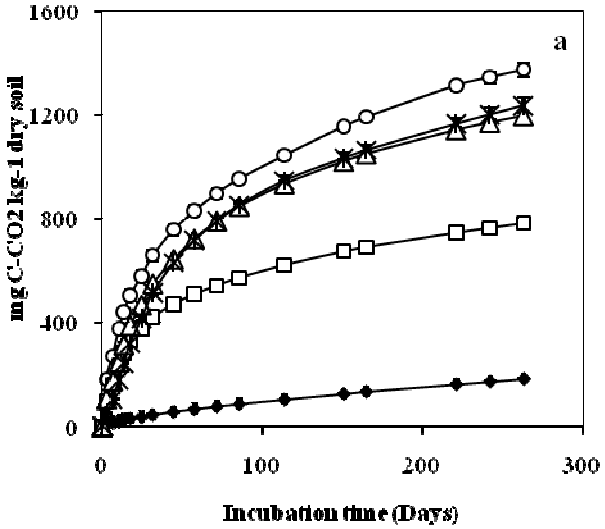

Figure 1. Kinetics of C mineralized in soils (a) without residues (control soil, *black diamond*) and after addition of rhizome (*white circle*), necrotized rhizome (*white triangle*), root (*white square*) (winter/0N treatment) and senescent leaf (*black star*). Data are means (n = 4)

Table 1. Correlation coefficient (r) between *Miscanthus* × *giganteus* residues initial chemical characteristics (rhizome, root, necrosis rhizome and senescent leaf from all treatments) and cumulative C mineralized. *, **, *, Significant at the 0.05, 0.01 and 0.001 probability levels respectively; "ns" means not significant.**

Days	3	7	14	32	58	86	114	200	263
% N	0.79***	0.81***	0.77**	ns	ns	ns	ns	ns	ns
NDS-soluble	0.82***	0.80***	0.83***	0.83***	0.71**	0.65**	0.62**	0.70**	0.68**
Total sugars	ns	ns	ns	0.57*	ns	ns	ns	ns	ns
Klason lignin	ns	ns	ns	-0.83***	-0.92***	-0.93***	-0.93***	-0.92***	-0.92***
(Klason lignin/total sugars) ratio	ns	ns	ns	-0.89***	-0.93***	-0.94***	-0.94***	-0.92***	-0.92***

Simple linear regressions were performed to establish relationships between the cumulative amounts of mineralized C over time and the initial chemical characteristics of the Miscanthus residues. To do this, rhizome, root, necrotic rhizome and senescent leaf from all treatments were considered together (Table 1). In the very short term (3 to 7 days), C-CO_2 is positively correlated with N concentration ($P \leq 0.01$) and with the NDS-soluble fraction. The correlation between NDS fraction and C-CO_2 remains highly significant up to day 32, then begins to decrease but remains significant until day 263 (P<0,01). There is no correlation between mineralized C and the total sugars fraction except at day 32 ($P \leq 0.05$). Mineralized C is strongly negatively correlated with Klason lignin from day 32 to day 263 ($P \leq 0.001$). Total sugars are positively and more clearly correlated with mineralized C at day 263 for rhizome ($R^2 = 0.66$) and necrotic rhizome ($R^2 = 0.79$) than for root ($R^2 = 0.33$), for which a negative correlation is obtained (data not shown).

Conclusions

We saw that a *Miscanthus giganteus* crop is characterized by a large amount of organic plant biomass that is potentially recycled in the soil, and that the amount, quality and therefore subsequent decomposition of these biomasses depend on harvesting strategy and to a lesser extent on N fertilization, if any. From an environmental point of view, harvesting the Miscanthus aerial biomass early (before plant maturity) in order to harvest plant biomass larger in amount and more easily enzyme-fractionable in term of biochemical quality would deprive the soil from the annual input of organic matter as leaves that fall during the winter, while also depriving the rhizomes of several months of accumulation of nutrients that are necessary for subsequent plant growth cycles.

Miscanthus leaves, roots and to lesser extent rhizomes are characterized by a high lignin content compared to other types of crop residues, inducing potentially low rates of mineralization, i.e. a high rate of organic C storage in the soil, which may be a positive criterion for this crop in terms of its impact on soil fertility. However, too few data are as yet available on Miscanthus residue quality and decomposition, particularly on the amount and extent of recycling of roots in the soil and on rhizome decay over the life of the Miscanthus plant. It also seems important to be able to predict the fate of the organic C stored in belowground parts when an old Miscanthus crop is destroyed.

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