Effects of manganese concentration on beech leaf litter decomposition: results from field and laboratory experiments

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Abstract
Lignin degradation is considered to be the rate limiting step of litter decomposition, a vital process in forest biogeochemical cycles. Mn concentration may play a key role in lignin degradation as it is essential for the activity of manganese peroxydase (MnP), a central enzyme of the lignin degrading system. We investigated the impact of Mn on litter decomposition by incubating for one year beech leaf litter of different Mn concentrations (40, 45, 60 and 120 mmol/kg_{DM}) in laboratory and in the field (litterbags). We determined carbon and nitrogen releases as well as the variation of leaf litter composition over time. With Mn concentration, leaf litters exhibited an increase in total CO_2 release, a decrease in total DOC release, along with a decrease in $NO_3^-/(NH_4^+ + NO_3^-)$ ratio and in carbon content and C/N ratio. In the light of our results, we hypothesize that higher Mn concentration (i) could improve biological activity and/or (ii) could promote ligninolysis. To support and confirm these hypotheses, we completed our study by considering the evolution of the content and the oxidation state of leaf litter lignin.

Key Words
Forest organic matter, lignin degradation, carbon and nitrogen releases, laboratory incubation, litterbags.

Introduction
Litter decomposition is of crucial importance for sustainable production in forest ecosystems, because it is responsible for carbon and nutrient cycling. Due to its complex and heterogeneous structure, lignin degradation is slow and exerts a major control on organic residues decomposition. Therefore, lignin concentration is generally considered to be an indicator of substrate quality (Melillo and Aber 1982). Among the factors affecting the degradation of lignin, Mn concentration may play a key role since Mn is essential for the activity of manganese peroxydase (MnP), the most wide-spread enzyme of the lignin degrading system secreted by the white-rot fungi (Hofrichter 2002). Kerem and Hadar (1995) demonstrated a positive effect of Mn on lignin degradation during the solid-state fermentation of a cotton fibre. Correlation between Mn concentration and litter carbon mineralization was also observed (Berg *et al.* 2007); nevertheless the role of Mn on litter decomposition has not yet been experimentally tested.
This study aims to assess the impact of Mn concentration in beech (*Fagus sylvatica* L.) leaf litter on carbon and nitrogen release (laboratory incubation) and on leaf litter chemical composition (field experiment) during the decomposition process.

Methods
Manganese concentrations in beech leaf litter
Leaf litters with identical composition, except Mn concentration, were obtained by dipping branches in Mn solutions (0 to 2.5 mM) for 10 days (Table 1). Manganese was transported through the transpiration stream and accumulated in leaves. Branches were sampled from beech trees from Bois de Lauzelle (Table 2) at the beginning of spring. We also examined beech leaf litter from the Transinne site (Table 2). Beech leaf litters from Bois de Lauzelle (Mn0 treatment) and from Transinne were identically concentrated in Mn but Transinne leaf litter exhibited a greater decomposition capacity (data not shown).

Laboratory experiment
Beech leaf litters were incubated in triplicate in columns hermetically closed for 350 days at 20°C. Carbon and nitrogen releases were determined during the incubation period. CO_2 was measured by back titration of a NaOH solution. Leachates were obtained by water extraction from beech leaf litter subsamples. On these leachates, dissolved organic carbon (DOC) was quantified using a carbon analyzer (Dohrman DC-180), absorbance (280 nm) was measured using a UV/VIS spectrometer UNICAM8625 and inorganic nitrogen ($NH_4^+$and NO_3^-) determined using a HPLC (Dionex LC20 with IONPAC AS 11 column).

Table 1. Mn concentrations in beech leaf litters subjected to different treatments [mmol/kg_{DM}].

		---------- Bois de Lauzelle ----------			
Treatment abbreviation	Transinne	Mn0	Mn1	Mn2	Mn3
Mn concentration in dipping solution [mM]	0	0	0.5	1	2.5
Leaf Mn concentration [mmol/kg_{DM}]	40	40	45	60	120

Field experiment (litterbags)

For each type of litter, the samples of about 2.5 g each were enclosed in separate litterbags (18 x 18 cm) made of polyester net with an upper mesh size of 2.4 mm and a lower mesh size of 0.5 mm. In November 2008, in the Forêt de Soignes (Table 2), litterbags were placed below the L layer in a randomized design. The procedure was replicated 4 times by leaving the litterbags in 3 different location to avoid pseudoreplication; the bags were then collected 5 (150 days, t_1), 10 (300 days, t_2) and 15 months later. Plant remains such as mosses and roots were removed. Then, the mass loss was determined by drying the sample at a constant temperature of 60°C. We quantified carbon and nitrogen contents using a NC-soil analyzer.

Table 2. Sites used in this study, their geographic location (Belgium), climate, soil and forest floor type.

	Bois de Lauzelle	Transinne	Forêt de Soignes
Location	Louvain-la-Neuve, 49°59'03"N, 5°11'31"E	Belgian Ardenne, 50°48'24"N, 4°28'04"E	Brussels, 50°40'44"N, 4°36'15"E
Mean annual temperature	9.4 °C	8.7 °C	9.4°C
Mean annual precipitation	816 mm	1035 mm	835 mm
Soil	Cambisol	Dystric cambisol	Podzol
Forest floor	Mor	Moder	Mor

Statistics

The differences between means were compared with Fisher's least significant difference (LSD) test. Using the laboratory results, the test was carried out by one-way variance analysis (Anova1), treatment being the factor. Then, using the field experiment results, the LSD-test was performed by two-ways variance analysis (Anova2), allowing us to take into account the variability introduced by the location factor and by its interaction with the treatment factor. All the statistical analyses were performed with the SAS software (SAS Institute 1999) with a significance level of 0.05.

Results

From laboratory experiment

At the end of the incubation period, the Mn3 leaf litter exhibited a significant a larger total CO_2 release than Mn0 and Transinne leaf litters (p-value of 0.0148). Except for Transinne leaf litter, leaf litter Mn concentration was higher while the total DOC release (p-value of 0.0001) was lower (Figure 1). Nevertheless, DOC in water extracts exhibited no significant difference of molar absorptivity (absorbance at 280 nm/DOC concentration) between the treatments.

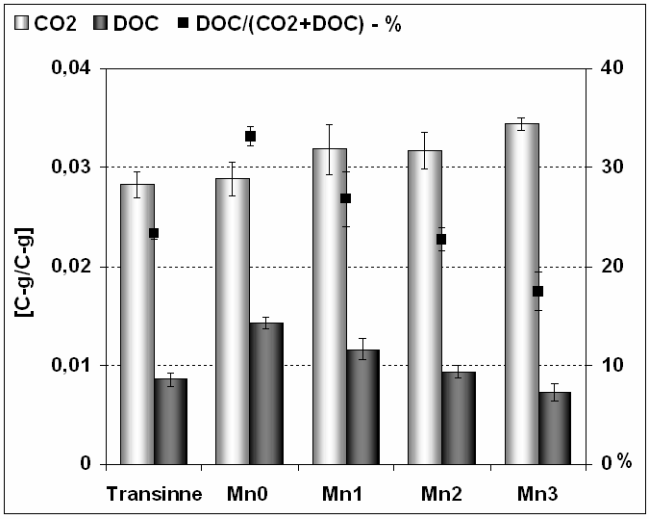

Figure 1. Cumulative release of carbon (CO_2 and DOC) over the incubation period (left Y-axis) and DOC/(CO_2 + DOC) ratio (right Y-axis).

Ammonium release was highest in Mn3 leaf litter water extract and the lowest in Transinne leaf litter extract (p-value of 0.0006). Nitrate releases were twice as low in Mn2 and Mn3 leaf litter extracts than in the water extracts of the other treatments (p-value of 0.0154). The nitrification degree ($NO_3^-/NH_4^+ + NO_3^-$) was significantly greater for nitrogen release from the Transinne leaf litter. For the other treatments, nitrification degree decreased with Mn concentration in leaf litter (Table 3).

Table 3. Cumulative release of nitrogen (NH_4^+ and NO_3^-) over the incubation period and nitrification degree ($NO_3^-/NH_4^+ + NO_3^-$). Means and their standard deviations are presented (n=3). Means with different associated letters are statistically different (α = 0.05).

Treatment	Transinne	Mn0	Mn1	Mn2	Mn3
NH_4^+ [N-mg/N-g]	0.012 ± 0.007	0.037 ± 0.001	0.030 ± 0.001	0.046 ± 0.005	0.078 ± 0.019
	c	b	bc	b	a
NO_3^- [N-mg/N-g]	0.014 ± 0.004	0.012 ± 0.004	0.012 ± 0.001	0.007 ± 0.001	0.005 ± 0.001
	a	ab	ab	bc	c
Nitrification degree - %	56.4 ± 7.4	24.7 ± 5.7	30.7 ± 11.4	13.5 ± 2.7	6.5 ± 1.6
	a	bc	b	cd	d

From field experiment

After 150 days in the field, we observed no significant difference in mass loss between leaf litters subjected to different treatments in litterbags the mass loss ranged between 10 and 20%. After 300 days, Transinne leaf litter exhibited a significant larger mass loss (30%) than the other leaf litters (20 – 25%) (p-value of 0.0383). After 300 days in the field, the leaf litter with the largest Mn concentration (Mn3) exhibited a decrease of carbon content significantly larger than the other leaf litters (p-value of 0.05) (Figure 2a). The leaf litter with the lowest Mn concentration (Mn0) exhibited the lowest increase of nitrogen content, already after 150 days (p-value of 0.0020 (t_1) and 0.0037 (t_2)) (Figure 2b). Over time, the decrease of C/N ratio in leaf litter material was lowest for Mn0 leaf litter and highest for Mn3 leaf litter (p-value of 0.0001 (t_1) and 0.0002 (t_2)) (Figure 2c).

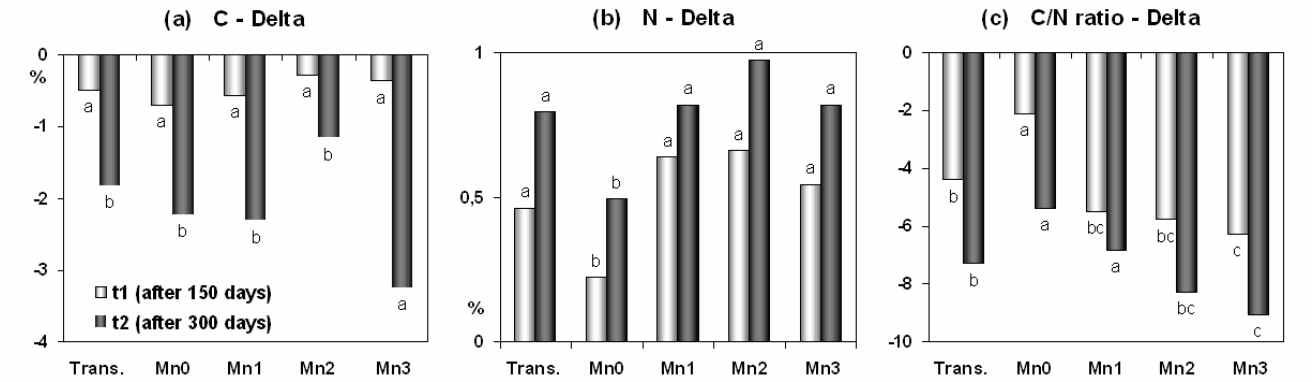

Figure 2. Difference in C-, N-content and C/N ratio between leaf litters sampled after 150 (t_1) or 300 days (t_2) and corresponding initial leaf litters (t_0).

Discussion

Carbon release

Our results suggest that Mn concentration affects carbon release. Total carbon (CO_2 + DOC) released by the leaf litters treated stayed constant. However, the form of released carbon evolved in favor of the mineralized form (CO_2) with Mn concentration in leaf litters. The DOC composition is complex and heterogeneous. The main sources of DOC are C-labile and ligninolysis products (hydrophobic components) (Guggenberger 1994). Our study suggests there was no variation in the composition of DOC released by the leaf litters treated, just as there was no variation in molar absorptivity. In fact, molar absorptivity estimates DOC aromaticity and indicates the quantity of hydrophobic components (Simonsson *et al*. 2005). In the field, leaf litter with the most Mn concentrated (Mn3) had the largest carbon loss. Field conditions (more moisture, involvement of micro- and macrofauna because of the litterbags design) could enhance biological processes.

We could hypothesize that Mn concentration (i) improves biological activity and/or (ii) promotes ligninolysis. In the first hypothesis, microorganisms could consume more C-labile (decrease of DOC release) and could produce more respiration (increase of CO_2 release). In the second hypothesis, lignin degradation could be more complete. Thus, microorganisms could have easier access to ligninolysis products.

Nitrogen release

Our results also suggest that the level of Mn concentration affects nitrogen release. According to the first hypothesis, the decrease in NO_3^- release could be explained by a larger consumption of microorganisms. In the second hypothesis, the decrease in $NO_3^-/NH_4^+ + NO_3^-$ ratio in leaf litters with large Mn concentration could be explained by depression in the nitrification process. Dissolved polyphenolic compounds produced by ligninolysis could inhibit nitrification (allelopathy) (Killham 1990).

Conclusions and perspectives

By reducing the C/N ratio, a classic indicator of litter decomposition (Figure 2c), Mn concentration impacts litter decomposition by improving biological activity or/and by promoting ligninolysis. To support and confirm these hypotheses, we will consider the evolution of the content and the oxidation state of leaf litter lignin in our laboratory and field samples. Moreover, we await results from the last collection of litterbags in order to study the temporal evolution of leaf litter composition.

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