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Discussions

Carbon losses from pyrolysed and original wood in a forest soil under natural and increased N deposition

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Abstract

Pyrogenic organic matter (PyOM) plays an important role as a stable carbon (C) sink in the terrestrial ecosystems. However, uncertainties remain about in situ turnover rates of PyOM in soil, the main processes leading to PyOM C and nitrogen (N) losses from the soil, and the role of N availability in PyOM cycling in soils.

We measured PyOM and native soil organic carbon losses from the soil as carbon dioxide and dissolved organic carbon (DOC) using additions of highly ^{13}C-labelled PyOM (2.03 atom %) and its precursor pinewood during one year in a temperate forest soil. The field experiment was carried out under ambient and increased mineral N deposition ($+60\,\text{kg N ha}^{-1}\,\text{yr}^{-1}$). The results showed that after one year: (1) 0.5 % of PyOM-C and 22 % of wood-C were mineralized as CO_2, leading to an estimate of minimum turnover time of 191 and 4 yr respectively, (2) the quantity of PyOM and wood lost as dissolved organic carbon was negligible (0.0004 ± 0.0003 % and 0.022 ± 0.007 respectively); and (3) N additions decreased cumulative PyOM mineralization by 43 %, but did not affect cumulative wood mineralization and did not affect the loss of DOC from PyOM or wood. We conclude that mineralization to CO_2 was the main process leading to PyOM losses during the first year of decomposition in a forest soil, and that N addition can decrease PyOM C cycling while leaving unaltered wood C cycling.

1 Introduction

Pyrogenic organic matter (PyOM) is the product of incomplete combustion of biomass (Goldberg, 1985) and is an important soil C pool because it can represent up to 45 % of soil organic carbon (Schmidt et al., 1999). Moreover, PyOM input from biomass burning is likely to increase in high-to-mid-latitude-regions in the future (Westerling et al., 2006; Moritz et al., 2012), because climatic conditions favouring fire will probably become more frequent. Due to its aromatic structure, PyOM has been hypothesized to be particularly resistant to microbial decomposition (Schmidt and Noack, 2000) and have

a centennial mean residence time (Schmidt et al., 2011; Singh et al., 2012). Several recent publications have investigated PyOM decomposition rate under controlled laboratory conditions (Hilscher et al., 2009; Kuzyakov et al., 2009; Abiven and Andreoli, 2010; Santos et al., 2012). However, only a few field experiments have been conducted, mainly because the decomposition rate of PyOM is difficult to detect without dedicated techniques such as isotopic tracers and/or biomarkers. Most field studies have been observational, comparing the PyOM content of archived soils or chronosequences. Hammes et al. (2008) compared the change in PyOM in a Chernozem sampled twice at 100 yr interval and found a loss of 25 % using the benzene polycarboxylic acid (BPCA) technique. Using a chronosequence of soils that underwent slash and burn at different times over the last 100 yr, Nguyen et al. (2008) found a loss of 30 % soil PyOM over this period; the authors used the NMR technique for analysing pre-oxidized handpicked samples. For the same samples using the BPCA technique, Schneider et al. (2011) observed no change in the PyOM content of soils that received PyOM deposition for 2 to 100 yr. This approach of using archived or chronosequence samples is limited by the lack of information regarding the amount of initial input of C to the soil and the changes in edaphic conditions during the decomposition. An alternative method that overcomes the need to analytically isolate PyOM is to use stable isotopes, which allows the fate of added PyOM to be traced over time. Major et al. (2010) used this approach in a field trial in a tropical ecosystem. They added PyOM produced from mango tree (C_3 plant) to a grassland soil in Colombia (soil organic matter derived from C_4 plants), and by measuring soil respiration and ^{13}C-CO_2 found that 2.2 % of the added PyOM-C was lost as CO_2 over two years. However, due to the slow rate of decomposition of PyOM, the difference in ^{13}C due to the plant photosynthetic pathways was not enough to observe a significant change in the ^{13}C content of CO_2 efflux, even though they observed an increase in soil respiration (SR). The study by Major et al. (2010), who found that losses as DOC were lower than 1 % of the initially added PyOM-C, is the only study to our knowledge, that has assessed the mineralization and leaching losses of PyOM.

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Singh et al. (2012) reviewed PyOM mean residence time by compiling a database with results from studies using different experimental designs. One clear message was that PyOM mean residence time was longer in field studies than in incubation studies, but the reason for that could not be reduced to one single factor. First, the constant temperature and moisture conditions in the laboratory may increase the decomposition of PyOM-C together with mixing soil and substrate at the beginning of the experiment. Second the short duration of incubation studies might overestimate turnover time of PyOM, because short-term experiments capture only the initial higher decomposition rate of PyOM (Hamer et al., 2004; Kuzyakov et al., 2009). Third, specific stabilisation processes, might need particular climatic conditions, for example freeze-thawing or disaggregation–aggregation, which usually occur under field conditions. The lack of short-term field manipulation experiments, does not allow disentangling this conundrum.

Losses of PyOM from soil have been shown to also occur as dissolved organic carbon (DOC), however there are contrasting evidences on the importance of this pathway for PyOM-C losses. Consistent losses of PyOM as DOC were observed by Leifeld et al. (2007) who reported that 21–69 % of PyOM moved below the ploughing depth (30 cm) over at least 50 yr, in a former agricultural soil where combustion residues were disposed. Substantial quantities of PyOM have been detected in riverine (Kim et al., 2004) and marine (Thorsten Dittmar, 2008; Ziolkowski and Druffel, 2010) DOC. Abiven et al. (2011) found that the soluble fraction in PyOM was $1\,\mathrm{g\,C\,kg^{-1}}$ PyOM-C, while PyOM aged in the field (10 yr) contained a much higher fraction of the soluble PyOM, with a value of $41\,\mathrm{g\,C\,kg^{-1}}$ PyOM-C. Major et al. (2010), reported PyOM losses as DOC of $0.2\,\mathrm{g\,C\,kg^{-1}}$ PyOM-C over a two year period. Moreover, the lack of data on losses of PyOM-C as CO_2 and DOC derived from same experiment leaves unresolved the question on the relative importance of the two processes (Bird et al., 1999; Hammes et al., 2008).

Also soil erosion can also cause losses of PyOM from a given location or watershed, mainly due to its low bulk density. For example Rumpel et al. (2006) found that PyOM-C

can represent up to 30 % of eroded carbon in a tropical watershed. However, erosion losses were not determined in the present study.

It has been also observed that PyOM may change the turnover rates of the native soil organic matter (SOM) already present in the soil. PyOM additions to soil has been found to increase (Wardle et al., 2008; Major et al., 2010; Luo et al., 2011), decrease (Liang et al., 2006; Cross and Sohi, 2011) or have no effect (Kuzyakov et al., 2009; Cross and Sohi, 2011; Santos et al., 2012) on the decomposition rate of the native soil organic matter in soils. Keith et al. (2011) found that PyOM increased the decomposition of native soil organic matter and decreased the decomposition of fresh organic matter added along with the PyOM. They suggested that small portions of labile PyOM may trigger the decomposition of the native soil organic matter, while fresh organic matter is incorporated in the microaggregates whose formation is promoted by PyOM (Liang et al., 2010). This change in the SOM decomposition rate, also called priming effect, is an important feedback mechanisms than can modify the carbon stocks in soils. Positive priming effect (an increase in SOM decomposition) has been related to the PyOM particles serving as a surface for microbial growth (Wardle et al., 2008), to the increased root inputs from plants following PyOM inputs (Major et al., 2010), or to the liming effect of PyOM addition (Luo et al., 2011). A negative priming effect has been explained by the adsorption of organic matter on PyOM surface in a way that protects it from mineralization (Liang et al., 2006; Cross and Sohi, 2011).

In addition to its chemical structure, PyOM decomposition may be affected by other drivers of the ecosystem, such as inorganic nitrogen (N) availability. Increased N deposition has been observed to have contradicting effects on soil organic matter dynamics, increasing (Burton et al., 2004) or decreasing (Pregitzer et al., 2007) decomposition rates. Where observed, reduced mineralization has been explained by a decrease in the activity of ligninolitic enzymes (Carreiro et al., 2000; Sinsabaugh, 2010) which could particularly affect PyOM given its aromatic structure. Alternatively a shift in microbial decomposers community toward more efficient C-user microbes has been suggested (Janssens et al., 2010) to be responsible for decreased soil respiration.

We added ^{13}C-labelled PyOM and ^{13}C-labelled wood parent material, *Pinus Ponderosa*, to the soil of a forest clearing, with two levels of N addition (0 and +60 kg N ha^{-1} yr^{-1}) to trace losses of C as CO_2 and as DOC, and to directly estimate priming effect. The focus was on the following research questions: (1) What is the CO_2 mineralization rate of PyOM compared to that of the un-pyrolized wood substrate? (2) How much PyOM is lost as DOC? (3) Does PyOM induce a priming effect on native soil organic matter? (4) What is the effect of N deposition on soil C and PyOM-C fluxes from the soil?

2 Materials and methods

2.1 Field study

The field experiment was located in a temperate forest adjacent to a CarboEurope forest tower on the Laegern mountain (CH-LAE, 47°28′42.0″ N; 8°21′51.8″ E, altitude is 700 m a.s.l., mean annual soil temperature 10 °C), 20 km from Zurich, Switzerland. The overstory vegetation consists mainly of beech (*Fagus sylvatica* L.), spruce (*Picea Abies* L.), ash (*Fraxinus excelsior* L.), fir (*Abies alba Mill.*), and small-leaved lime (*Tilia Cordata Mill.*), and understory of *Allium ursinum* (L.) (Ruehr et al., 2009). The soil is a Cambisol (sand: 45 %, silt: 24 %, clay: 31 %), rich in organic C (34 g C kg^{-1}, Table 1).

The experimental design was a 3 × 2 factorial experiment, we tested three organic input treatments (control – corresponding to no input, wood, and PyOM) and two N addition levels (ambient and +60 kg N ha^{-1} yr^{-1}). The experiment was set-up in three blocks each containing all the possible combinations of input treatment and N addition, i.e. the experiment was composed of three replicates. The experimental blocks were located in a forest gap of approximately 0.5 ha. We installed 18 mesocosms, each consisting of 20 cm long, 10 cm diameter polyethylene cylinders which were inserted in the soil down to a depth of ca. 15 cm with 5 cm above the soil surface. Each mesocosm had an open bottom and had two 0.7 mm-mesh covered "windows" (4 cm diameter) placed

at distances of 7.5 cm and 12.5 cm distance from the bottom. These windows were added to the mesocosms to allow biological, chemical, and climatic equilibration with the external environment, without coarse roots entering. Mesocosms were installed in April 2009 and labelled organic input addition occurred in October 2009.

Any grass and roots growing inside the mesocosms were removed by clipping each time CO_2 was sampled (see the section on CO_2 efflux) to avoid including autotrophic respiration in the measurements.

Half of the mesocosms received N fertilization (60 kg N-NH$_4$NO$_3$ ha^{-1} yr^{-1}) with 5 kg ha^{-1} applied every month (10 kg ha^{-1} was applied exceptionally three times to catch up with application delay due to the accessibility of the research site). N was first added to soil in March 2010, 6 months after the application of organic inputs. In each block at a depth of 5 cm we placed a sensor measuring temperature and soil moisture (5TM soil moisture sensor, Decagon, USA). The data collected prior to March 2010 were thus not affected by the N fertilization.

2.2 Organic inputs

The pine wood and PyOM added to the soil mesocosms were isotopically enriched in ^{13}C (see Table 1). Two-years-old pine saplings (*Pinus Ponderosa*) were labelled with ^{13}C-CO_2 under controlled greenhouse conditions. They were exposed to ten photoperiods of enriched ^{13}C-CO_2 (10 atom %) during the course of their growth (Bird and Torn, 2006). PyOM was produced by pyrolyzing the labelled wood at 450 °C for 5 h under N$_2$ atmosphere, as described in (Santos et al., 2012). The PyOM and wood were subsequently milled (< 2 mm) and their C, N, and ^{13}C content analysed (see Table 1). The equivalent of 397 g C m of PyOM and 189 g C m of wood were placed at a soil depth of 1 cm within mesocosms, and the soil was mixed. The soil in the control mesocosms was also mixed to 1 cm. The detailed chemical composition, structure, and isotopic content of the pine wood and PyOM used in this study are described in (Chatterjee et al., 2012). Chatterjee et al. (2012) report that the pyrolisis of wood lead to a depoly-

merisation of hemicelluloses and cellulose which were less present in the char than in the wood. Unfortunately their study did not allow concluding on the fate of ligneous components of wood, which may be partially or totally transformed into new aromatic compounds during the charring process.

2.3 Soil respiration

Soil respiration (SR) was measured 16 times during one year using a Li-Cor 8100 equipped with a chamber (10 cm diameter, 854.2 cm volume) on the following sampling dates: 6, 13, 20 November in 2009; 31 March, 8 and 29 April, 18 May, 4 and 22 June, 9 and 20 July, 4 and 26 August, 16 September, 4 October, 4 November in 2010. The hiatus between November and March 2009 is due to snow covering the soil.

Soil respiration was measured three times per mesocosm on each sampling date for 90 s. To allow the re-equilibration of the CO_2 concentration between the chamber and the atmosphere measurements were taken 90 s apart. At the same time, we also collected samples to measure the ^{13}C enrichment of soil respired CO_2. A Keeling plot approach (Keeling, 1958) was used to estimate the ^{13}C enrichment of the soil-respired CO_2 and to calculate the ^{13}C excess efflux from enriched PyOM and wood C. An 800 cm^3 chamber was placed on top of the mesocosms and CO_2 was sampled with a syringe three times at regular intervals over 9 min while the CO_2 increased. To produce each keeling plot we took three gas samples, collected after 0, 4.5, and 9 min, corresponding to a total average interval of 246 ppm between the gas sample taken at minute 0 and the one taken at minute 9. We sampled 17 mL of gas from the chamber, which was injected into a 12 mL vial (*Exetainer, Labco, UK*) using overpressure as suggested in Joos et al. (2008). Prior to sampling, the gas vials were flushed with N_2 three times, and stored under N_2. The ^{13}C enrichment of the CO_2 samples was measured using an isotope ratio mass spectrometer (IRMS) (*Delta-S, Finnigan MAT-Thermofscher scientific,Waltham, USA*). Keeling plots were accepted if r of the regression line was higher than 0.9, if $r^2 < 0.9$ the data were discarded. A correction of $\delta^{13}C$ −4.4 ‰ was

applied to the soil ^{13}C-CO$_2$ efflux for isotopic discrimination processes occurring during CO$_2$ diffusion from the soil as suggested by Mortazavi et al. (2004). Where data for soil respiration and ^{13}C-CO$_2$ were missing due to technical failure they were replaced by the average of the same day of the corresponding treatment.

2.4 Dissolved organic carbon

Suction lysimeters (multilayer borosilicate 1 µm pore 10 cm diameter, *Ecotech, DH)* were placed at the bottom of the mesocosm (at a depth of 15 cm) and kept under constant vacuum at 650 to 750 mbar. Soil water was collected 9 times over the study period, on the following dates:

27 November, and 17 December in 2009, 14 January, 26 February, 13 May, 22 June, 24 August and 26 November in 2010.

Water samples were filtered using a 0.45 µm cellulose acetate membrane. DO^{13}C measurement failed on two dates (27 May and 22 June) and since not enough material was left to repeat the measurement, these two sampling dates were discarded. The solution pH and electrical conductivity were measured (*Metrohm, 620 pH meter, Switzerland, and WTW tetracon, 325, Austria)* and samples after filtration stored at 4 °C for maximum three weeks. Total organic carbon content was measured on a 20 mL subsample of the leachate. A second subsample of 60 mL was freeze-dried after removing potential carbonate by lowering pH to 2.8 ± 0.1. Freeze-dried DOC (2–4 mg) samples were analyzed for total C using an elemental analyzer (*Shimatzu, Asi-v, Kyoto, Japan*) and ^{13}C enrichment was measured with an IRMS *(Delta-S, Finnigan MAT-Thermofscher scientific,Waltham, USA)*, coupled to an elemental analyzer (*EA 1100, Carlo Erba, Italy*). Where data for DOC and DO^{13}C were missing due to technical failure they were replaced by the average of the same day of the corresponding treatment. If the fraction derived from the substrate (Eq. 1 was below 0), then this was set to 0.

2.5 Calculations

The partitioning of the CO_2 and DOC fluxes between native SOM-derived and organic-input-derived (wood and PyOM) was calculated using Eq. (1) from Balesdent and Mariotti, (1996):

$$f = 1 - (\delta^{13}C_{mix} - \delta^{13}C_{organic\ input})/(\delta^{13}C_{control} - ^{13}\delta C_{organic\ input}) \tag{1}$$

Where f = fraction of CO_2 flux derived from the organic input; $\delta^{13}C_{mix}$ = the isotopic signature of soil CO_2 or DOC in organic inputs treatments, $\delta^{13}C_{organic\ input}$ = the isotopic signature of the added organic inputs (PyOM and wood), and, $\delta^{13}C_{control}$ = the isotopic signature of soil CO_2 or DOC in the control treatment. The priming effect, i.e the change in native SOM mineralization due to the organic input was calculated for each sampling date as in Eq. (2):

$$PE = (SR_{native} - SR_{control}) \tag{2}$$

Where $SR_{control}$ = soil respiration in the control treatment and SR_{native} = mineralization rate of the native soil organic matter in PyOM or wood treatment, calculated as: $SR_{total} - (SR_{total} \cdot f)$ where SR substrate is the total soil respiration in the substrate treatment.

To interpolate soil respiration between sampling dates, we modelled the CO_2 fluxes according to the method proposed by Fang and Moncrieff (2001) (Eq. 3). Kammer et al. (2011) successfully applied this model to the same site area as our study, and Ruehr et al. (2009) showed that the soil moisture affects soil respiration only when moisture is lower than 15 volume %. Soil moisture was always higher than this value during our study period, so soil moisture dropped out of our model and soil respiration was calculated as:

$$SR_{interpolated} = a \cdot (T - T_{min}) \cdot b \tag{3}$$

where T = the soil temperature (°C) measured at a depth of 5 cm (30 min measurement interval), T_{min} is the temperature at which SR is supposed to be 0 (in this case fixed

to −20 °C) a, and b are model parameters calculated for each single mesocosm. The simulated soil respirations for different treatments are shown in Fig. 1. PyOM mineralization measurements were linearly interpolated between sampling dates using Eq. (4) assuming that the fraction derived from PyOM varied linearly between dates:

$$\text{PyOM mineralization rate} = SR_{interpolated} \cdot f_{interpolated} \tag{4}$$

where $f_{interpolated}$ values were calculated by interpolating between subsequent sampling date values obtained from Eq. (1) as in Ngao et al. (2005) and Kammer and Hagedorn (2011). The modelled soil respiration (Fig. 1) and the fraction derived from the labelled organic substrate in the period November 2009–March 2011 in the increased N deposition treatments was made equal to the ones occurring in the ambient N in the correspondent period to stress the effect of N addition.

We fitted the initial and the remaining quantity (Table 2) of PyOM at the end of the experiment, calculated by cumulating values from Eq. (4), to a first-order decay model to estimate the Mean Residence Time (MRT) of PyOM and pine wood, as in Singh et al. (2012). Decomposition at time t in the first order decay model is expressed as follows:

$$-\mathrm{d}C/\mathrm{d}t = k \cdot C_t \tag{5}$$

where C is the size of PyOM-C or the wood-C pool. From this formula it is possible to derive the pool size at time t using Eq. (6), assuming that the inputs to the system were equal to 0 and that losses as DOC were negligible:

$$C_t = C_{t=0}e^{-kt} \tag{6}$$

Where k is the constant decay rate, and then calculate the mean residence time (MRT), for each mesocosm as:

$$MRT = 1/k \tag{7}$$

2.6 Statistical analyses

The effects of treatment, N addition, and time were tested on the following variables: soil respiration, the fraction derived from added substrate in soil respiration and DOC, DOC daily production rate, soil water conductivity and soil water pH, using repeated measures ANOVA and individual ANOVA procedures on individual sampling dates.

Two different repeated measures ANOVA were performed: one over the time period November 2009–November 2010 to test the effect of different organic inputs and time, and their interactions, and another was performed over the period March 2010–November 2010 (during the N addition period, started in March 2010). Individual comparisons within the same organic treatment and the N-addition treatment were performed using Tukey's comparison to test differences among sampling dates.

T tests were performed on individual sampling dates (ambient and increased N treatment pooled together) to test whether the fraction derived from CO_2 was significantly different from 0.

Repeated measures ANOVA was performed using *SPSS Statistics v.18* (IBM, New York, USA) and Tukey's comparison tests were performed using *R* (version 2.10), extended by the "agricolae" package.

3 Results

3.1 Soil respiration rates, organic input mineralization, and priming effect

The average soil respiration rate (SR) in the control treatment (no additions) during the year was $2.0 \pm 0.1\,\mu\text{mol}\,CO_2\,\text{m}^{-2}\,\text{s}^{-1}$. Neither N nor organic input addition significantly affected the soil respiration rates whereas time did, with higher respiration rates measured in July and September (repeated measures ANOVA $p < 0.001$, Figs. 1 and S1).

The fraction of soil respiration derived from the substrate was significantly higher in the wood treatment than in PyOM for all sampling dates (Post-Hoc Tukey test $p < 0.05$, Fig. 2). Nitrogen addition did not have a significant effect on the fraction derived from the added substrate.

Under ambient N the fractions of soil respiration derived from PyOM on 6 November 2009 was significantly different from that measured on 18 May 2010. Under increased N, during the period March 2010–November 2010, we observed no differences in the PyOM derived fraction of soil respiration among sampling dates (Post-Hoc Tukey test $p < 0.05$, Fig. 2a).

For the wood treatment under ambient N, the wood derived fraction of the 6 and 20 of November 2009 was significantly higher than the one measured between June and November 2010. Under increased N, during the period March–November 2010, only the fraction derived from wood on the 31 March was significantly different from the following ones (Post-Hoc Tukey test $p < 0.05$, Fig. 2b).

The PyOM decomposition rate was on average $0.0022 \pm 0.0003\%$ and $0.0011 \pm 0.0002\%$ of PyOM-C d^{-1} added under ambient and increased N, respectively. Wood decomposition rate was 0.077 ± 0.008 and $0.081 \pm 0.008\,d^{-1}$ of $\%$ wood-C added under ambient and increased N, respectively.

N addition did not significantly affect the decomposition of the substrates. On individual sampling dates N addition significantly decreased PyOM decomposition only on the 20 July, while wood decomposition was not affected by N addition at any stage (Fig. 3). No significant difference in mineralization rate was observed among sampling dates.

The mineralization of native soil organic matter, calculated as soil respiration minus organic substrate-derived CO_2 in the substrate-addition treatment or raw soil respiration in the control treatment, was not significantly affected by either the addition of the organic inputs or the addition of N, i.e. no priming was observed (Fig. 4, repeated measures ANOVA, $p < 0.05$). However, under ambient N the PyOM induced priming effect tends to be higher.

Using Eq. (4) we estimated the quantity of PyOM-C and wood-C lost as CO_2 over one year. PyOM-C losses were $0.5 \pm 0.1\,\%$ of initial PyOM-C and wood-C losses were $22 \pm 3\,\%$ of initial wood-C input. N addition significantly decreased the PyOM-C decomposition to $0.3 \pm 0.1\,\%$, (t test, $p < 0.05$), while wood decomposition was not affected by N addition (Fig. S2, Table 2). The MRT calculated according to Eq. (7) was 191 ± 24 and $430 \pm 146\,yr$ for PyOM-C for ambient and increased N, respectively and $4 \pm 1\,yr$ for Wood-C for both ambient and increased N treatment.

3.2 Dissolved organic carbon

DOC production rate did not significantly differ with treatment and different levels of N added, and nor did its pH, conductivity, or volume (data not shown). The fraction of DOC derived from the substrate was significantly different between the wood and PyOM (repeated measures ANOVA, $p < 0.05$), under increased and under ambient N. Cumulative losses of PyOM-C were $0.0004 \pm 0.0003\,\%$ of the initial input-C, while wood-C losses were $0.022 \pm 0.007\,\%$ of the wood-C, and under increased N addition losses were $0.0002 \pm 0.0001\,\%$ for PyOM, and $0.03 \pm 0.01\,\%$ for wood (Fig. S3; Table 2). Nitrogen did not affect the cumulative losses of DOC neither in the PyOM and nor in the wood treatment.

4 Discussion

4.1 PyOM and wood mineralization

In our study, we estimated that PyOM in the field has a turnover time of 191 yr, while wood had a turnover time of 4 yr. PyOM turnover time in our field study was higher than the turnover time for wood derived PyOM found in incubation studies (Hamer et al., 2004; Hilscher et al., 2009; Singh et al., 2012). Using the same substrate in an incubation experiment, Santos et al. (2012) found a decomposition rate of 0.39 % after 180 days compared to 0.08 % in our study after 180 days. The increased turnover time

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for PyOM decomposition than observed in the laboratory incubation studies indicate that higher and more constant temperature and soil moisture conditions are likely to increase the decomposition rate of PyOM compared to our field studies, whose mean annual soil temperature was 10 °C and moisture was 30 volume %.

The PyOM mean residence time calculated here is closer to the values reported by Nguyen et al. (2008), who found a mean residence time of 264 yr under tropical climate and from Hammes et al. (2008) who found a mean residence time of 347 yr in a boreal steppe, indicating that irrespective of the climate, the quantification method and the length of the experiment PyOM has a mean residence time ranging in the centennials, when measured in field conditions. On the other hand in a two-years field experiment carried out in a plant-soil system in a tropical savanna, Major et al. (2010) found a turnover time of 89 yr. However in their experiment the presence of rhizodeposition, due to the increase in biomass (observed in the first year) following PyOM addition, may have primed the PyOM decomposition. In fact easily decomposable compounds like glucose or rhizodeposition can increase the PyOM decomposition rate, since enzymes produced for their decomposition can contribute also to the decomposition of PyOM (Hamer et al., 2004; Kuzyakov et al., 2009). A similar effect was observed by Keith et al. (2011), who found an increase in PyOM decomposition as result of the addition of fresh organic matter to the soil.

The PyOM mineralization rate did not decrease significantly with time (Fig. 3). Kuzyakov et al. (2009) found that the PyOM mineralization rate decreased over the first two to three months before levelling off. Likewise, in an incubation in quartz medium, the PyOM mineralization rate was twice as fast in the first month as in the second month (Hamer et al., 2004). In our experiment PyOM decomposition rate tended to decrease (not significantly) over the first three weeks, and increased in summer, suggesting that temperature might have influenced PyOM decomposition (Fang et al., 2011). However the setup of our study did not allow distinguishing between the effect of the season and of time.

The fraction of PyOM-derived C in the soil respiration did not vary along the time of decomposition (Fig. 2a). The fraction was higher for the first three sampling date, but was rapidly levelled off. This confirms findings from Smith et al. (2010) who observed that the PyOM fraction in soil respiration rapidly decreased over one week in an incubation experiment. Using the fraction derived from PyOM as an indicator for PyOM decay relative to native soil organic carbon, our study indicates that the stability of PyOM relative to native soil organic matter was constant over time, and was not affected by the higher temperature, not confirming the theory proposed by Hartley and Ineson (2008) on the higher temperature sensitivity of resistant organic matter. Cumulative wood decomposition after one year was equal to 22 % of the initially added wood-C. On the same site Kammer and Hagedorn (2011) found similar values for beech twigs, 22–26 % mineralization over one year at the soil surface. Using the same substrate Santos et al. (2010) found wood decomposition of 22 % in an andesitic soil and 30 % in a granitic soil over 180 days, while in our study only 10 % of the initially added wood-C was decomposed over the same period; these results confirm that similar to the PyOM, the decomposition of wood-C is slower in field conditions than in laboratory incubation experiments. The fraction of total soil respiration coming from wood decreased with time (Fig. 2b). This agrees with findings from Kammer et al. (2011).

4.2 Priming effect

We did not observe a significant change in native soil organic matter mineralization due to the addition PyOM or wood despite large differences observed between the means (Fig. 4). This is mainly due to the large variability in soil respiration measured in the field as well as the limited number of replicates used in our study. However, we observed a trend towards positive priming effect for both organic inputs under ambient N deposition, and no effect under increased N deposition.

Neither Abiven and Andreoli (2010) who incubated PyOM and litter in the same soil, nor Santos et al. (2012) who incubated the same substrate in different soils observed a priming effect. In contrast, Wardle et al. (2008) reported increased losses of native C

in a 10 yr litterbag experiment mixing PyOM and humus and Major et al. (2010) found that PyOM addition increased native soil organic matter respiration. One important difference between these studies is the addition rate, Wardle et al. (2008) added PyOM in a 50 : 50 ratio to humus litter bags, while Major et al. (2010) had an addition rate 6 times higher than our study. Also Keith et al. (2011), in an incubation experiment, found a positive priming effect on native soil organic matter adding a higher quantity of char to soil, in fact they added 20 mg PyOM g^{-1} soil, a quantity ten times higher than ours, considering a soil depth of 15 cm.

The amount of new substrate added compared to the initial soil organic matter amount should explain partially the changes in microbial activity in the soil (Fontaine et al., 2003). So we propose that the addition rate of PyOM might play a role in the size of priming effect.

4.3 Dissolved organic carbon

In our study, the losses of PyOM and wood as DOC were three orders of magnitude lower than their respective losses as CO_2 after one year (0.0004 % vs. 0.5 % and 0.02 % vs. 22 % of initially applied PyOM-C and wood-C, respectively; Fig. S3). The losses of wood-C as DOC were 50 times higher than the DOC losses of PyOM. In a field study in a savanna Oxisol, Major et al. (2010) found that DOC losses were 0.003 % of the initial PyOM-C at a depth of 15 cm, i.e. ten times higher than observed in the present study. This discrepancy can be partially explained by the different operational definition of DOC used in the two studies. In fact while Major et al. (2010) considered as DOC particles with a size < 0.7 μm, we considered as DOC particles with a size < 0.45 μm. Major et al. (2010) found that PyOM increased the soil water fluxes, but we observed no such increase in our study, maybe because their PyOM application rate was 6 times higher than ours. Also the rainfall regime of Savanna, characterised by high and concentrated annual rainfall might have contributed to increase the level of PyOM lost by leaching, compared to temperate forest where rainfall are low and more distributed during the year. Finally, Major et al. (2010) studied the

movement of PyOM in an Oxisol, that is characterised by high water infiltration rates (Major et al., 2010; Soil Survey Stuff, 1999), while the soil in our field site was rich in clay that may have prevented water from quickly percolating through the soil profile. The DOC movements are probably comparatively restricted in our soil, and so are the PyOM DOC. This would indicate that soil properties determine the leaching of PyOM DOC. A similar conclusion was drawn by Leifeld et al. (2007) who observed consistent vertical movements of PyOM along the profile of three grassland soils (21–69 % of PyOM-C moved below 30 cm over 50 yr). They suggested that such high vertical movement is due to the high hydraulic conductivity of the soil, which is characterised by a high porosity as it was formerly a peatland.

Our findings on limited losses of PyOM derived DOC confirmed previous studies showing that only a small portion of fresh PyOM was soluble in water (Abiven et al., 2011). However, the soluble fraction of PyOM may increase with the degree of surface oxidation and ageing of PyOM (Hockaday et al., 2006; Abiven et al., 2011). Alternatively, it is possible that part of the dissolved PyOM is absorbed into the first layers of the soil, characterised by high clay content, and released in the following years. Therefore due to the PyOM ageing and partial release of the PyOM adsorbed on clay minerals, it is reasonable to expect that larger proportion of PyOM will be released as DOC in the future.

In a nearby experiment, Kammer et al. (2011) observed wood losses as DOC equivalent to 1.5 % of initially applied wood-C over one year, however in their experiment wood was applied on the soil surface and therefore directly exposed to the rainfall, while in our case wood was incorporated in the first cm of the soil. Moreover they observed that wood-derived DOC was strongly decreasing from the litter layer to 5 cm depth. Such a decrease of DOC fluxes along the profile indicates that the adsorption of DOC on mineral surfaces could be an important mechanism in preventing the wood-derived DOC from percolating down to 15 cm (Kaiser and Georg Guggenberger, 2000).

4.4 Effect of nitrogen addition

The addition of N had a significant effect on substrates decomposition only on one sampling date (Fig. 3). However N decreased the cumulative PyOM decomposition by 47 % after one year (Fig. S2; Table 2) but had no effect on cumulative wood decomposition. Santos et al. (2012) incubated the same PyOM in different soils, and found no effect of N addition on PyOM decomposition. These different results could be explained by the difference in the setup (3 times lower N addition level and in a single addition at the beginning of the incubation). Soil properties may also play a role, Santos et al. (2012) suggest that the added N may have been adsorbed in the interlayers of the vermiculite present in their soil preventing it from being available to microbes.

Carreiro et al. (2000) and Sinsabaugh (2010) observed that N addition decreased phenol-oxidase and peroxidase activities. Such enzymes are involved in the cleavage of aromatic compounds, and thus could affect breakdown of PyOM. However this hypothesis was not confirmed by a decrease of wood decomposition that is also supposed to be affected by the activity of oxidative enzymes.

Our results agree with the N mining theory (Craine et al., 2007). N addition might depress the decomposition of added substrate by fulfilling the N demand of microbes "mining" the recalcitrant fraction of the substrate to obtain available N. Confirming this hypotheses a substantial mineralization of PyOM-N by microbes was observed by Santos et al. (2012) and Hilscher and Knicker (2011).

Alternatively N addition might have induced a shift in microbial community, by lowering the fungal : bacterial ratio (Frey et al., 2004). However very little is known on the microorganisms responsible for PyOM decomposition Lehmann et al. (2011).

Wood decomposition was not affected by N deposition confirming findings of Hagedorn et al. (2003) who measured beech twigs decomposition in a neighbouring site. However they found that N addition altered the decomposition pattern of wood by depressing the wood decomposition from six to twelve months after N addition (i.e. in the same time frame of N addition in our experiment), confirming findings of Berg and

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Matzner, (1997). Such different result may be explained by the shorter duration of N addition in our experiment, six months instead of one year, supporting findings of Knorr et al. (2005) who observed that the effect of N addition was extremely low when lasting for less than six months.

5 Conclusions

We added ^{13}C-labelled PyOM or pine wood to a temperate forest soil with and without added inorganic N. In the first year we observed that:

- PyOM C mineralized at a rate of 0.5 % of applied C per year.

- PyOM losses as dissolved organic carbon (DOC) were three orders of magnitude smaller than their losses as CO_2.

- N addition depressed the decomposition of PyOM by 43 % but did not alter wood decomposition neither the losses by leaching from wood or PyOM.

- There was no significant increase in soil CO_2 respiration of native SOC after PyOM or wood was added to soil.

Therefore, we conclude that this pine-derived PyOM-C has a centennial scale mean residence time, that N can decrease PyOM mineralization and that mineralization to CO_2 is the main process leading to PyOM losses in the first after PyOM addition.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/11/1/2014/bgd-11-1-2014-supplement. pdf.

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Table 1. Chemical and physical properties of the soil (0–15 cm depth) at the field site, and of PyOM and wood. The values presented are means of 3 replicates ± standard errrors.

	Texture %			Bulk density g cm^{-3}			pH	CEC mmol kg^{-1}	Elemental analysis g kg^{-1} soil			C/N (mass ratio)	^{13}C (atom %)	Application rate (g C m^{-2})
	Sand	Silt	Clay	0–5 cm	5–10 cm	10–15 cm			C	H	N			
Soil	45.5 ±3.5	24.2 ±4.4	31.5 ±2.4	1.20 ± 0.14	1.21 ± 0.32	1.60 ± 0.32	5.9 ± 0.5	74.3 ± 14.9	33.7 ± 4.8	8.9 ± 0.7	2.4 ± 0.2	14		
PyOM									799	7.1	34.3	110	2.03	397
Wood									499	4.3	66.2	115	2.05	189

Table 2. Average total C losses over the study period ± standard error ($n = 3$). The losses as DOC and DOC from the added inputs correspond to the cumulated losses over a year. The losses as CO_2 and DOC from the added input are calculated using Eq. (3). Letters indicate where treatments are significantly different (pair wise paired t test, $p < 0.05$).

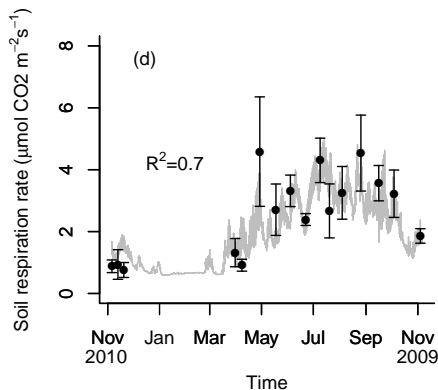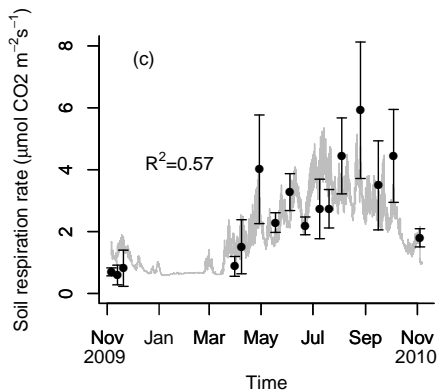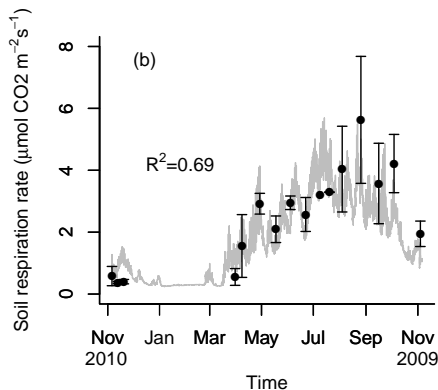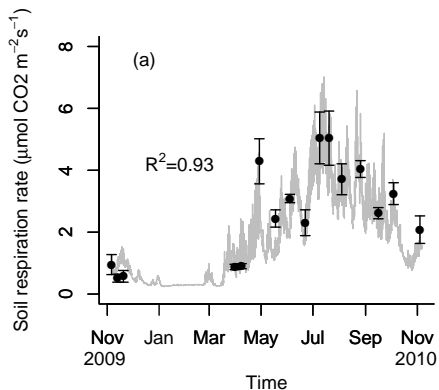|	Total DOC	Cumulated losses from the added input as DOC	Total losses as CO_2	Cumulated losses over one year from the added input as CO_2
	($g\,C\,m^{-2}$)	(% initial input-C)	($g\,C\text{-}CO_2\,m^{-2}$)	(% initial input)
Ambient N				
Control	2.1 ± 0.7 a		503 ± 100 a	
PyOM	1.8 ± 0.3 a	0.0004 ± 0.0003 a	653 ± 21 a	0.54 ± 0.07 a
Wood	1.7 ± 0.3 a	0.022 ± 0.007 b	726 ± 189 a	22 ± 3 b
Increased N				
Control	1.5 ± 0.2 a		638 ± 33 a	
PyOM	1.6 ± 0.6 a	0.0002 ± 0.0001 a	650 ± 119 a	0.28 ± 0.07 c
Wood	2.4 ± 2.2 a	0.03 ± 0.01 b	715 ± 125 a	22 ± 3 b

Fig. 1. Measured (full symbols) vs. modelled (continuous line) soil respiration rate (in $\mu mol\, CO_2\, m^{-2}\, s^{-1}$), for PyOM under ambient N **(a)**, PyOM under increased N **(b)**, wood under ambient N **(c)** and wood under increased N **(d)**. Error bars represent the standard error of the mean ($n = 3$) of the measured soil respiration.
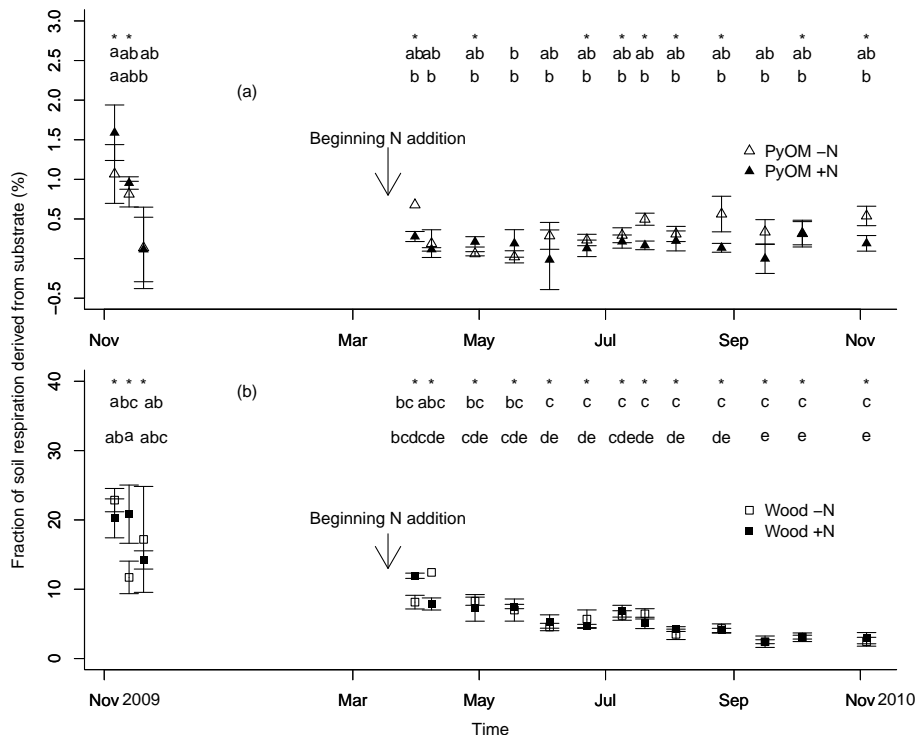
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Fig. 2. Fraction of soil respiration derived from PyOM **(a)** and from wood **(b)**. The sampling dates within the same treatment with different letters (on the top) are significantly different ($p < 0.05$ TukeyHSD test). The first line of letters refers to the ambient N treatment and the second to the increased N treatment. Error bars represent the standard error of the mean ($n = 3$). Asteriscs (*) indicate when the fraction is significantly different from 0 (ambient and increased N treatment were pooled together, $n = 6$, $p < 0.05$).
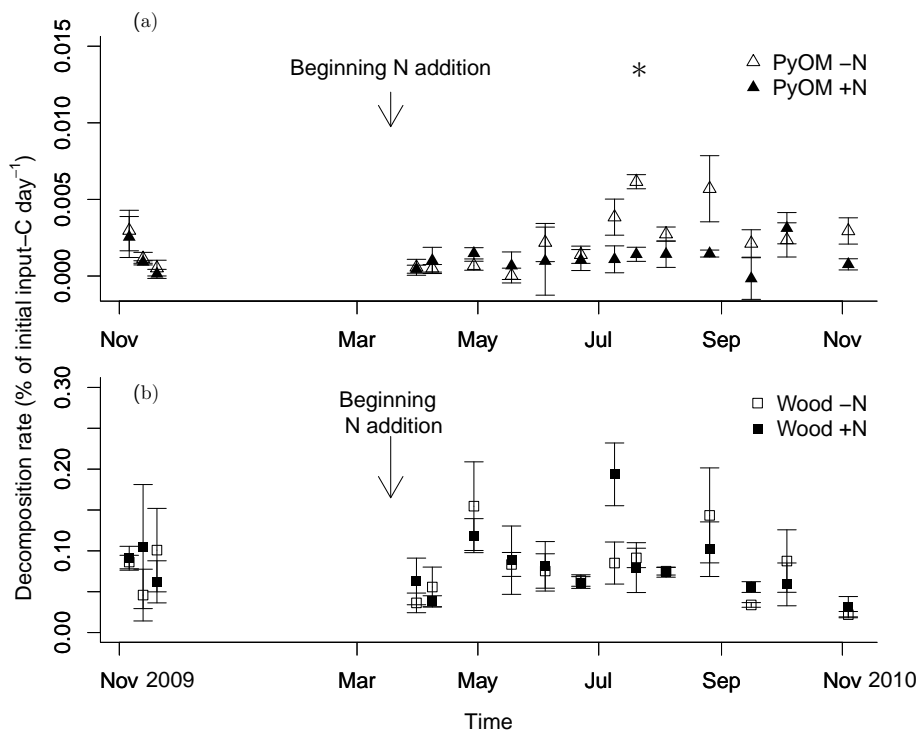
Fig. 3. Mineralization rate (in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$) of wood **(a)** and PyOM **(b)**. Empty symbols represent the treatment under ambient N and full symbols under increased N. Asteriscs (*) indicate the dates when the addition of N significantly affected substrate decomposition (paired t test, $p < 0.05$). Error bars represent the standard error of the mean ($n = 3$).

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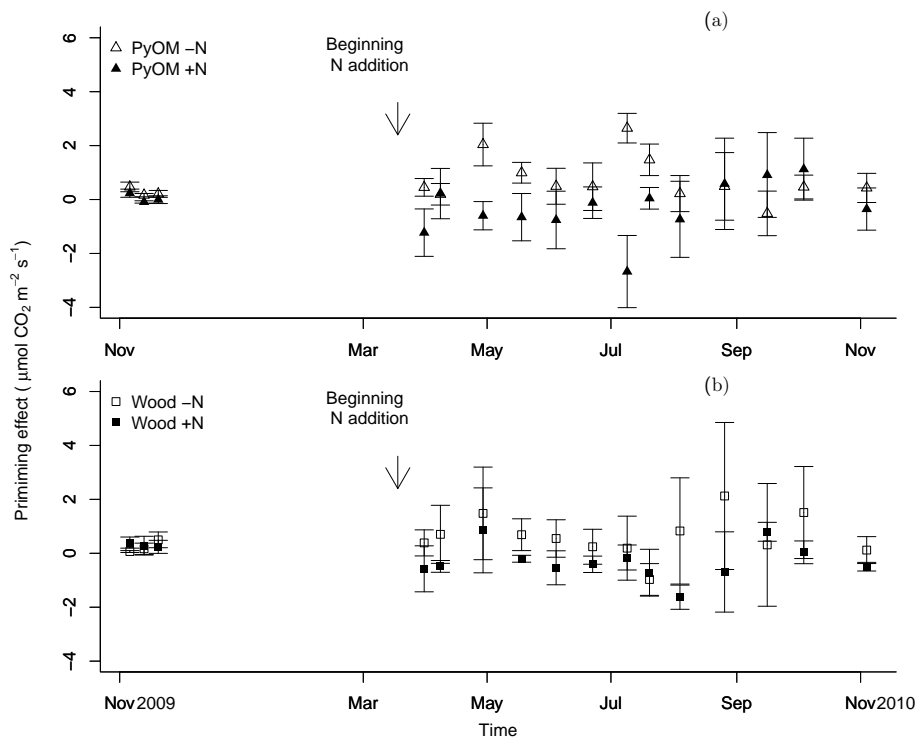
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Fig. 4. Priming effect induced by PyOM **(a)** and wood **(b)** in $\mu mol\, CO_2\, m^{-2}\, s^{-1}$, derived from Eq. (2). Full symbols represent the treatment under ambient N and the empty symbols represent the treatment under increased N. Error bars represent the standard error of the mean ($n = 3$).