

# Effects of liming and mineral N on initial decomposition of soil organic matter and post harvest root residues of poplar



Christine Wachendorf

Department of Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

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## ABSTRACT

Short-term potential carbon (C) and nitrogen (N) mineralization of soil organic matter and belowground plant litter was determined in the top soil layer of a sandy soil of a short-rotation coppice (SRC) and the effect of addition of lime, mineral nitrogen and poplar roots was determined. Lime addition was equivalent to  $1.2 \text{ mg CaCO}_3 \text{ g}^{-1}$ , mineral N and root litter were added corresponding to  $6.8 \mu\text{g N g}^{-1}$  and  $13.8 \text{ mg roots g}^{-1}$  soil respectively. Partitioning of carbon dioxide ( $\text{CO}_2$ ) derived from lime and soil organic matter (SOM) was calculated by using stable isotope analysis of the  $\text{CO}_2$ . In addition, particulate organic matter (POM) as well as microbial biomass carbon and nitrogen were analyzed after 51 days of incubation.

Addition of lime decreased  $\text{CO}_2$  derived from organic matter in all treatments, but the level of decrease varied with N and root litter addition. Microbial biomass C and N increased with N addition in soil without root litter but decreased with root litter, nevertheless an immobilization of mineral N was especially high in soil amended with root litter. Particulate organic matter (POM) decreased by 41% of its dry weight in root litter amended soil. Liming decreased decomposition of POM in these soils, whereas no effect was observed after addition of mineral nitrogen or mineral nitrogen and lime. These results show that liming has the potential to decrease C mineralization of humified organic matter as well as plant residues and that decreased mineralization of organic matter occurred after liming, even if mineral N was added.

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## 1. Introduction

Recultivation of short rotation coppices (SRC) into arable land meets multiple challenges. Cultivation of rapidly growing trees often results in a decline of the soil pH (Jug et al., 1999; Kahle et al., 2007; Baum et al., 2009; Petzold et al., 2010). During reconversion of these soils to arable land liming is therefore required. Furthermore, a large amount of above-ground and belowground harvest residues accrues, which makes intensive tillage necessary (Toenshoff et al., 2013a). Both liming and intensive soil tillage may increase the turnover of soil organic matter thus offsetting the potential positive effect of SRC on the soil carbon (C) pool. Moreover, the accumulation of woody plant residues from trees makes it likely that nitrogen (N) immobilization occurs after incorporation of harvest residues (Toenshoff et al., 2014). Therefore, N fertilization would appear to be essential to avoid a shortage of N for the succeeding plants. The short term N immobilization capacity of plant litter with low nitrogen content (Recous et al., 1999) is counteracted by nitrogen addition, which often results in a higher C mineralization of residues, since it releases the N limitation for microorganisms (Recous et al., 1995). On the other hand, decomposition of woody plant residues may decrease as a result of N inputs, as formation of lignolytic enzymes is suppressed

in the presence of mineral nitrogen (Keyser et al., 1978; Tien and Myer, 1990; Wang et al., 2004). Therefore, N fertilization and liming may have an adverse effect on the C-sequestration potential of soils reconverted from SRCs to arable land.

Although liming is a common agricultural practice and the effect on soil properties has been extensively studied, the effect on soil organic matter (SOM) stocks is still poorly known (Paradelo et al., 2015). This is due to the fact that multiple factors may result in different net effects on SOM. Liming increases the soil biological activity, thus favoring the mineralization of organic matter (Anderson, 1998). On the other hand liming increases the stability of organic matter linkages (Kaiser et al., 2012), thus decreasing the mineralization of organic matter (Muneer and Oades, 1989a). Furthermore liming improves plant growth, thus increasing the C-input to soil (Paradelo et al., 2015). In microcosm experiments excluding the effect of lime on growing plants, an increased production of  $\text{CO}_2$  after liming is often observed (Haynes and Swift, 1988; Badalucco et al., 1992; Priha and Smolander, 1994). The increase is often a short-term effect (Shah et al., 1990), but long-term effects are also observed (Lohm et al., 1984). Nevertheless a reduced C mineralization in soils amended with Ca salts was observed Muneer and Oades (1989a). The decrease is probably due to  $\text{Ca}^{2+}$  ions forming covalent bonds between clays and humified OM (Muneer and Oades, 1989b). Therefore, the stabilizing effect of lime may apply only for humified organic matter in mineral soils (Clough and Skjemstad, 2000).

E-mail address: [c.wachendorf@uni-kassel.de](mailto:c.wachendorf@uni-kassel.de).

Decomposition of plant residues is often accelerated by liming, because the rising pH may increase the activity of the microorganisms (Zelles et al., 1987; Badalucco et al., 1992). These diverse results suggest that the effect of liming may be different on humified organic matter and plant residues.

Similar to C mineralization, adverse effects of liming on N mineralization are observed. Results depend upon the type of organic matter, with increased N mineralization in O horizons of an acid forest soil but no effect on the N mineralization of the mineral soil (Nohrstedt, 2002; Corre et al., 2003). N mineralization of limed soils may be influenced by indirect effects, like changes in amount and quality of litter. These effects are excluded in incubation experiments. Nevertheless only few laboratory studies are available in which the interaction between liming and mineral nitrogen addition on C and N mineralization was observed. Interaction of lime and N addition depends on soil type and quality of organic matter. Lime additions decreased potentially mineralizable N during aerobic incubation of a fen peat, but there was no effect of N addition, whereas nutrient additions in a bog peat increased cumulative N mineralization over 30 weeks, but lime addition had no effect (Chapin et al., 2003). Shah et al. (1990) observed that liming caused a rapid and substantial increase in CO<sub>2</sub> evolution of the upper mineral soil layer of a podzol under grassland. Nevertheless, gross N mineralization of limed and unlimed soils did not differ after N addition, although limed soils showed lower N mineralization rates. Results of these studies demonstrate that the effects of lime and N addition on C and N mineralization may reverse.

Application of lime to a soil leads to a net emission of CO<sub>2</sub> during dissolution (West and McBride, 2005; Sanderman, 2012). Therefore estimates about the turnover of the organic matter in limed soils may be overestimated, because CO<sub>2</sub> may originate from organic matter as well as dissolution of the lime. But only few studies distinguish between the two sources. A differentiation between biotic respiration of organic matter and abiotic C efflux derived from dissolution of carbonate is possible by stable isotope analyses of the CO<sub>2</sub>, as the two sources differ in their <sup>13</sup>C signature. With this methodological approach, Biasi et al. (2008) showed in a short term experiment that biotic respiration rates were similar in limed and unlimed soils, although total carbon dioxide release in limed peat was significantly higher than in unlimed peat. Similar results were reported by Bertrand et al. (2007). She estimated that the contribution of soil carbonates to CO<sub>2</sub> production led to an overestimation of organic C mineralization by up to 35%. Furthermore, the addition of crop residues increases the rate of CO<sub>2</sub> production in soils and may result in higher soil CO<sub>2</sub> concentrations, thereby favoring CaCO<sub>3</sub> dissolution and overestimation of organic C mineralization. Nevertheless, this effect could not be proven, as it was masked by ten times greater respiration of residue amended soils (Bertrand et al., 2007).

The short-term effect of liming on the dynamics of organic matter after reconversion of SRC has not been investigated before, but it is assumed that the effect is different from the effect observed in forest and arable soils. Reconverted soils differ from arable soils in the high input of woody plant residues, with coarse harvest residues accounting for 16 to 30 t C ha<sup>-1</sup> in the topsoil (Toenshoff et al., 2013a). And unlike in forest soils, where plant residues often accumulate in the litter layer, plant residues are intensively mixed into the soil by tillage. Knowledge of the effects of management factors on the dynamics of organic matter during reconversion will improve our capacity to predict the sustainability and environmental impact of short rotation coppices beyond the period of wood fuel production.

To explore the effects of lime and mineral nitrogen on C mineralization and N mineralization, a topsoil from an SRC with single, double or triple mixtures of poplar root litter, lime and mineral fertilizer was incubated. Partitioning of CO<sub>2</sub> derived from lime and organic matter was considered by analyzing the <sup>13</sup>C signature of the evolved CO<sub>2</sub>. The following hypotheses were tested: (1) Liming accelerates initial decomposition of poplar root litter, but decreases decomposition of humified

organic matter; (2) Addition of mineral N increases short-term C mineralization in root litter amended soils, but this increase is absent if lime and mineral N are applied at the same time; and (3) N immobilization in root litter amended soil is higher in limed than in unlimed soils.

## 2. Materials and methods

### 2.1. Incubation experiment

Soil samples from the upper mineral soil layer (0–30 cm) of a poplar SRC were taken shortly before clear cutting and conversion to arable land. Soil was collected from a deep-plowed Gleyic Podzol with a soil texture of silty sand (76% sand, 20% silt, 4% clay) described by Toenshoff et al. (2013a). The soil was sieved (2 mm mesh size) and the root litter was removed by sieving and by hand picking. Sieved soil samples were stored at 4 °C until further processing. The incubation experiment was performed under standard conditions (20 °C, 40% water holding capacity; WHC). Soil samples were conditioned in the dark at 20 °C for three days. Root litter material was collected in a 21-year-old poplar stand before final harvest of the trees and dried at 60 °C. Roots were excavated on a 1.4 × 1.4 m square, centered around five selected trees to a depth of 30 cm and were separated into coarse (>5 mm) and fine (<5 mm) roots (Toenshoff et al., 2013a). As the CN ratio between coarse and fine roots differs only slightly, coarse and fine roots were mixed at a ratio of 70 to 30, as previously determined in the field, and contained 47% C and 0.50% N (CN ratio 94). Root samples were shredded by using a cutting mill resulting in particle size of 1–5 mm. Moist soil was amended with root litter, equivalent to 6.6 mg C g<sup>-1</sup> soil, pulverized lime equivalent to 0.14 mg C g<sup>-1</sup>, N as NO<sub>3</sub>NH<sub>4</sub> equivalent to 6.8 μg N g<sup>-1</sup>, and the samples were shaken vigorously. The calculated addition rates, based on a bulk density of 1.0 for the Ah horizon and soil depth of 15 cm, correspond to a rate of 2.07 kg root litter m<sup>-2</sup>, which was the estimated root biomass under SRC at harvest (Toenshoff et al., 2013a). The rate of lime and mineral N corresponds to 0.18 kg CaCO<sub>3</sub> m<sup>-2</sup> and 1 g N m<sup>-2</sup>, respectively. A low N rate was chosen to mimic the mineral N content in soil solution remaining after plant uptake. Root litter (R), lime (L) or mineral N (N) was added individually to the soil, and root litter and mineral N (R + N), root litter and lime (R + L), lime and mineral N (L + N) or root litter, mineral N and lime (R + N + L) were added together. The control consisted of untreated soil. The incubation experiment was conducted with four replicates.

### 2.2. Soil analysis

For soil analysis at the beginning and during the incubation, 100 g soil was weighed in 250 ml PE flasks for each treatment and incubated for 51 days. For each treatment and sampling date, four replicates were prepared, giving 16 samples per treatment. At each sampling date, 4 replicates of each treatment were sampled destructively. The pH was measured in 0.01 M CaCl<sub>2</sub> (10 g soil: 25 ml) at room temperature. The water content of the soil samples was determined from 10 g of fresh soil on an oven dry weight basis (105 °C). The dried soil was milled to fine powder for analysis of C and N and δ<sup>13</sup>C.

### 2.3. Determination of microbial biomass and analyses of mineral N

Determination of microbial biomass was carried out by the fumigation–extraction method as described by Vance et al. (1987). Soils were fumigated with chloroform for 24 h at 20 °C. Fumigated and non-fumigated soils were treated with 0.05 M K<sub>2</sub>SO<sub>4</sub> (soil K<sub>2</sub>SO<sub>4</sub> ratio 1:5), shaken for 30 min on a horizontal shaker at 200 rev. min<sup>-1</sup>, centrifuged at 2000 g and filtered. In order to remove any dissolved carbonate, one drop of 2 M HCl was added before determination of organic C and N concentration using a CN analyzer (Dimatec 100, Essen). Microbial biomass C and N were calculated as the difference between fumigated and non-

fumigated samples using a  $k_{EC}$  value of 0.45 (Wu et al., 1990) and a  $k_{EN}$  value of 0.54 (Brookes et al., 1985) to account for the non-extractable part. Mineral N was analyzed in  $K_2SO_4$  extracts also used for estimating microbial biomass. Ammonium-N and nitrate-N in the extracts were measured with a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Ainring, Germany).

#### 2.4. Determination of particulate organic matter

Particulate organic matter (POM) was determined for treatments with added root litter and the control soil only. 100 g of moist soil was initially dispersed in 400 ml 5% NaCl, shaken by hand and allowed to stand for 45 min (Magid and Kjærgaard, 2001). Then, the samples were poured onto a sieve of 0.4 mm mesh size and washed with tap water. The material retained on the sieves was transferred into a beaker. Tap water was added, the beaker was swirled and organic material was separated from the mineral material by flotation–decantation. Swirling and flotation–decantation was repeated until plant residues were no longer visible in the mineral fraction. The mineral fraction was discarded and the remaining POM fraction was dried at 40 °C for 48 h, weighed and ground for total C and N analysis by dry combustion (Elementar Vario El, 156 Heraeus, Hanau, Germany). The recovery rate of added root litter in the POM was estimated by subtracting POM-C of the soils with added litter from the POM-C of the control.

#### 2.5. CO<sub>2</sub> evolution

CO<sub>2</sub> evolution was determined using 300 g of field-moist soil samples incubated in closed 1600 ml jars according to the method of Isermeyer (1952) as described by Alef (1991). The CO<sub>2</sub> production after 2, 6, 10, 14, 22, 29, 37, 44, and 51 days of incubation was estimated by titration of 0.5 M NaOH with 0.5 M HCl after adding 0.5 M BaCl<sub>2</sub> and phenolphthalein indicator. Another set of glass jars equipped with a three-way stopcock was prepared to sample gas for isotopic analysis. 10 ml gas samples were taken using an airtight syringe and transferred into a pre-evacuated exetainer vial. The isotopic signature of CO<sub>2</sub> released in soils with sole root litter addition and in all soils treated with lime was determined as described below. After each titration and gas sampling, jars were ventilated for 2 min with compressed air.

#### 2.6. Stable isotope analysis

The  $\delta^{13}C$  signature, C and N of the soil samples and lime were analyzed by using the isotope ratio mass spectrometry (IRMS) interfaced with an elemental analyzer (EA, Thermo Finnigan, Bremen, Germany) via the open split interface (Conflow III, Thermo Finnigan, Bremen, Germany) (Table 1). The  $\delta^{13}C$  of CO<sub>2</sub> after 2, 6, 19, and 51 days was analyzed by a continuous-flow isotope ratio mass spectrometer (IRMS; Thermo Finnigan DELTA Plus, Bremen, Germany) coupled with a Trace GC and a GP interface. Separation of CO<sub>2</sub> from N<sub>2</sub>O was performed with a Pora PLOT Q column (25 m length; 0.32 mm i.d.; Varian) at 22 °C using He as the carrier gas. Sample loading was done with a sample loop swept by the carrier stream using PAL autosampler (CTC Analytics AG, Zwillingen, Switzerland). Samples were injected with a gas-tight syringe (Hamilton). Each sample measurement cycle was started and ended with six reference gas injections, CO<sub>2</sub> concentration of the reference gas varied from 360 to 3000 ppm.  $\delta^{13}C$  of the reference gas

was  $-28.8 (\pm 0.2)$ . The analytical precision of the  $\delta^{13}C$  measurements of the gas samples was  $\pm 1.7$ . The isotopic values are expressed relative to the Vienna-Pee Dee belemnite (V-PDB) reference as  $\delta^{13}C$ . The CO<sub>2</sub> concentration and isotopic content were corrected for the amount of compressed air in the jars, the concentration of which was  $482 \pm 16 \text{ ml l}^{-1}$ , and the  $\delta^{13}C$  was  $-13.8 \pm 0.37\%$ . Assuming that the carbonates in soil solution were in isotopic equilibrium with the solid carbonates, the contribution of organic C to the total amount of CO<sub>2</sub> produced during a time interval in soil without root litter amendments was calculated as follows:

$$\text{lime derived CO}_2\text{-C}(\%) = \frac{\delta^{13}\text{CO}_2 - \delta^{13}\text{soil}}{\delta^{13}\text{lime} - \delta^{13}\text{soil}} * 100 \quad (1)$$

where  $\delta^{13}CO_2$  is the  $^{13}C$  signature of the produced CO<sub>2</sub>-C during the time interval of the incubation and  $\delta^{13}\text{lime}$  and  $\delta^{13}\text{soil}$  are the isotopic signature of the C of the lime and the soil organic C, respectively. The  $^{13}C$  signature of the soil organic C in the control soil differed slightly from the signature of the root litter (Table 1). Therefore, the relative contribution of root litter C to the total CO<sub>2</sub> evolution was calculated in solely root litter amended soils as follows:

$$\text{root litter derived CO}_2\text{-C}(\%) = \frac{\delta^{13}\text{CO}_2 - \delta^{13}\text{soil}}{\delta^{13}\text{root} - \delta^{13}\text{soil}} * 100 \quad (2)$$

where  $\delta^{13}CO_2$  is the  $^{13}C$  signature of the CO<sub>2</sub>-C produced during the time interval of the incubation and  $\delta^{13}\text{root}$  and  $\delta^{13}\text{soil}$  are the isotopic signature of the C of the root litter and the soil organic C, respectively. Lime derived CO<sub>2</sub>-C in root litter amended soil was calculated by using a  $\delta^{13}C$  value for the soil root litter mixture of  $-28.58$  instead of the  $\delta^{13}C$  soil value in Eq. (1), considering that root litter derived C was between 94 and 82% of evolved CO<sub>2</sub> in the litter amended soils.

#### 2.7. Statistical analysis

Data of C and N-mineralization, microbial biomass and pH, were statistically analyzed by a three factorial analyses of variance (ANOVA) for effects of L (liming), N addition (N) and root litter addition (R) and their interaction. As particulate organic matter was only analyzed in control and all root amended treatments, root litter addition was analyzed by a single factorial ANOVA and lime and N addition in root litter amended soil was analyzed by a two factorial ANOVA. The prerequisites for ANOVA, normality of the residuals and homogeneity of variances, were tested. LS Means were computed for the display of results. If higher order interactions were significant main effects or lower order interactions were not interpreted, even if significant. Statistical analyses were performed using SAS (SAS Institute, 2014).

### 3. Results

#### 3.1. CO<sub>2</sub> evolution from lime and organic matter

Total cumulative CO<sub>2</sub> evolution was significantly affected by addition of lime, mineral nitrogen and root litter (Table 2). Addition of root litter and lime controlled the effect of mineral N addition. In soil without root litter but with lime addition mineral nitrogen increased total cumulative CO<sub>2</sub> evolution, whereas in soils added with root litter this effect was reverse (Fig. 1). Lime decreased CO<sub>2</sub> evolution derived from organic matter (OM), but this effect varied with N and root litter addition. In root amended soil this decrease was higher in soils amended with N than without N addition. The opposite was observed in soil without root litter amendments.

**Table 1**  
Mean C and N content and  $\delta^{13}C$  of lime, soil and poplar root litter ( $\pm$  standard deviation).

	C (mg g <sup>-1</sup> )	N (mg g <sup>-1</sup> )	C/N	$\delta^{13}C$ (‰)
Lime	119.9 ( $\pm 1.8$ )	n.d.	n.d.	$-0.64 (\pm 0.69)$
Soil	40.7 ( $\pm 3.9$ )	2.2 ( $\pm 0.2$ )	20	$-28.32 (\pm 0.09)$
Root litter	476.7 ( $\pm 4.7$ )	5.2 ( $\pm 0.1$ )	92	$-28.61 (\pm 0.07)$

n.d.: not detectable.

**Table 2**  
Results of the three factorial analyses of variance (ANOVA) for effects of L (liming), N addition (N) and root litter addition (R) and their interaction on cumulative total CO<sub>2</sub> (total CO<sub>2</sub>) and cumulative CO<sub>2</sub> derived from organic matter (CO<sub>2</sub> d OM), pH, microbial biomass C and N (Cmic and Nmic) and mineral nitrogen (Nmin) after 51 days of incubation.

Treatment/interaction	Total CO <sub>2</sub>		CO <sub>2</sub> d OM		pH		Cmic		Nmic		Nmin	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
L	11.13	0.0028	7.27	0.0126	20.97	0.0001	2.80	0.1074	6.55	0.0172	7.20	0.0130
N	115.68	<.0001		NS		NS		NS		NS		
R	80824.8	<.0001	186.81	<.0001	23.49	<.0001	16.13	0.0005	5.13	0.0328	187.39	<.0001
L * N	149.43	<.0001		NS		NS		NS		NS		NS
L * R	9.03	0.0061	4.61	0.0420		NS		NS		NS	4.75	0.0393
N * R	79.97	<.0001		NS		NS	11.51	0.0024	13.69	0.0011		NS
L * N * R	297.91	<.0001		NS		NS		NS		NS		NS

NS: not significant.

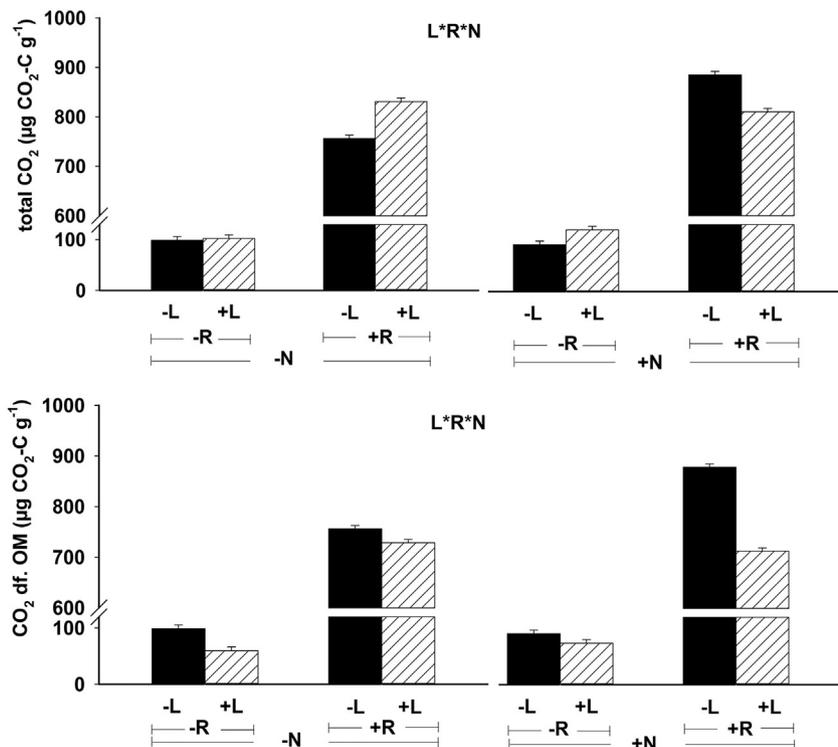
F-values were omitted for non-significant effects.

### 3.2. Treatment effects on pH, soil microbial biomass and mineral nitrogen

Lime as well as root addition increased pH significantly, although changes were small (Table 2 and Fig. 2). Liming decreased microbial biomass C and N (Fig. 3). Regardless of liming, mineral nitrogen increased microbial biomass C and N in soil without root litter, whereas the opposite effect was observed in soils with root litter. During the first seven days of incubation, N immobilization was observed in all treatments (Fig. 4). N immobilization was higher in root litter amended soil and it further increased with N addition. From days 8 to 21, N immobilization decreased in soil with added root litter, and N was mineralized in soil without root litter addition. At the end of the incubation period, the content of mineral N was very low in the root amended soils regardless of lime addition, whereas lime decreased mineral N in soil without root litter (Fig. 5).

### 3.3. Treatment effects on POM

Particulate organic matter was significantly higher in root litter amended soils ( $P < 0.001$  at days 0 and 51, Fig. 6). The amount of C recovered in POM at the beginning of the incubation experiment was  $6.8 \text{ mg C g}^{-1}$  soil in root litter amended soils, which is 82% of the added root litter-C. During 51 days of incubation, POM decreased by 41% of its dry weight in root litter amended treatments. Liming decreased the decomposition of POM in these soils ( $P = 0.011$ ). Remaining POM-C was higher in limed but not N amended soil, whereas with mineral N this effect was not observed ( $P = 0.015$  for  $L * N$  interaction). The CN ratio of POM in soil without root litter was 25 and did not change during incubation, whereas in soil with single addition of root litter the CN ratio increased from 119 to 186. Addition of lime and mineral nitrogen significantly affected the CN ratio of POM at the end of the



**Fig. 1.** Mean and standard error of the mean (bar) of total CO<sub>2</sub>-emission (upper row) and CO<sub>2</sub>-emission derived from organic matter (df. OM) (lower row) of soil amended with lime, mineral nitrogen and poplar root litter and control soil after 51 days of incubation. Only significant interaction of lime (–L and +L), mineral N (–N and +N), and root (–R and +R) is shown.

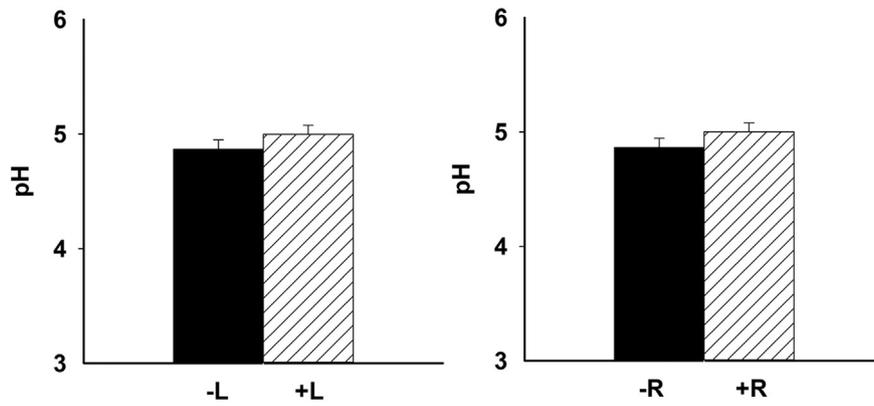


Fig. 2. Mean and standard error of the mean (bar) of pH (CaCl<sub>2</sub>) of soil amended with lime, mineral nitrogen and poplar root litter and control soil after 51 days of incubation. Only significant effects, lime (–L and +L) and root litter (–R and +R) are shown.

incubation period ( $P < 0.001$  for  $L * N$  interaction). The CN ratio of POM decreased within the root litter treatments when lime and mineral N were given at the same time, whereas without mineral N this decrease was minor.

#### 4. Discussion

##### 4.1. CO<sub>2</sub> evolution affected by liming and mineral N addition

The results reveal that lime decreases CO<sub>2</sub> evolution derived from soil organic matter. A stabilizing effect of lime on SOM was postulated by Baldock and Skjemstad (2000). They explained the effect of liming on soil structure by the high concentration of Ca<sup>2+</sup> increasing the flocculation and cementing of soil aggregates. In this way, organic matter may be occluded and become inaccessible for microorganisms. Clough and Skjemstad (2000) showed that organic matter was protected in calcareous soils, regardless of whether the calcium occurred naturally or was an amendment. The decreased C mineralization of humified

organic matter in the presence of Ca<sup>2+</sup> ions also may be due to calcium ions cross linking with SOM (Kaiser et al., 2012). The latter process is probably more important in the sandy soil used in this experiment, as the soil is characterized by its low aggregate stability (Toenshoff et al., 2013b). Stabilization of organic matter may also be affected by the pH value, because of its influences on the humification rate. This effect is probably due to a faster reaction of quinones with amino compounds, which is a major humification pathway in soil (Jastrow et al., 2007). Nevertheless, as the change of pH after liming was small and the duration of the experiment was short, this effect was probably minor in the present experiment. It is assumed that the expected pH increase by the dissolution of lime was partially compensated by the acidification due to the nitrification in the soil (Hamilton et al., 2007).

Contrary to the results in mineral soils, liming of forest soils induces high C mineralization rates of forest humus layers (Persson et al., 1995). However, in contradiction to the hypothesis, CO<sub>2</sub> evolution derived from soil organic matter in root litter amended soil decreased after liming and the decrease was especially high with mineral N addition.

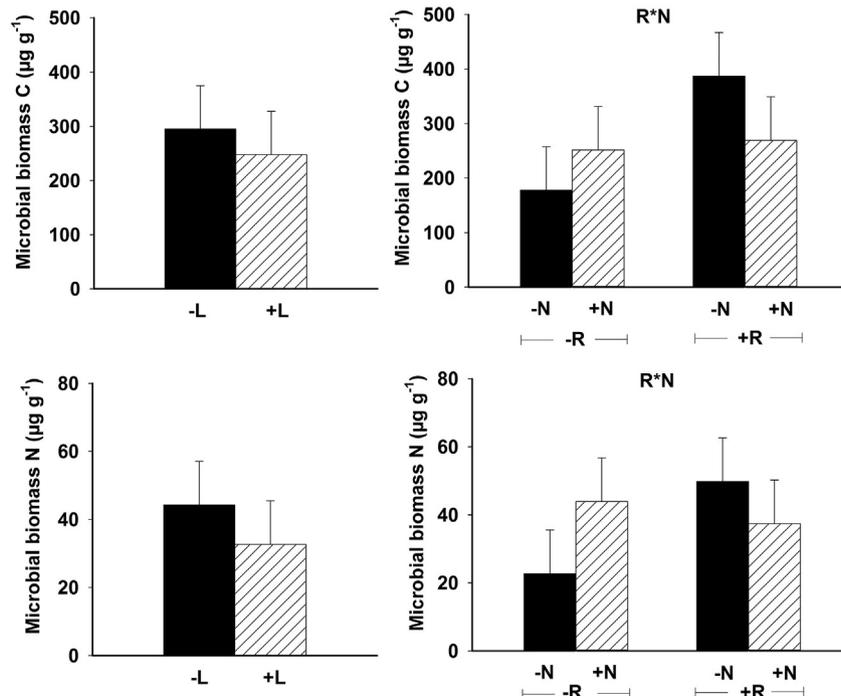
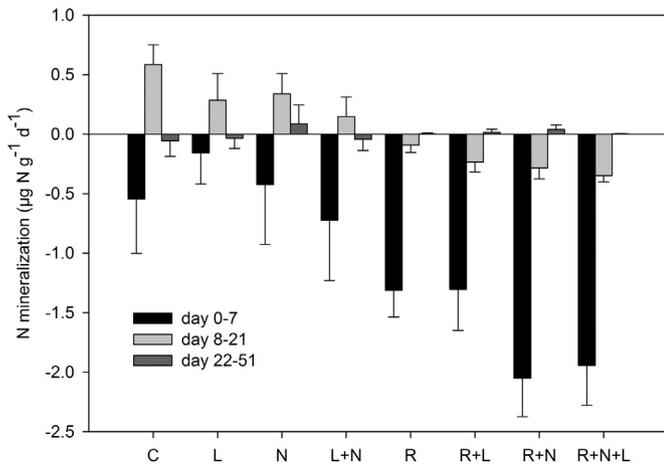


Fig. 3. Mean and standard error of the mean (bar) of microbial biomass C (upper row) and microbial biomass N (lower row) of soil amended with lime, mineral nitrogen and poplar root litter and control soil after 51 days of incubation. Only significant effect of lime (–L and +L) and significant interaction of mineral N (–N and +N), and root litter (–R and +R) is shown.

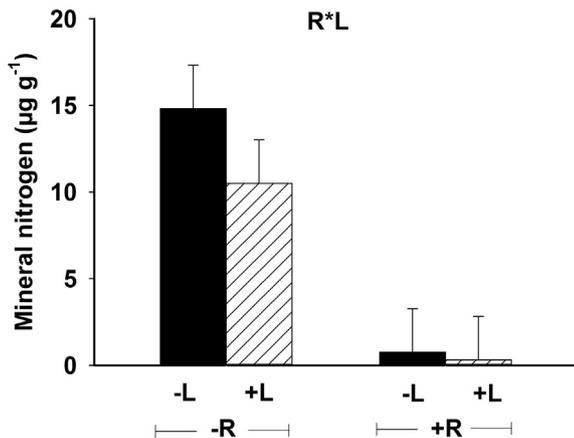


**Fig. 4.** Mean and standard error of the mean (bar) of mineralization and immobilization rate of nitrogen in control soil (C), soil amended with lime (L), mineral nitrogen (N), lime and mineral nitrogen (L + N) and in soil with root litter (R) and soil amended with root litter and lime (R + L), root litter and mineral nitrogen (R + N) and root litter and mineral nitrogen and lime (R + N + L) during three intervals of incubation.

Furthermore the results demonstrate that the increased C mineralization of root litter by addition of mineral N was extinguished with liming. Lime and mineral N increased total CO<sub>2</sub> evolution in soil without root litter addition, whereas it decreased in soil amended with root litter. These results reveal, that even if CO<sub>2</sub> derived from lime is not considered, the decrease of C mineralization with simultaneous application of lime and mineral N in root litter amended soil can be proven. Further experiments are necessary to identify the cause for this observation. Both, a decreased decomposition of root litter, as well as a decreased priming effect induced by addition of litter with a high CN ratio might explain the observed reduction in C mineralization.

#### 4.2. N mineralization and microbial biomass affected by liming, mineral N and root litter addition

In root litter amended soils, N was immobilized as has been shown for other crop residues with a high CN ratio (Trinsoutrot et al., 2000; Cayuela et al., 2009; Muhammad et al., 2011). N stimulates the decomposition of non-lignified tissue and the degradation of unshielded holocellulose of fresh litter, whereas degradation of lignin and lignified tissue is hampered by high N content (Berg, 2014). Hobbie et al. (2012) showed that the hydrolytic degradation of holocellulose, dominating in



**Fig. 5.** Mean and standard error of the mean (bar) of mineral nitrogen of soil amended with lime, mineral nitrogen and poplar root litter and control soil after 51 days of incubation. Only significant interaction of lime (-L and +L) and root litter (-R and +R) is shown.

the early stage of decomposition, increased after N additions. In this experiment, sole addition of N to soil had no effect on C mineralization derived from organic matter. Nevertheless, in all root litter amended soils, N immobilization was accompanied by increased C mineralization. In contrast, Toenshoff et al. (2014) observed no increase of C mineralization amended with mineral N and the same root litter in a different soil. Toenshoff et al. (2014) added 20 µg N g<sup>-1</sup> soil, which was threefold the amount added in this incubation experiment, resulting in a complete immobilization of N only in soil with added woody harvest residues from crowns of poplar trees with a much higher CN ratio than the root litter. In our experiment, root litter addition induced an almost complete immobilization of N, thus N addition in these soils increased C mineralization. Results of both investigations suggest that a N effect on C mineralization is due to the CN ratio of added plant material as well as the level of mineral N in the soil.

In contrast to field investigations of acidic forest soils, where liming increased N mineralization in O horizons (Nohrstedt, 2002; Corre et al., 2003), all treatments decreased N mineralization in this short-term incubation experiment and the decrease was highest for root litter amended soil. This is probably due to the high CN ratio of the added root litter and the resulting immobilization capacity of the residues. The influence of the CN ratio of the organic matter on the N mineralization was shown by (Persson et al., 1989). They observed an increased net N mineralization after liming of the forest humus layer with a CN ratio of 31 but no effect when CN ratio of the forest humus layer was 44.

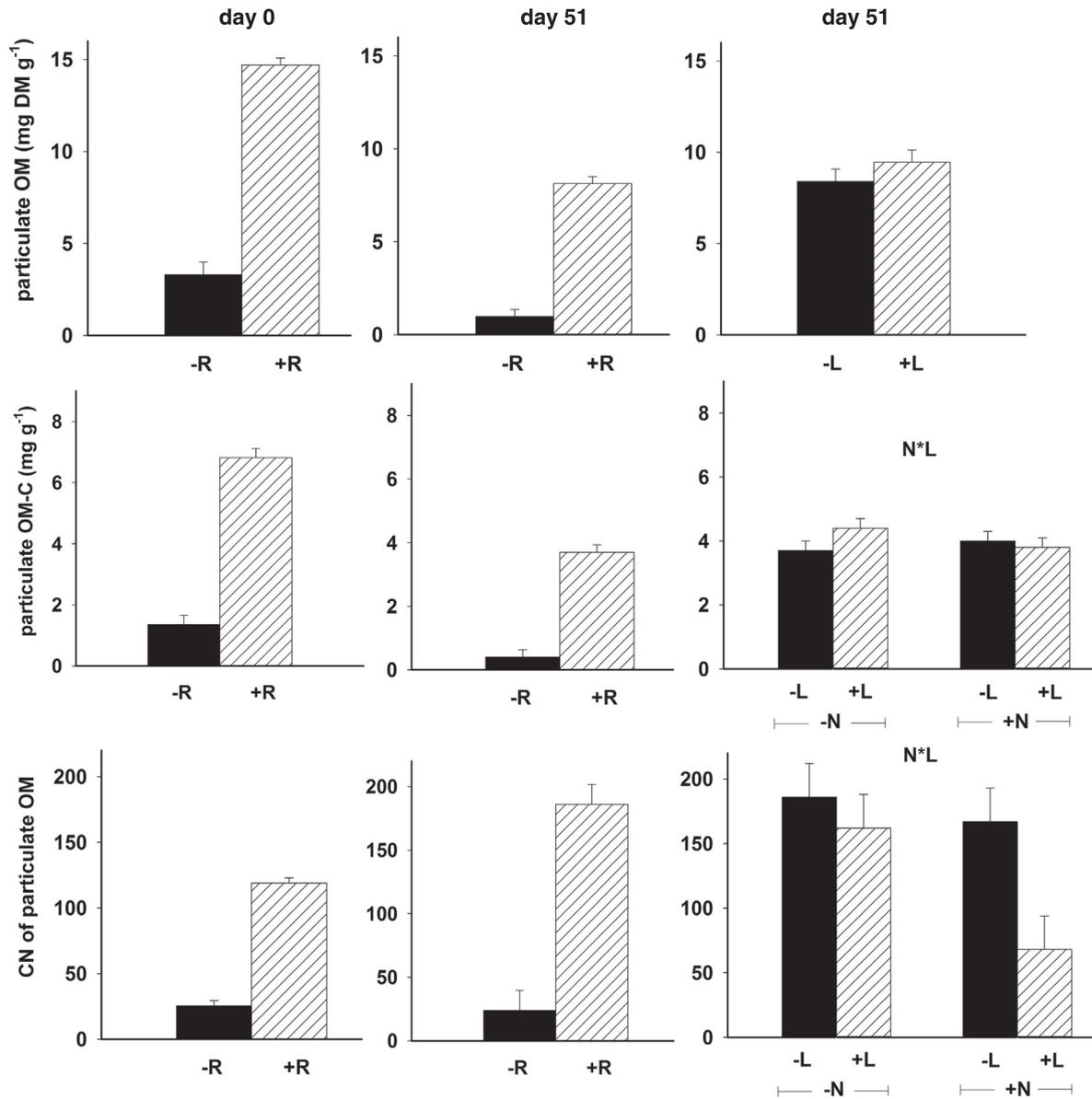
Microbial biomass decreased by liming, although the pH slightly increased. After liming a short term increase of available C substrate often increase microbial activity, but with depletion of these substrates microbial biomass does not change or even decrease (Badalucco et al., 1992). Considering the results of the C mineralization, the stabilizing effect of lime was probably the dominating process observed during incubation of this soil.

#### 4.3. Particulate organic matter affected by liming and mineral N addition

No attempt was made to distinguish between root litter and soil carbon by δ<sup>13</sup>C analysis of POM, because the difference of δ<sup>13</sup>C values between soil and root litter is very small. However, it is deduced that POM consisted mainly of root litter, considering that POM extracted from root litter amended treatments is characterized by a mean C content of 456 mg g<sup>-1</sup> soil and a CN ratio of 104 at the start of the incubation experiment. Liming decreased the decomposition of POM or root litter, but this decrease did not occur if mineral N was added simultaneously. This demonstrates that lime induced reduced decomposition of root litter in this soil can be alleviated by N addition.

Treatment effects on POM and CO<sub>2</sub> evolution derived from organic matter differed. The decrease of POM displayed decomposition of root litter to a size smaller than 0.4 mm. Therefore the decreased amount of POM-C is only partially due to mineralization to CO<sub>2</sub>, it is also due to a decrease in particle size of the root litter during decomposition. These results differed from findings of Yakovchenko et al. (1998), who stated that POM and respiration gave similar results. They extracted POM with a size of 75 to 250 µm from a former pasture followed by 9 years of arable cropping, which resulted in a POM fraction with a much lower CN ratio of 13. The results highlight the fact that the importance of POM in mineralization processes and the significance as a soil quality indicator depends on the origin, particle size and stage of decomposition, which is reflected in the quality of the POM.

In this experiment the CN ratio of the POM increased with incubation time in the single root litter treatment. This increase was probably due to the preferential decomposition of N rich components of the root litter. In contrast, a decrease of the CN ratio with proceeding decomposition frequently observed in depth profiles of humus layers was observed when root litter and lime were added simultaneously. The decrease is often due to the relative increase of N with increasing C mineralization. However, the decreased CN ratio of POM with liming,



**Fig. 6.** Mean and standard error of the mean (bar) of particulate organic matter (OM) dry mass, organic C mass and CN ratio in control soil and single root litter amended soil at day 0 and after 51 days of incubation (columns one and two) and significant effect of lime ( $-L$  and  $+L$ ) and interaction of mineral N ( $-N$  and  $+N$ ), and lime ( $-L$  and  $+L$ ) after 51 days of incubation in root amended soils (column three). Only significant effect of root litter ( $-R$  and  $+R$ ), lime ( $-L$  and  $+L$ ) and significant interaction of mineral N ( $-N$  and  $+N$ ), and lime ( $-L$  and  $+L$ ) is shown.

particularly observed with N addition, was not a function of the C-loss of POM, as the amount of POM-C did not differ between both treatments. The much lower CN ratio of the POM in the root litter, mineral N and lime added soil was probably due to immobilized N in microbial biomass concentrated on the POM fraction. Studies have demonstrated that plant residues represent hot spots of microbial activity (McMahon et al., 2005; Nicolardot et al., 2007) and that POM clearly differed in the structure of their bacterial communities compared to the bulk soil (Blaud et al., 2014). However, an analysis of the microbial community or immobilized N in the microbial biomass of the surrounding POM would be necessary to resolve the cause of the differing dynamics of the CN ratio of the POM.

#### 4.4. Conclusions

C mineralization of organic matter in recently limed soil is overestimated if lime-derived CO<sub>2</sub> is not considered. Based on results of previous studies, it is well accepted, that liming and N-fertilization

increases mineralization of soil organic matter. In contrast to these studies moderate liming and low N addition of a sandy soil with a high content of organic matter formerly cultivated with SRC, decrease the mineralization of organic matter. Thus, both management treatments are recommended, not only to increase the pH and alleviate the immobilization capacity, but also to increase the C sequestration of this soil. The results highlight the fact that the quality of the POM is highly variable, depending on the origin and stage of decomposition of organic matter. Therefore, the significance of POM as a soil quality indicator may be increased if quality is also considered.

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