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Mineral fertilization did not affect decay of old lignin and SOC in a ^{13}C-labeled arable soil over 36 years

A. Hofmann[1], A. Heim[1], P. Gioacchini[2], A. Miltner[3], M. Gehre[3], and M. W. I. Schmidt[1]

[1]Department of Geography, University of Zurich, Zurich, Switzerland
[2]Institute of Agricultural Chemistry, University of Bologna, Bologna, Italy
[3]UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany

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Abstract. Retardation of soil organic carbon (SOC) decay after nitrogen addition to litter or soil has been suggested in several recent studies and has been attributed to a retardation in lignin decay. With our study we tested the long-term effect of mineral fertilization (N+P) on the decay of the SOC component lignin in arable soil. To achieve this, we tracked ^{13}C-labeled lignin and SOC in an arable soil that is part of a 36-year field experiment (conversion from C_3 to C_4 crops) with two mineral fertilization levels. We could show that fertilization neither retarded nor enhanced the decay of old SOC or lignin over a period of 36 years, proposing that decay of lignin was less sensitive to fertilization than previously suggested. However, for new, C_4-derived lignin there were indications that decay might have been enhanced by the fertilization treatment, whereas decay of new SOC was unaffected.

1 Introduction

Mineral fertilization, specifically nitrogen fertilization, was already discussed in the 1950s as a potential means to raise soil organic matter concentrations in arable soils by increasing the amount of plant biomass returned to the soil (Allison, 1955). However, net storage of SOC is a balance between biomass input and decay (mineralization). Because soil microorganisms compete for nutrients with plants, the addition of mineral fertilizer might not only increase plant biomass production but also microbial biomass and microbial activity (Wang and Bakken, 1997). The latter effect could enhance the decay of soil organic matter. This hypothesis has recently been supported by the study of Khan et al. (2007) who showed that high mineral fertilization (NPK) led to significant losses of soil organic carbon during 51 years of continuous maize cropping at the Morrow plots (Illinois, USA). Even so, the evidence for changes in SOC due to fertilization with nitrogen is contradictory – especially for agricultural soils, whereas in forest soils nitrogen enrichment seems to suppress carbon loss (Reay et al., 2008). Fog (1988), Henriksen and Breland (1999) and Kuzyakov et al. (2000) give examples for a retardation of SOC decay under nitrogen fertilization, which was termed "negative priming effect" (Kuzyakov et al., 2000). In his review, Fog (1988) found that nitrogen has either a retarding or no effect on microbial activity and SOC decay in the long term. Retardation of decay after nitrogen addition was mainly reported for organic matter with high C/N ratios and high lignin contents. Fog (1988) suggested three explanations for the retardation of decay after nitrogen addition: (I) nitrogen might promote certain types of decomposer microorganisms on the expense of others, (II) nitrogen might block the production of certain enzymes of decomposer microorganisms, (III) amino compounds might condense with polyphenols to form toxic or inhibitory products. Especially the second explanation seems well supported by the literature. Already Keyser et al. (1978) found that the basidiomycetes *Phanerochaete chrysosporium* and *Trametes versicolor* produced lignin-degrading enzymes only when nitrogen levels were low. Similarly, Carreiro et al. (2000) found that the activity of lignin-degrading phenol oxidase declined in response to nitrogen. On the basis of these findings by Keyser et al. (1978), Fog et al. (1988) and Carreiro et al. (2000), recent studies suggested that a retardation of SOC mineralization under nitrogen additions might be due to the retardation of lignin decay (Hagedorn et al., 2003; Foereid et al., 2004). However, a direct effect of nitrogen, or mineral fertilization in general, on lignin decay has not yet been shown in long-term field experiments. Our objective was therefore to test if mineral fertilization might reduce lignin decay over a long time period in a field experiment.

Correspondence to: A. Heim
(alexander.heim@geo.uzh.ch)

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Recently, the study of lignin decay on a molecular level has improved through compound-specific stable isotope analysis of isotopically labeled lignin biomarkers (Dignac et al., 2005; Heim and Schmidt, 2007a). This method provides the opportunity to study the decay of the SOC component lignin in long-term field experiments (Bahri et al., 2006; Hofmann et al., 2009). To achieve this, we tracked ^{13}C-labeled lignin and SOC in an arable soil that is part of a 36-year arable soil field experiment with two mineral fertilization levels.

2 Materials and methods

2.1 Soils and treatments

For our study we made use of archived soil samples from a long-term field experiment of continuous silage maize cropping initiated in 1966 by Giovanni Toderi (Cadriano field experiment, University of Bologna, Italy; 44°32′51″ N, 11°23′56″ E, mean annual temperature 11°C, mean annual precipitation 650 mm). The crops grown on this site previous to the start of the experiment were plants with a C_3-photosynthetic pathway (e.g. wheat, alfalfa, beets), which have a lower natural abundance of the stable carbon isotope ^{13}C in comparison with plants that have a C_4-photosynthetic pathway such as maize. The continuous cropping of maize therefore introduced naturally ^{13}C enriched organic carbon to the soil (Balesdent et al., 1987), which can be used as a label to distinguish new, C_4-derived (= maize-derived) and old, C_3-derived SOC. The experimental soil was classified as Typic Udochrept (USDA, 1975). Gioacchini et al. (2007) provide chemical and physical characteristics of the soil: pH (H_2O) 6.9; soil organic carbon 8.5 g kg^{-1}; total nitrogen 1.1 g kg^{-1}; cation exchange capacity 16.5 cmol$_c$ kg^{-1}; sand 56%, silt 16%, clay 28%. The experiment includes two treatments, (I) conventional mineral fertilization (300 kg N ha^{-1}a^{-1}, 150 kg P ha^{-1}a^{-1}), which will be called "fertilized treatment" in the following and (II) no mineral fertilization (0 kg N ha^{-1}a^{-1}, 0 kg P ha^{-1}a^{-1}), which will be called "non-fertilized treatment". Atmospheric nitrogen (N) deposition ranges between 10 to 25 kg N ha^{-1}a^{-1} in the region (for the time period 1978–1994, Holland et al., 2005), thus the non-fertilized treatment does not represent a total absence of N inputs but rather an input by atmospheric deposition as it might be typical for large parts of Europe (Holland et al., 2005). The fertilization treatment was carried out with half the dose at sowing and the other half at the four-leaf stadium of maize plants.

2.2 Sampling of soil and plant biomass

The experiment was started in 1966 with two replicate plots per treatment. The plots were sampled in the years 1973, 1980, 1985, 1997, 2002 and the samples were archived. As no archived sample of the plots was available for year 1966, we used the sample of 1973 from a simultaneous continuous wheat experiment at the same experimental site to represent the initial conditions (C_3-plant input) in 1966. The soils were sampled after harvest (end of September for maize plots, mid-July for wheat plots) within the plow horizon with an electric auger to a depth of 25 cm until 1992, afterwards to a depth of 35–40 cm. For each plot four sub-samples were taken. The sub-samples were mixed, air-dried, ground and sieved to 2 mm. Plowing depth at the experimental field site was relatively deep (40–50 cm), as it is common in Mediterranean agriculture. Plant samples were collected at plant physiological maturity during harvest, when also total grain and stover yields were recorded. Unfortunately only aboveground plant biomass was sampled and archived, therefore no samples from belowground plant biomass were available for analysis.

2.3 Chemical analysis

Soil and plant samples were analyzed for carbon and nitrogen concentrations with an elemental analyzer (EA, Thermo Electron EA 1110, Germany). Stable carbon isotope composition (δ^{13}C) was determined using an elemental analyzer (EA, Thermo Electron EA 1110, Germany) coupled to a continuous flow-isotope ratio mass spectrometer (CF-IRMS, Delta Plus Thermo Electron, Germany). Lignin was extracted from soil and plant samples by cupric oxide oxidation (Hedges and Ertel, 1982; Goñi and Montgomery, 2000; as adapted by Heim and Schmidt, 2007a), which is a standard method for lignin analysis in soils and sediments. The oxidation products (lignin monomers vanillyl (V), syringyl (S) and cinnamyl (C) phenols) were quantified in the extracts by gas chromatography using a flame ionization detector (GC-FID, Agilent Technologies HP 6890N Plus, USA). The oxidation products are lignin-specific and their sum can therefore be used as an indicator for lignin (VSC lignin). Multiplication with the carbon content of the individual lignin oxidation products yields the amount of lignin carbon (C_{VSC}). The stable carbon isotope composition of the lignin monomers was determined in the extracts by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS, Goñi and Eglinton, 1996; gas chromatograph Agilent Technologies HP 6890N Plus, USA, interface Combustion III, Finnigan Thermoquest, Germany and IRMS MAT 252, Finnigan, Germany). To volatilize lignin monomers in the extracts, the derivatization agent BSTFA/TMCS 99:1 was added 1:1 (vol.) prior to injection into the gas chromatograph. The shift in the isotopic composition due to the addition of trimethylsilyl carbon from BSTFA/TMCS 99:1 was corrected according to the mass balance equation given by Dignac et al. (2005) as described in Hofmann et al. (2009). For further details of the method please see Hofmann et al. (2009) and Heim and Schmidt (2007a). The proportions of old (C_3-derived) and new (C_4-derived) soil organic carbon and lignin were

calculated by adapting the formula established by Balesdent and Mariotti (1996), as suggested by Dignac et al. (2005) and Heim and Schmidt (2007a) (Eq. 1).

$$F_{\text{new}} = \frac{\Delta\ \delta^{13}C_{\text{soils}}}{\Delta\ \delta^{13}C_{\text{plants}}} \quad (1)$$

F_{new} is the fraction of new, C_4-derived SOC or lignin, $\Delta\delta^{13}C_{soils}$ is the difference between the delta values (‰V-PDB; determined by GC-C-IRMS) of SOC or lignin in the soil before and after the conversion to C_4-vegetation, and $\Delta\delta^{13}C_{plants}$ is the difference between the delta values of organic carbon or lignin extracted from the two different kinds of input vegetation (C_3- or C_4-derived). Quantities of C_4-derived SOC or lignin were calculated by multiplication of F_{new} with the SOC or lignin concentration determined by elemental analyses or GC-FID. C_3-derived SOC or lignin is the difference between total SOC or lignin and C_4-derived SOC or lignin. C_3-derived SOC or lignin can only decrease over the course of the experiment because new biomass input is exclusively from the new label, the C_4-vegetation. Therefore C_3-SOC or lignin can be used to describe the decay of old C_3-derived SOC or lignin, with "old" referring to the time before the experiment was started (1966).

2.4 Statistical analysis

In order to estimate change rates, we performed linear regressions of concentrations (lignin carbon C_{VSC}, soil organic carbon SOC) against time. The slope of the regression is the estimator of the change rate. We tested if the change rates were significantly different from zero with a t-test (p=0.05; df=4). To assess if nitrogen fertilization had an effect on C_{VSC} and SOC concentrations, we used a paired t-test for comparing measured concentrations of each date between the two treatments (p=0.05; df=8). Additionally, also with a t-test, we tested if mineral fertilization had a direct effect on the change rates (p=0.05; df=8).

3 Results

3.1 Total soil lignin carbon and soil organic carbon concentrations

Total lignin carbon (C_{VSC}) concentrations in soil ranged between 69 and 132 μg C_{VSC} g^{-1} soil (Fig. 1a) which corresponded to 9.6 and 17.0 mg C_{VSC} g^{-1} SOC (Table 1). Applying linear regressions, we found that C_{VSC} change rates were not significantly different from zero (Table 2: total C_{VSC}), suggesting that total lignin carbon concentrations remained constant during the experiment. Differences in total C_{VSC} concentrations between treatments were not consistent and not significant over time (Fig. 1a, Table 2: total C_{VSC}, fertilization effect). In addition, also the change rates were not significantly different between treatments (Table 2: total C_{VSC}, fertilization effect). From these results we can conclude that mineral fertilization had no effect on total soil lignin carbon concentrations in this field experiment.

Total SOC concentrations in this arable soil were relatively low, ranging between 7.0 and 8.7 mg SOC g^{-1} soil (Fig. 1d). Total SOC concentrations decreased slightly in both treatments over the course of the experiment (Fig. 1d, Table 2: total SOC). Similar to the results for total C_{VSC}, also for total SOC, no significant effects of mineral fertilization (Fig. 1d, Table 2: total SOC, fertilization effect) were observed during 36 years.

3.2 Accumulation of new, C_4-derived lignin carbon and C_4-derived OC in soil

New, C_4-derived (from maize biomass) lignin carbon (Fig. 1b) as well as C_4-derived SOC (Fig. 1e) accumulated slowly but significantly during the 36 years of the experiment (Table 2). This low accumulation rate was likely due to the relatively small amounts of aboveground plant residues returned to the soil with silage maize cropping. A similarly low accumulation rate of C_4-derived organic carbon was found in the silage maize experiment studied by Flessa et al. (2000). While we could show that there was no difference in the accumulation of new, C_4-derived lignin between the fertilization treatments (Table 2, fertilization effect C_4–C_{VSC}), we could also demonstrate that new C_4-SOC was significantly accumulated with mineral fertilization (Fig. 1e; Table 2, fertilization effect C_4-SOC). This result points out potential differences in the retention of new SOC and lignin. The enhanced accumulation of new, C_4-derived SOC was the only significant effect of mineral fertilization on C stocks in the soil of the studied field experiment.

3.3 Decay of old, C_3-derived lignin carbon and C_3-derived OC in soil

During the experiment, no fresh C_3-derived carbon from plant biomass was added to the soil. Thus the pre-existing C_3-derived SOC originating from the start of the experiment (age $\geq$36 years in the sampling year 2002) could be tracked for decay over time. Mineral fertilization had no effect on the decay of neither old, C_3-derived lignin nor C_3-SOC. Concentrations of old, C_3-derived lignin were similar between fertilization treatments (Fig. 1c; Table 1). The rate at which old, C_3-derived lignin carbon was lost from the soil was not affected by mineral fertilization (-1.8 ± 0.5 μg C_3–C_{VSC} g^{-1} soil a^{-1} in the non-fertilized treatment vs. -1.2 ± 0.6 μg C_3–C_{VSC} g^{-1} soil a^{-1} in the fertilized treatment, Table 2). Also old C_3-SOC concentrations seemed to be unaffected by the fertilization treatment (Fig. 1f). From the change rates (Table 2) it can be concluded that after 36 years on average about 44% of the initial lignin carbon concentration decomposed in comparison to only 24% of the initial SOC concentration, suggesting

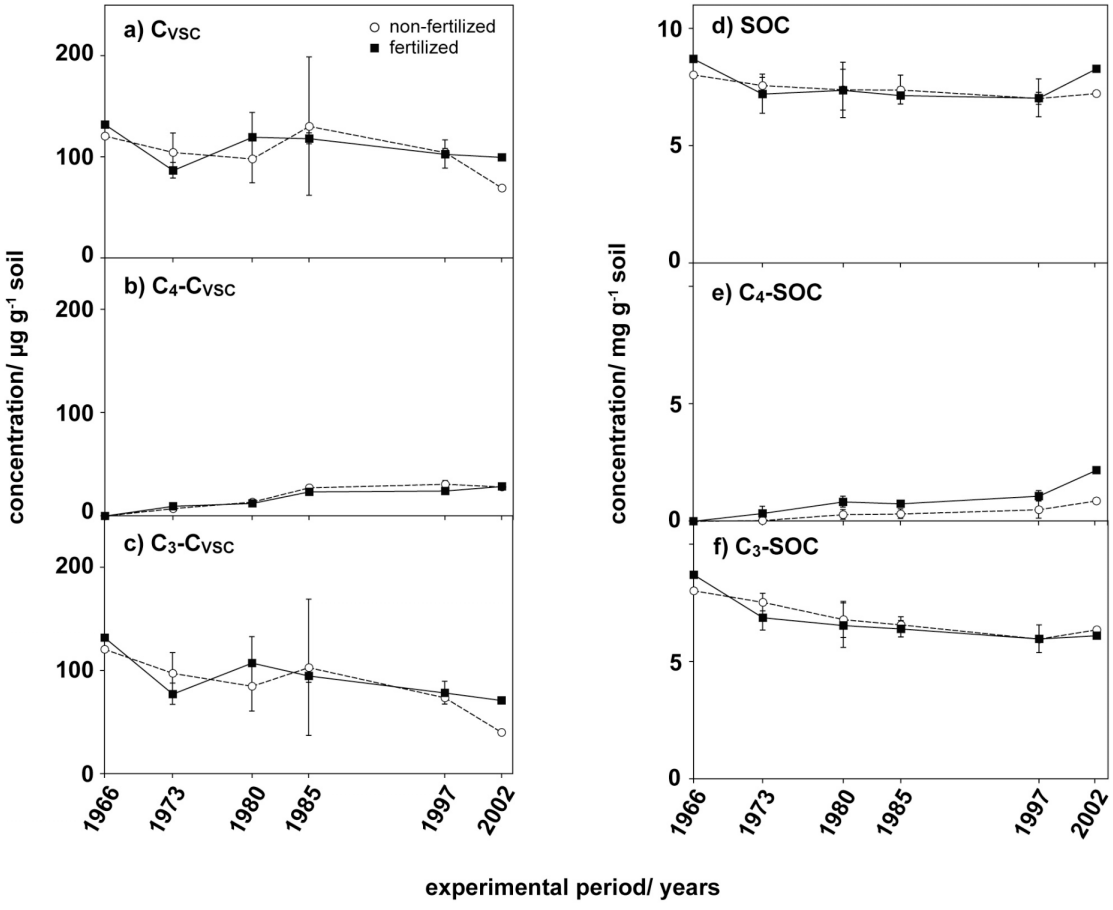

Fig. 1. Lignin carbon (C_{VSC}) and soil organic carbon (SOC) in archived soil of the continuous silage maize fertilization experiment at Cadriano (University of Bologna, Italy). Error bars denote the standard error of two field replicates. The starting year is represented by archived soil samples of the parallel continuous wheat plots.

that decay of old lignin was faster than decay of old bulk SOC.

3.4 Quality of new plant biomass input

Aboveground maize plant material (stover, i.e. stems, leaves, and husks) produced during the fertilization experiment had an average lignin carbon concentration of approximately 28 mg C_{VSC} g^{-1} plant dry weight (Table 3). This corresponded to an average of 63 mg C_{VSC} g^{-1} plant organic carbon (OC), as calculated from Table 3. Dignac et al. (2005) give similar concentrations for maize plant material, also measured after CuO oxidation: 55 mg C_{VSC} g^{-1} plant OC in the leaves (107 C_{VSC} g^{-1} plant OC in the stems and 103 C_{VSC} g^{-1} plant OC in the roots). While we found no significant differences in lignin concentrations between the treatments, OC concentrations were significantly higher in plant material from fertilized vs. non-fertilized plots (Table 3). In Fig. 2 we give quality parameters of aboveground maize biomass such as C/N and C_{VSC}/N ratios (Fig. 2a, b) for which increased values were found in non-fertilized maize plant material, indicating lower degradability. The quality of lignin, as assessed by ratios between lignin monomeric units, remained constant in fertilized plant material, while lignin in maize stover of non-fertilized plants showed relatively high variability (Fig. 2c, d).

4 Discussion

The fertilization treatment of the long-term field experiment was nitrogen and phosphorus (300 kg N ha^{-1}a^{-1}, 150 kg P ha^{-1}a^{-1}) in combination. All possible effects of the fertilization treatment on the decay of SOC and lignin (enhancing, retarding, no effect) would thus be due to both nutrients. However, the focus of our study was on the effect of nitrogen fertilization on lignin decay because nitrogen was suggested to reduce enzymatic lignin decay by Keyser et al. (1978), Fog et al. (1988), Carreiro et al. (2000), Hagedorn et al. (2003), Foereid et al. (2004). Phosphorus has not been

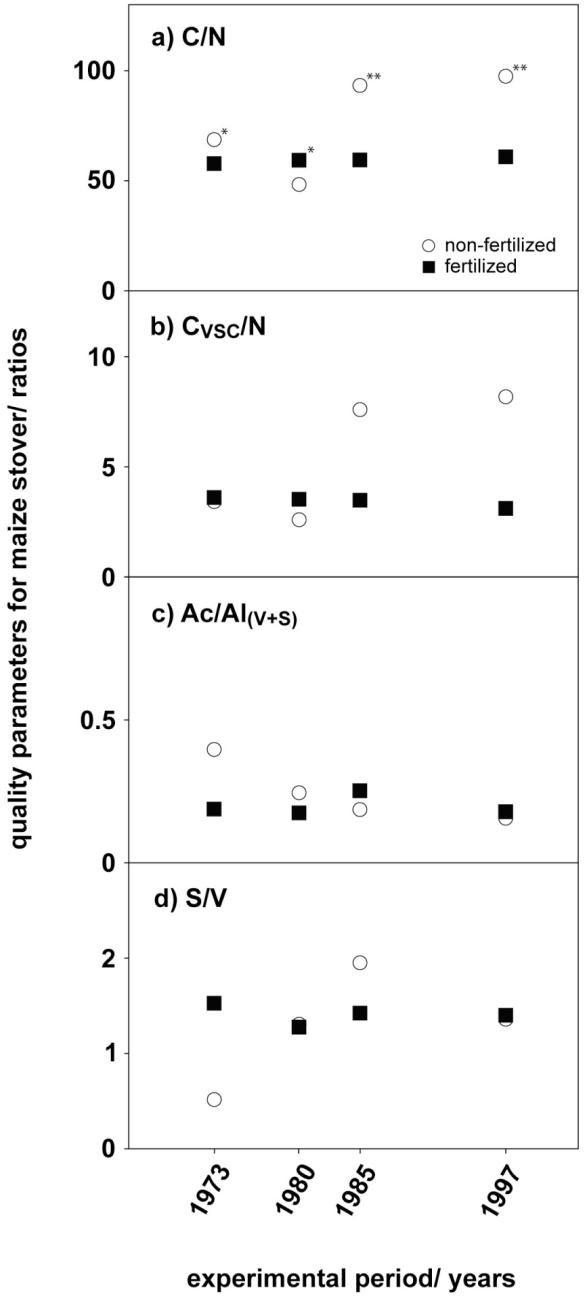

Fig. 2. Quality parameters for aboveground maize plant material (stover) of the continuous silage maize fertilization experiment at Cadriano (University of Bologna, Italy). Maize stover quality of non-fertilized plants showed relatively high variability, whereas the quality of fertilized plant material remained constant. Differences in means could statistically be proven for C to N ratios **(a)** for which n=3 analytical replicates existed. Levels of significance: $^{*}P < 0.05$ significant, $^{**}P < 0.01$ very significant. For parameters on the state of lignin decomposability **(b–d)** only one analytical replicate existed.

suggested in this context so far. We found only few studies on the effect of phosphorus on SOM decay. For an 18-year field experiment Han et al. (2006) show a smaller net loss of SOC when N+P were fertilized, instead of N alone. The field experiment of which we used archived soil samples was originally designed as a long-term maize cultivation experiment with a conventional agricultural fertilization treatment. We accepted the compromise that not only nitrogen but also phosphorus was fertilized because we were interested in this experiment for the unique long-term natural ^{13}C-labeling in combination with the fertilization treatment. Additionally, nitrogen fertilization alone in a long-term experiment might have induced phosphorus limitation both for maize plants as well as for microbial communities.

4.1 Does mineral fertilization reduce the decay of old lignin?

Our initial hypothesis that mineral fertilization reduces lignin decay for the studied long-term field experiment has to be rejected as the fertilization treatment did neither significantly affect lignin carbon concentrations (Fig. 1a), nor the decay of C_3-labeled old lignin carbon (Fig. 1c) in the soil over 36 years. Variations in the quality of plant lignin have been suggested to alter its degradability (Bahri et al., 2006). The absence of a fertilization effect on lignin decay is therefore in line with the constant quality of lignin inputs (Fig. 2c, d), which we assessed by comparing monomer abundances as introduced by Hedges and Mann (1979). Since we did not detect a fertilization effect on C_3-SOC decay during 36 years, we could not test if lignin might be a cause for retardation of C_3-SOC decay as it had been suggested by Fog (1988). We suggest that in those experiments that found retarding effects on SOC decay by mineral fertilization, lignin concentrations should be measured before such an effect can be attributed to lignin. Alternatively, as done by Keeler et al. (2009), studying the activity of lignin-degrading enzymes (phenol oxidase, peroxidase) might provide the results independently from analysis of lignin concentrations.

4.2 Why do we not find fertilization effects on decay in this field experiment?

One reason for not detecting significant differences between the mineral fertilization treatments might be the fact that field conditions were influenced by atmospheric nitrogen deposition (Holland et al., 2005). With field conditions, no total absence of nitrogen input can be achieved in the way it could be simulated in laboratory or semi-field experiments as conducted by Keyser et al. (1978), Magill and Aber (1998), Carreiro et al. (2000), Hagedorn et al. (2003) and Foereid et al. (2004). The difference between nitrogen levels might not have been large enough in the studied field experiment to induce significant differences in decay dynamics. This is supported by the findings of Henriksen

Table 1. Lignin carbon concentrations in SOC, carbon to nitrogen ratios (C/N) and lignin carbon to nitrogen ratios (C_{VSC}/N) of soil samples from two nitrogen fertilization treatments of the Cadriano continuous silage maize fertilization experiment (University of Bologna, Italy). Soil samples are from the ploughing horizon (0–ca. 45 cm), sampling depth was 25 cm in 1973, 1980, 1985 and 40 cm in 1997 and 2002. Results are given as the mean with the standard error of two field replicates.

Treatment	Crop	Sample year	Lignin carbon (C_{VSC}) /mg g^{-1} SOC	C_4-lignin carbon (C_4-C_{VSC})	C_3-lignin carbon (C_3-C_{VSC})	C/N	C_{VSC}/N
non-fertilized	Wheat	1973	15.0±n.a.[a]	0	15.0±n.a.[a]	7.1±n.a.[a]	0.11±n.a.[a]
non-fertilized	Maize	1973	13.7±1.9	1.0±0.2	12.8±2.1	7.1±0.4	0.10±0.02
		1980	13.1±1.7	1.8±0.3	11.3±2.0	8.6±1.0	0.11±0.03
		1985	17.0±7.8	3.7±0.0	13.3±7.8	8.0±0.9	0.14±0.07
		1997	14.9±1.1	4.4±0.7	10.5±0.4	7.4±0.3	0.11±0.00
		2002	9.6±n.a.[a]	3.9±n.a.[a]	5.5±n.a.[a]	7.7±n.a.[a]	0.07±n.a.[a]
Change rate[b]/a^{-1}			−0.1±0.1	0.1±0.0	−0.2±0.1	0.1±0.2	0.00±0.01
P change rate[c]			0.376 n.s.	0.007 **	0.028 *	0.677 n.s.	0.553 n.s.
fertilized	Wheat	1973	15.2±n.a.[a]	0	15.2±n.a.[a]	8.1±n.a.[a]	0.12±n.a.[a]
fertilized	Maize	1973	12.3±2.5	1.3±0.2	11.0±2.7	8.0±1.3	0.10±0.01
		1980	16.1±0.7	1.7±0.4	14.4±1.1	8.3±1.6	0.13±0.03
		1985	16.6±1.6	3.3±0.0	13.4±1.6	7.5±0.5	0.12±0.01
		1997	14.6±0.3	3.5±0.0	11.1±0.3	8.1±1.2	0.12±0.02
		2002	12.0±n.a.[a]	3.5±n.a.[a]	8.6±n.a.[a]	9.6±n.a.[a]	0.12±n.a.[a]
Change rate[b]/a^{-1}			0.0±0.1	0.1±0.0	−0.1±0.1	0.3±0.2	0.00±0.00
P change rate[c]			0.607 n.s.	0.008 **	0.088 n.s.	0.259 n.s.	0.906 n.s.
Fertilization effect:							
P paired t-test[d]			0.457 n.s.	0.191 n.s.	0.312 n.s.	0.178 n.s.	0.256 n.s.
P t-test for change rates[e]			0.692 n.s.	0.435 n.s.	0.428 n.s.	0.542 n.s.	0.553 n.s.

[a] No replicate sample available.
[b] Slope of linear regression.
[c] Probability of error. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.
[d] Pairs are the results of treatments for individual sample years; tests the effect of mineral fertilization on the variable.
[e] Tests if mineral fertilization had an effect on the change rates.

and Breland (1999) who showed that mineralization might be retarded only at rather high nitrogen concentrations. According to the model proposed by Schimel and Weintraub (2003), it requires low amounts of N to maintain a maximal rate of microbial decomposition. These results are supported by Keeler et al. (2009), who, in their recent paper on the topic of microbial enzyme activity and OC decomposition, did not find an effect of nitrogen fertilization on lignin degrading enzyme activity in grassland and forest ecosystem field plots. In summary, it becomes clear that, although lab experiments are essential for studying mechanistic relationships, they should be complemented by field experiments. In addition to direct, mechanistic effects which might be predictable from lab studies, the relevant actual effect under field conditions on the total agro-ecosystem is potentially additionally controlled by feedback effects such as a preferential microbial decomposition of fresh maize biomass input instead of older soil organic carbon moieties.

A second reason for not detecting significant differences might be the fact that in this long-term field experiment the fertilization treatment was nitrogen and phosphorus (300 kg N $ha^{-1}a^{-1}$, 150 kg P $ha^{-1}a^{-1}$) in combination, and not nitrogen alone as in previous laboratory or semi-field experiments (Keyser et al. (1978), Magill and Aber (1998), Carreiro et al. (2000), Hagedorn et al. (2003) and Foereid et al. (2004)). Nitrogen fertilization alone in a long-term experiment might have induced phosphorus limitation both for maize plants as well as for microbial communities, which would have been an undesirable side effect and is therefore avoided in conventional agricultural practice. Also the

Table 2. Results of linear regression for organic carbon and lignin carbon concentrations in soil samples of the Cadriano continuous silage maize fertilization experiment (University of Bologna, Italy).

Treatment	total SOC	C_4-SOC	C_3-SOC	total C_{VSC}	C_4-C_{VSC}	C_3-C_{VSC}
non-fertilized						
Change rate[a]/μg g^{-1} soil a^{-1}	−22.2±6.0	22.6±3.4	−51.8±11.2	−0.9±0.6	0.9±0.2	−1.8±0.5
P change rate[b]	0.021 *	0.003 **	0.010 *	0.205 n.s.	0.008 **	0.018 *
fertilized						
Change rate[a]/μg g^{-1} soil a^{-1}	−9.9±25.1	50.9±10.7	−60.7±21.0	−0.5±0.6	0.8±0.1	−1.2±0.6
P change rate[b]	0.715 n.s.	0.009 **	0.045 *	0.452 n.s.	0.004 **	0.106 n.s.
Fertilization effect:						
P paired t-test[c]	0.443 n.s.	0.031 *	0.574 n.s.	0.531 n.s.	0.302 n.s.	0.416 n.s.
P t-test for change rates[d]	0.646 n.s.	0.036 *	0.719 n.s.	0.588 n.s.	0.644 n.s.	0.452 n.s.

[a] Slope of linear regression.
[b] Probability of error. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.
[c] Tests the effect of mineral fertilization on the variable.
[d] Tests the effect of mineral fertilization on the change rates.

Table 3. Total organic carbon and lignin carbon concentrations in aboveground plant material from two nitrogen fertilization treatments of the Cadriano continuous silage maize fertilization experiment (University of Bologna, Italy) and corresponding yield data for maize grain and stover (stem and leaves). Results are given as the mean with the standard error of two field replicates, if available.

Treatment	Crop	Sample year	Organic carbon (OC) /mg g^{-1} dry weight	Lignin carbon (C_{VSC}) /mg g^{-1} dry weight	/mg g^{-1} OC	Grain yield /t dry weight ha^{-1}	Stover yield /t dry weight ha^{-1}
non-fertilized	Wheat	1980	389.0±2.0	34.0±0.6	87.4±10.1	n.a.[a]	n.a.[a]
non-fertilized	Maize	1973	406.7±7.9	20.3±n.a.[a]	49.9±n.a.[a]	3.2±n.a.[a]	3.1±n.a.[a]
		1980	379.0±0.4	20.4±n.a.[a]	53.9±n.a.[a]	0.8±n.a.[a]	1.9± n.a.[a]
		1985	397.1±3.2	32.4±n.a.[a]	81.5±n.a.[a]	2.4±n.a.[a]	4.3±n.a.[a]
		1997	405.1±2.2	34.0±n.a.[a]	84.0±n.a.[a]	0.8±n.a.[a]	1.5±n.a.[a]
		2002	n.a.[a]	33.1±3.4	n.a.[a]	2.5±n.a.[a]	2.3±n.a.[a]
fertilized	Wheat	1980	397.8±6.8	37.7±0.1	94.8±19.6	n.d.[a]	n.d.[a]
fertilized	Maize	1973	421.4±0.1	26.3±n.a.[a]	62.5±n.a.[a]	7.4±n.a.[a]	4.3±n.a.[a]
		1980	415.7±2.8	24.7±n.a.[a]	59.5±n.a.[a]	6.1±n.a.[a]	6.5±n.a.[a]
		1985	424.4±1.3	24.9±n.a.[a]	58.6±n.a.[a]	8.5±n.a.[a]	9.5±n.a.[a]
		1997	431.0±1.5	22.1±n.a.[a]	51.2±n.a.[a]	7.7±n.a.[a]	5.0±n.a.[a]
		2002	n.a.[a]	40.3±3.3	n.a.[a]	6.6±n.a.[a]	6.0±n.a.[a]
Fertilization effect:							
P paired t-test[b]			0.010 **	0.926 n.s.	0.455 n.s.	0.001 ***	0.006 **

[a] No sample or no replicate sample available.
[b] Pairs are the results of treatments for individual sample years; tests the effect of nitrogen fertilization on the variable. P Probability of error. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.

long-term experiment discussed by Khan et al. (2007) received a full conventional fertilization treatment and was not able to observe a retarding effect of fertilization on SOC decomposition as previous "N-only" experiments had done. This raises the question whether previously observed retarding effects of N on decomposition could have been indirect effects by inducing P limitation in decomposer communities, but this question cannot be answered with our study.

4.3 Estimates of the belowground input of new, maize-derived organic carbon and lignin

According to the review by Amos and Walters (2006) we can assume a net belowground carbon deposition (root biomass and rhizodeposition) of 29 ±13% of shoot (= stover) biomass carbon for maize at physiological maturity. From the data on stover yield in Table 3 we can therefore estimate the belowground carbon deposition was $757 \pm 344\,\mathrm{kg\,OC\,ha^{-1}a^{-1}}$ in the fertilized plots. Taking into account that root/shoot ratios increase by 41.6 ±8.6% under nitrogen deficiency (Amos and Walters, 2006), net belowground carbon deposition in the non-fertilized plots was $421 \pm 242\,\mathrm{g\,OC\,ha^{-1}a^{-1}}$ (calculated from Table 3). With a lignin concentration of 10.3% in organic carbon of maize roots (Dignac et al. 2005) as a basis, the belowground lignin carbon deposition from belowground maize biomass could be estimated as $79\,\mathrm{kg\,C_{VSC}\,ha^{-1}\,a^{-1}}$ in the fertilized plots versus $44\,\mathrm{kg\,C_{VSC}\,ha^{-1}\,a^{-1}}$ in the non-fertilized plots (calculated from Table 3). From these calculations, we suggest a doubled net belowground carbon and lignin input in fertilized plots in comparison to non-fertilized plots. Information on belowground carbon input is important because in this experiment the maize crop was harvested as silage (all aboveground plant parts were removed from the field) and only roots and stubbles remained as effective C_4-labeled maize biomass input.

4.4 Mineral fertilization might have enhanced the decay of new lignin

The increased biomass production in the fertilized treatment can be related to the actual SOC accumulation in the soil, so that conclusions on decay of fresh biomass input can be drawn. While we estimated that the input of total belowground maize biomass carbon approximately doubled in the fertilized plots (Sect. 4.3), we also measured a doubled actual accumulation of C_4-derived SOC (Table 2, change rates, C_4-SOC; Fig. 1e). This result suggests that the decay of fresh maize-derived organic carbon was similar in the two fertilization treatments. In contrast, C_4-lignin accumulation (Fig. 1b, Table 2, C_4–C_{VSC}) was not matching the estimated doubled input (Sect. 4.3) under mineral fertilization, proposing that decay of lignin from fresh biomass input might have been enhanced in fertilized plots. This interpretation relies on the assumption that lignin concentrations in root derived organic matter were not affected by fertilization, similarly to what we could show for aboveground plant material (Table 3). We propose that decay of new lignin might be enhanced by mineral fertilization in contrast to no effect on decay of old lignin. This however would contrast studies stating that it is rather the limitation of nitrogen that increases litter decomposition (Craine et al., 2007). To explain the opposing findings it would be necessary to analyse lignin concentrations in experiments where clear fertilization effects (either enhancement or retardation) on SOC were found. In those cases lignin concentrations might help to explain the effect on total SOC.

4.5 Lignin decay was faster than SOC decay

Our results support earlier evidence that lignin might decompose faster than bulk SOC (Kiem and Kögel-Knabner, 2003). With 56% of the initial C_3-lignin carbon and 76% of the initial C_3-SOC still measurable in the soil after 36 years, we found an overall slow mineralization of C_3-SOC and C_3-lignin in the studied long-term field experiment. This result is in accordance with our finding in a previous study where we proposed that about two thirds of the initial C_3-lignin was stabilized during 18 years (Askov continuous silage maize, Hofmann et al., 2009). Similarly, 64% of C_3-SOC was detected after 23 years in another arable soil experiment (Boigneville, Heim and Schmidt, 2007a). A possible explanation for the overall slow mineralization of lignin and SOC could be an intensive cropping of the soils before the experiments were initiated. Intensive cropping, including aeration of the soil with ploughing, could have resulted in mineralization of most old C_3-carbon, leaving only carbon that was already stabilized. This suggestion is supported by the result from a field experiment where only 28% of initial C_3-lignin was stabilized over the duration of 23 years (Rotthalmünster, Heim and Schmidt, 2007b). The fast mineralization found in this study might be related to the fact that the arable soil had been established on former cultivated grassland, and thus might not have been in equilibrium for carbon stocks in contrast to arable soils that have been intensively worked for decades.

5 Conclusions

In a natural agro-ecosystem, decay of old, stabilized lignin was less sensitive to mineral fertilization than previously suggested. Mineral fertilization neither retarded nor enhanced the decay of old, C_3-labeled SOC or lignin over a period of 36 years.

Mineral fertilization might have had an effect on new, non-stabilized lignin carbon. For fresh C_4-labeled biomass there were indications for SOC and lignin decay being decoupled. Decay of C_4-labeled lignin from fresh biomass might have been enhanced by mineral fertilization, whereas decay of C_4-SOC was not. Mineral fertilization might thus have neither

an effect on already stabilized old SOC or lignin nor on new SOC, but might affect new, non-stabilized lignin.

5.1 Author contributions

The study was proposed and supervised by A. Heim, M. W. I. Schmidt and A. Miltner. P. Gioacchini provided the samples and conducted the EA-IRMS measurements. M. Gehre supervised the GC-C-IRMS analyses. Lignin extraction, GC-FID, GC-C-IRMS, data analysis and paper writing was completed by A. Hofmann with contributions of all co-authors.

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