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

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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 nitrogen fertilization 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 with two mineral nitrogen fertilization levels. We could show that nitrogen 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 nitrogen fertilization than previously suggested. However, for fresh biomass there were indications that lignin decay might have been enhanced by nitrogen fertilization, whereas decay of SOC was unaffected. A retardation of SOC decay due to nitrogen addition, as found in other experiments, can therefore only be explained by effects on lignin decay, if lignin was actually measured.

1 Introduction

Nitrogen fertilization in agriculture, and atmospheric nitrogen deposition resulting from fossil-fuel combustion add large amounts of nitrogen to the world's ecosystems, accelerating the global nitrogen cycle (Gruber and Galloway, 2008). Available soil nitrogen is a limiting factor for plant biomass production. Nitrogen fertilization consequently might enhance plant growth and could thus reduce the atmospheric CO_2 level through an increased photosynthetic carbon fixation (Reich et al., 2006). However, soil microorganisms compete for soil nitrogen with plants. Hence, if nitrogen is deficient, its addition 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 (mineralization) of soil organic matter. This hypothesis has recently been supported by the study of Khan et al. (2007) who show that high mineral nitrogen fertilization led

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6, 1657–1675, 2009

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 under nitrogen addition 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. 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 recalcitrant organic matter with a high C/N ratio and high lignin contents. Fog (1988) proposed that the effect of adding nitrogen may depend on the microflora present and suggests 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 on lignin decay has not yet been shown in long-term experiments.

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 directly test the long-term effect of mineral nitrogen fertilization on the decay of the SOC component lignin. 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 nitrogen 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 (Cadriano field experiment, University of Bologna, Italy; 44° 32′51.26″ N, 11°23′55.92″ E). 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%. Climate at the site is temperate with a mean annual temperature of 11°C and a mean annual precipitation of 650 mm. 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 "nitrogen-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 (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). Fertilization in the nitrogen-fertilized 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 is 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 horizont 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

Soils were analyzed for SOC concentrations and stable carbon isotope composition ($\delta^{13}C$) using an elemental analyzer (EA 1110, Thermo Finnigan, Bremen, Germany) coupled to a continuous flow-isotope ratio mass spectrometer (CF-IRMS; Delta Plus, Thermo Electron, Bremen, Germany).

Lignin was extracted from soil and plants 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 (vanillyl (V), syringyl (S) and cinnamyl (C) phenols) were quantified in the extracts by gas chromatography using a flame ionization detector (GC-FID; HP

6890N Plus, G1530N, Agilent Technologies, Wilmington DE, USA). The oxidation products are lignin-specific and their sum can therefore be used as an indicator for lignin (VSC lignin). The stable carbon isotope composition of lignin was determined in the extracts by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS; Goñi and Eglinton, 1996; gas chromatograph HP 6890N Plus, Agilent Technologies, Wilmington DE, USA, interface Combustion III, ThermoQuest-Finnigan, Bremen, Germany and IRMS MAT 252, Finnigan, Bremen, Germany). To volatilize lignin monomers in the extracts, the derivatisation 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).

$$F_{\text{new}} = \frac{\Delta\ \delta^{13}\text{C}_{\text{soils}}}{\Delta\ \delta^{13}\text{C}_{\text{plants}}} \tag{1}$$

F_{new} is the fraction of new, C_4-derived SOC or lignin, $\Delta\ \delta^{13}\text{C}_{\text{soils}}$ 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 $\Delta\delta^{13}\text{C}_{\text{plants}}$ 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-SOC or lignin can only decrease over the course of the experiment because new biomass input is only 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.

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 nitrogen 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}, Nitrogen effect). In addition, also the change rates were not significantly different between treatments (Table 2: total C_{VSC}, Nitrogen effect). From these results we can conclude that nitrogen fertilization had no effect on total soil lignin carbon concentrations in the 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 in the course of the experiment (Fig. 1d, Table 2: total SOC). Similar to the results for total C_{VSC}, also for total SOC we can show that there are no significant effects of nitrogen fertilization (Fig. 1d, Table 2: total SOC, Nitrogen effect) during 36 years.

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

New, C_4-derived lignin carbon from maize biomass (Fig. 1b) as well as C_4-derived SOC from maize biomass (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 find no difference in the accumulation of new C_4-lignin between the fertilization treatments (Table 2, nitrogen effect C_4-C_{VSC}), we found that the accumulation of C_4-SOC was significantly enhanced with nitrogen fertilization (Fig. 1e; Table 2, nitrogen effect C_4-SOC). This result points out possible differences in the accumulation of C_4-SOC and C_4-lignin. The enhanced accumulation of C_4-derived SOC was the only significant effect of nitrogen fertilization on C stocks in the soil of the studied field experiment.

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

During the experiment, no new 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 ≥36 years in the sampling year 2002) could be tracked for decay over time. Nitrogen 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 2, nitrogen effect C_3-C_{VSC}). Neither was the change rate of old C_3-derived lignin carbon affected by nitrogen fertilization, as the rates were similar for both treatments (−1.8±0.5 vs. −1.2±0.6, Table 2, C_3-C_{VSC}). Also C_3-SOC concentrations seemed to be unaffected by the fertilization treatment (Fig. 1f; Table 2, C_3-SOC). The change rates predict 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 (Table 2, C_3-C_{VSC}, C_3-SOC). The change rate for C_3-SOC was about 0.7% of the year 0 value (8.0 mg g^{-1} soil, Fig. 1; Table 2) and about 1.5% of the year 0 value (120 μg g^{-1} soil, Fig. 1; Table 2) for C_3-C_{VSC}, suggesting that C_3-lignin decay was faster than bulk C_3-SOC decay. It is remarkable that in both treatments a substantial proportion of old lignin (56%) and old SOC (76%) did not decompose during the experimental period of 36 years and thus must have been stabilized in the soil.

3.4 Plant biomass

Aboveground maize plant material (stover, i.e. stems, leaves, and husks) had an average lignin carbon concentration of 2.8%, which means that 6.3% of aboveground plant organic carbon were lignin carbon (Table 3, C_{VSC}). We found no significant differences in lignin concentrations between plant material from nitrogen-fertilized and non-fertilized plots. The concentrations are in accordance with the values given for maize stems and leaves by Dignac et al. (2005). Nitrogen-fertilized plots produced on average three times more aboveground maize biomass (grain and stover) in comparison to non-fertilized plots (5 vs. 14 t dry weight ha^{-1}, calculated from Table 3). When assuming a net belowground carbon deposition (root biomass and rhizodeposition) of 29±13% of shoot (=stover) biomass carbon for maize at physiological maturity (Amos and Walters, 2006), we can estimate the belowground carbon deposition was 757±344 kg OC ha^{-1} a^{-1} in the nitrogen-fertilized plots (calculated from Table 3). Taking into account that root/shoot ratios inrease by 41.6±8.6% under nitrogen deficiency (Amos and Walters, 2006), net belowground carbon deposition in the non-fertilized plots was 421±242 kg 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 44 kg C_{VSC} ha^{-1} a^{-1} in the non-fertilized plots versus 79 kg C_{VSC} ha^{-1} a^{-1} in the nitrogen-fertilized plots (calculated from Table 3). From these calculations, we can propose a doubled net belowground carbon and lignin input in nitrogen-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 Discussion

4.1 Nitrogen fertilization had no effect on C_3-lignin and C_3-SOC decay

From our results we can state that nitrogen fertilization did neither affect total SOC or lignin concentrations in the studied long-term field experiment (Fig. 1a, d), nor the decay of C_3-labeled old SOC or lignin (Fig. 1c, f). Our result thus adds to the controversy about nitrogen effects on SOC in agricultural systems by suggesting no change in SOC under nitrogen enrichment (Reay et al., 2008). Since there was neither an enhancement nor a retardation effect on C_3-SOC decay detected over 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) and supported by Keyser et al. (1978), Magill and Aber (1998), Carreiro et al. (2000), Hagedorn et al. (2003) and Foereid et al. (2004). One reason for not detecting significant differences between the nitrogen fertilization treatments might be the fact that field conditions are 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 studies. The difference between nitrogen levels might therefore not have been large enough in the studied experiment to induce significant differences in decay dynamics. This is supported by the findings of Henriksen and Breland (1999), who showed that mineralization might be retarded only at rather high nitrogen concentrations. Additionally, it has to be considered that with a field experiment we rather measure the relevant actual effect of nitrogen on the total agro-ecosystem and not merely the direct effect of nitrogen on decay of SOC or lignin. In the field feedback effects might occur such as a preferential microbial decomposition of fresh maize biomass input instead of older SOC and lignin.

4.2 Nitrogen fertilization might have increased the decay of C_4-lignin carbon

The increased biomass production in the nitrogen-fertilized treatment can be compared to the actual SOC accumulation in the soil, and conclusions on decay of fresh biomass input might be drawn. While we estimated that the input of total belowground maize biomass carbon approximately doubled in the nitrogen-fertilized plots (Sect. 3.4), we measured that the actual accumulation of C_4-derived SOC was doubled as well (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 fertilization treatments. In contrast, C_4-lignin accumulation (Fig. 1b, Table 2, C_4-C_{VSC}) was not matching the estimated doubled input (Sect. 3.4) under nitrogen fertilization, proposing that decay of lignin from fresh biomass input might have been enhanced in nitrogen-fertilized plots. This interpretation relies on the assumption that lignin concentrations in root derived organic matter are not affected by nitrogen fertilization, like we could show for aboveground plant material (Table 3). We propose that decay of fresh lignin might be enhanced by nitrogen in contrast to no effect on decay of old lignin. This however would contrast studies stating that it is rather nitrogen limitation 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 nitrogen effects (either enhancement or retardation) on SOC were found. In those cases lignin concentrations might help to explain the effect on total SOC.

4.3 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

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

2. For fresh C_4-labeled biomass there are indications that SOC and lignin decay were not closely linked. Decay of C_4-labeled lignin from fresh biomass might have

been enhanced by nitrogen fertilization, whereas decay of C_4-SOC matched the accumulation, reflecting the higher biomass production with nitrogen fertilization.

3. Retardation or enhancement of SOC decay, as observed in other experiments, can only be explained by effects on lignin decay if lignin was actually measured in these experiments. Future work should concentrate on studies where an effect of nitrogen on SOC has been observed and systematically analyse lignin in these studies.

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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Table 1. Lignin carbon concentrations in SOC of bulk lignin carbon (C_{VSC}), new maize-derived lignin carbon (C_4-C_{VSC}), old C_3-derived lignin carbon (C_3-C_{VSC}), 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.
nitrogen-fertilized	Wheat	1973	15.2±n.a.[a]	0	15.2±n.a.[a]	8.1±n.a.[a]	0.12±n.a.[a]
nitrogen-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.
Nitrogen 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 for changes. 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 nitrogen fertilization on the variable. [e] Tests if nitrogen fertilization had an effect on the change rates.

Table 2. Results of linear regression for organic carbon and lignin carbon concentrations in 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 *
nitrogen-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.
Nitrogen 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 for changes. 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 nitrogen fertilization on the variable. [d] Tests the effect of nitrogen 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, where 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	1973	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]
nitrogen-fertilized	Wheat	1973	397.8±6.8	37.7±0.1	94.8±19.6	n.d.[a]	n.d.[a]
nitrogen-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]
Nitrogen effect: *P* paired t-test[d]			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 for changes. Levels of significance: $P>0.05$ not significant n.s., $P<0.05$ significant *, $P<0.01$ very significant **, $P<0.001$ highly significant ***.

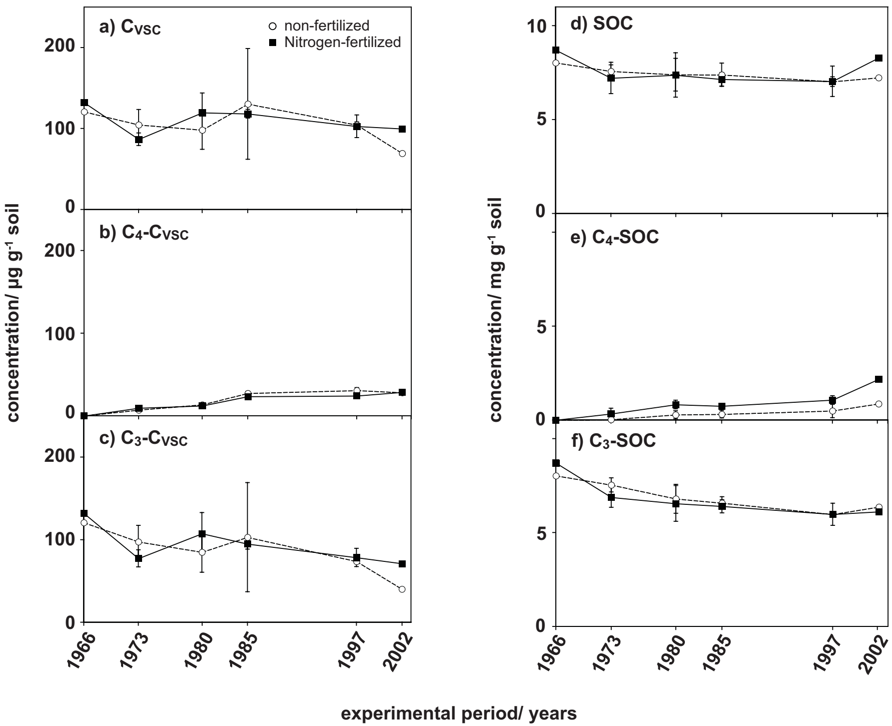

Fig. 1. Lignin carbon (C_{VSC}) and soil organic carbon (SOC) in archived soil of the continuous silage maize fertilization experiment at Cadriano (University of Bologna, Italy). Error bars denote the standard error of two field replicates. The starting year 1966 is represented by an archived soil sample of the parallel continuous wheat plots.