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# Does maize and legume crop residue mulch matter in soil organic carbon sequestration?

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ABSTRACT

Soil management techniques, such as mulching, are used to enhance soil organic carbon sequestration. However, we demonstrate that the potential of crop residue mulching to increase soil organic carbon (SOC) sequestration varies by cropping system and soil type in Austrian agricultural soils. Effects of mulch (as harvested crop residues applied at 1.0 t C $ha^{-1}$) on soil and microbial carbon (C) and nitrogen (N) and soil $\delta^{13}C$ were measured in an Austrian Cambisol field experiment with sole maize or vetch or vetch-maize rotation cropping systems after five years with or without mulching to elucidate how SOC is affected. The direct role of mulch on SOC in different soil types was also investigated in a similar greenhouse mesocosm study with controlled moisture using the same Cambisols and an Austrian agricultural Chernozem. Only sole maize cropping in the field experiment resulted in higher SOC with mulching and when legumes were included in a legume-maize rotation SOC did not improve. Mulching in the field experiment only resulted in higher SOC in the top 0–5 cm of soils with sole maize cropping (by 22%) compared to soils without mulch. Although mulch did not increase SOC in vetch-maize rotation, the $\delta^{13}C$ of SOC was less negative with mulch indicating larger C contribution from maize than vetch mulch. After four years of annual soybean-maize rotation in the mesocosm experiment, no significant differences in SOC were observed in Cambisols with or without mulch. Again, $\delta^{13}C$ of both soil types was less negative with mulching indicating a larger C contribution from maize than soybean mulch. No relationships between microbial biomass C and N and SOC were observed in either experiment and only soil N concentration was positively correlated with SOC. Together these studies indicate that maize can increase SOC when crop residues are applied in Austrian Cambisols but that inclusion of legume production and legume mulch in rotation can mute these benefits.

## 1. Introduction

Mulching, such as with crop residue retention, is considered a sustainable soil management technique that prevents soil erosion, retains water, buffers temperature fluctuations, restores biodiversity increases fertility and improves soil structure- all of which are important for improving plant growth (for review see Erenstein, 2003). Additionally, mulching with crop residues increases soil organic carbon (SOC) and carbon (C) storage thus reducing anthropogenic greenhouse gas emissions to the atmosphere (Smith *et al.*, 2008). These benefits of plant residue retention are of significance in agricultural systems, as roughly 70% of agricultural land around the globe is moderately to highly degraded (Delgado, 2010; FAO, 2011). However, the effectiveness of mulching in improving SOC stocks varies depending on the type of mulch and environment, both of which can affect rates of decomposition and retention (Mando and Stroosnijder, 1999; Ossoml et al., 2001). The benefits of mulching are also often very slow, with no observable improvements even after six growing seasons or longer (Affholder et al., 2009; Kihara *et al.*, 2012).

In agricultural systems, growth of different types of crops can affect rates of SOC sequestration (Zhang et al., 2010) and the type of residue (associated with crop type) applied as mulch can also greatly influence SOC retention. Specifically, it has been proposed that maize crop residues may increase buildup of SOC (Mathew *et al.*, 2017) but that legume crop residues might be more likely to stabilize soil C (Drinkwater et al., 1998). This is because the magnitude of SOC increase depends upon the quantity of organic matter produced and returned to soil (Studdert and Echeverria, 2000; Brandani *et al.*, 2015) as well as the quality of these C sources that can affect microbial activity and decomposition (Balesdent et al., 1988; De Clercq et al., 2015). Model simulations support this theory and indicate that long-term increases in SOC due to crops and crop residues may in fact be higher in cereal-

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legume intercrops compared to sole maize crops (Oelbermann et al., 2017). However, other studies such as a meta-analysis by West and Post (2002) suggest that enhanced rotation complexity from maize to maize-soybean may not result in a significant increase in SOC due to a decrease in residue production and carbon input in maize-soybean rotation compared to continuous maize systems.

Because the effects of mulching vary depending on the type of plant material and environment and often develop slowly, it is important to measure the effectiveness of this sustainable practice thoroughly in different agricultural settings and to evaluate it over longer terms. However, it is difficult to issue a method to evaluate the effectiveness of this soil management technique due to the typically slow development of mulch effects. Isotopic techniques can help elucidate potential long-term effects of mulching before shifts in soil C pools can be observed. For over three decades stable isotopes have been used to evaluate the turnover of C and organic matter in soils (Balesdent et al., 1988; Natelhoffer and Fry, 1988). Studies measuring the isotopic composition of C in soil components at natural abundance levels can elucidate contribution of plant residues and roots to SOC (Spaccini et al., 2000; Jin et al., 2017). Furthermore, soil $\delta^{13}C$ analysis can identify differences in plant contribution of C to soils by different plants, particularly when there is a shift in plant composition between plants with $C_3$ photosynthesis and $C_4$ photosynthesis (Barthès et al., 2004; Sisti et al., 2004; Christensen et al., 2011). Because $C_3$ plants have a $\delta^{13}C$ of approximately −28‰ as opposed to $C_4$ plants that have a $\delta^{13}C$ of approximately −14‰ (O'Leary and Osmond, 1988), an increase or decrease in soil $\delta^{13}C$ that follows a change in $C_3$ and $C_4$ plant composition can be indicative of each plant type's contribution to soil C.

We used these isotopic techniques in combination with classic soil analytical techniques to elucidate how SOC levels are affected by maize and legume residue mulch. This was done by evaluating the effects of mulch on soil and microbial C and N and soil $\delta^{13}C$ in Austrian field and greenhouse studies with maize and legume monocrops as well as rotation cropping. We hypothesized that mulching would improve SOC and N status, since the two are tightly linked (Cleveland and Liptzin, 2007), in all cropping systems but that more SOC would be stored in soils with mulch and legume-maize rotation due to the relatively higher production of organic material by maize as well as higher quality, low C:N residues produced by legumes. We also hypothesized that the soil with lower organic matter and a higher potential to accumulate C in the mesocosm study would improve SOC content with mulching more than the soil with higher organic matter. Furthermore, we predicted that mulching in legume-maize rotation would result in a soil $\delta^{13}C$ more similar to legumes since it has a higher quality residue that may be more stabilizing than maize mulch.

## 2. Materials and methods

### 2.1. Field experiment

The field experiment was conducted at the AGES (Austrian Agency for Health and Nutritional Security) agricultural site located in Ruprechtshofen, Austria (48°08′N,15°13′E). The site is dominated by $C_3$ native grasses, has a mean annual precipitation range of 600–700 mm (Kilk and Konecny, 2013) and the soil can be classified as a Eutric Cambisol (IUSS-WRB, 2014) with a pH of 6.7, no inorganic C and a bulk density in the top 15 cm of 1.31 g $cm^{-3}$ (for methods on measuring bulk density, see IAEA, 2016). Our experiment was established in March of 2012 in three experimental blocks (10 × 12 $m^2$ per block) (Fig. 1). Initial SOC and total N in soils from 0 to 5 and 5–15 cm for each block is described in Table 1 and SOC $\delta^{13}C$ was approximately -27.4‰. Each of the three blocks contained six plots (6.25 $m^2$ per plot); one with an annual sole vetch monocrop with mulch, an annual sole maize monocrop with mulch and an annual vetch-maize rotation crop with mulch and another three plots with the same cropping systems without mulch.

Prior to each planting, soils were prepared with a rotary cultivator to a depth of approximately 10 cm. Annual vetch (*Vicia sativa*, a $C_3$ legume species) was planted in April and maize (*Zea mays* L., a $C_4$ species) was planted in June. Vetch was grown in plots at 120 kg seed $ha^{-1}$ and maize was grown at 102 kg seed $ha^{-1}$. Phosophorous (P) and potassium (K) fertilizer was applied at the time of vetch planting at 33 kg P $ha^{-1}$ (as triple superphosphate) and 95 kg K $ha^{-1}$ (as $K_2SO_4$) and N, P and K fertilizer was applied at 120 kg N $ha^{-1}$ (as urea), 43 kg P $ha^{-1}$ and 190 kg K $ha^{-1}$ at the time of maize planting. Fertilizer application was kept similar to that typically used at the agricultural site.

Vetch was harvested before maize planting in June and maize was harvested at the end of September. At each harvest, vetch was cut with a sickle bar mower and maize cobs were first removed by hand and maize was cut with sickles. Fresh weight of harvested leaf and stem material (crop residues) was measured on site before being mechanically chopped to make mulch (Viking GB 460 C; STIHL Company m.b.H., Austria). Fresh vetch and maize mulch was applied to mulch treated soils immediately after harvest at 9.7 kg (2.7 t dry mulch $ha^{-1}$, or 1.0 t C $ha^{-1}$ and 0.7 t N $ha^{-1}$) and 6.6 kg (2.2 t dry mulch $ha^{-1}$, or 1.0 t C $ha^{-1}$ and 0.3 t N $ha^{-1}$) per plot, respectively, in their associated treatments. Soils that were not treated with mulch were left bare during fallow periods.

A subsample of annual harvested mulch was also dried at 65 °C to record the dry weight to calculate plant yield (tons dry biomass per hectare) and was then submitted for C and N analysis. Mean C and N concentration of maize and vetch mulch was 0.44 ± 0.002 g C and 0.012 ± 0.0004 g N and 0.38 ± 0.008 g C and 0.025 ± 0.0001 g N per gram of mulch, respectively. In 2017, after five years of crop production with or without mulch treatments, five 2.5 cm diameter soil cores were collected from each plot and 0–5 cm and 5–15 cm soils were placed on ice for transport back to the laboratory for further soil sample processing and analysis of C and N.

### 2.2. Greenhouse mesocosm experiment

A similar study as that described in the field experiment was performed in a controlled greenhouse setting to determine the direct effect of mulch on soils with consistent soil moisture. This was done using two soils, the Cambisol from the field experiment as well as another Austrian Chernozem with relatively higher organic matter concentration to determine if soil response to mulch treatments was consistent regardless of the quality of organic matter. The second soil was collected from the International Atomic Energy Agency Laboratories experimental fields in Seibersdorf, Austria (47°58′ N, 16°30′ E). The site was also originally dominated by $C_3$ native grasses, has a mean annual precipitation range of 400–500 mm (Kilk and Konecny, 2013) and the soil is characterized as a haplic Chernozem (IUSS-WRB, 2014) with a pH of 6.8, and inorganic C as $CaCO_3$.

Topsoils from 0 to 30 cm were collected in 2012 from the Cambisol and Chernozem field sites, homogenized and sieved to 4 mm. Mesocosms with a 49 cm diameter were then filled with soils to a height of 70 cm with a bulk density of 1.4 g $cm^{-3}$ and were set up in three blocks with four mesocosms per block in a greenhouse. Each mesocosm contained either the Cambisol or Chernozem soil and was used to produce an annual soybean-maize rotation with or without mulch, with 3 replicates per soil type and mulch treatment following a randomized complete block design (Fig. 2). Soybean (*Glycine max* (L.) Merr, a $C_3$ legume species) and maize (*Zea mays* L., a $C_4$ species) were grown annually in rotation with 16 soybean planted in April and one maize planted in June in each mesocosm. Due to logistical reasons, we only focused on a legume-maize rotation and did not include mesocosms with sole maize or legume monocrops. Additionally, soybean was used instead of vetch due to the smaller size of the mesocosms compared to field plots. Similar to the field experiment, fertilizer was applied when soybeans were first planted at 50 kg P $ha^{-1}$ (as triple superphosphate) and at 120 kg K $ha^{-1}$ (as $K_2SO_4$) and at 43 kg P $ha^{-1}$, 190 kg K $ha^{-1}$ and 120 kg N $ha^{-1}$ (as urea) at the time of maize planting. Soil moisture was

**Fig. 1.** Mature maize plants in experimental plots at Grabenegg, Austria. In 2012, the field experiment was established on a Cambisol soil, following a randomized complete block design including three blocks.

monitored with soil probes to 15 cm depth and was kept at 15–20% daily to maintain similar moisture across soils and mulch treatments. Soil disturbance was minimal with no-till and hand seeding and harvesting.

Soybean was harvested before maize planting in June and maize was harvested in September. At the time of each harvest, the fresh weight of leaf and stem material was recorded per mesocosm and then material was combined by treatment and hand cut into approximately 2 cm pieces for mulch (similar to the size of mechanical chopping in the field experiment). Cut mulch was then dried at 65 °C. Dry weight was recorded to calculate plant yield (tons dry biomass per hectare) and a subsample was submitted for C and N analysis. Mean C and N concentration of maize and soybean mulch was 0.43 ± 0.002 g C and 0.019 ± 0.0005 g N and 0.44 ± 0.002 g C and 0.038 ± 0.001 g N per gram of mulch, respectively. Dried mulch was reapplied to mesocosms with the mulch treatment at 40 g per mesocosm (2 t dry mulch $ha^{-1}$, or 0.9 t C $ha^{-1}$ and 0.08 t N $ha^{-1}$ for soybean mulch and 0.9 t C $ha^{-1}$ and 0.04 t N $ha^{-1}$ for maize mulch). Mesocosms without the mulch treatment were left with bare soil during the fallow periods. In 2016, after four years of annual soybean-maize rotation with or without mulching, three 1.8 cm diameter soil cores were harvested from each mesocosm and 0–5 and 5–15 cm soils were collected for further soil sample processing and analysis of C and N.

Initial soil C and N analysis was also performed at the beginning of the greenhouse mesocosm study and SOC of the Cambisol and Chernozem soils was and 26.5 mg C $g^{-1}$ soil, respectively, and $\delta^{13}$C of soil organic carbon was −26.78 and −26.49‰, respectively. Total soil N concentration for Cambisol and Chernozem soils was 1.29 and 2.62 mg N $g^{-1}$ soil, respectively.

### 2.3. Soil sample processing

Soil samples that were collected in the field and greenhouse mesocosm study were placed on ice until transport to a laboratory for storage at 4 °C. Within 24 h of collection, soils were homogenized by hand kneading in bags and then sieved to 2 mm. Two 5 g soil aliquots were then removed for measurement of soil soluble and microbial biomass C and N and another 5 g of soil was dried at 100 °C for over 48 h to calculate gravimetric water content. Soil soluble C and N were extracted from 5 g of soil with 25 mL 0.05 M $K_2SO_4$ on an orbital shaker for 1 h then filtered with Whatman (1001 090) filter paper. Microbial biomass plus soil soluble C and N were also extracted with $K_2SO_4$ as described above following a 24 h vacuum-incubation with chloroform (Brookes et al., 1985). Extracts were then dried at 65 °C and submitted for C and N analysis. An additional fresh soil subsample was dried at 40 °C and ground for homogenization prior to submission for C and N analysis.

For greenhouse mesocosm soils, ground soils that were submitted to our stable isotope laboratory for C and N analysis were first treated with HCl to remove inorganic C in Chernozems as well as potential inorganic C that could have been added to all mesocosm soils during watering (due to low concentration of carbonates). Approximately 0.10 mg soil was loaded into silver capsules with 30 μL distilled water and fumigated with 35–38% HCl for at least 6 h prior to drying at 60 °C for at least another 6 h prior to C and N analysis.

### 2.4. Stable isotope laboratory C and N analysis

Ground, homogenized plant and soil samples were submitted for C and N analysis in the Soil and Water Management & Crop Nutrition Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Seibersdorf, Austria. All samples were analyzed for C and N concentration and bulk soil and plant samples were also analyzed for $\delta^{13}$C using an Elementar Vario Isotope Select Elemental Analyzer coupled to an Isoprime 100 IRMS. The stable isotope abundance of C was calculated as

**Table 1**
Analysis of SOC and total soil N as well as $\delta^{13}$C of Cambisol 0–5 cm and 5–15 cm soils from experimental design blocks 1–3 at the time of set up in 2012. Each value represents the mean of 3 replicates and standard errors. No significant differences by block were observed ($p > 0.05$).

| Soil depth | Index | Block 1 | Block 2 | Block 3 |
|---|---|---|---|---|
| 0–5 cm | Total mg OC $g^{-1}$ soil | 13.94 ± 0.90[a] | 12.30 ± 0.08[a] | 12.32 ± 0.42[a] |
| 0–5 cm | Total mg N $g^{-1}$ soil | 1.61 ± 0.11[a] | 1.43 ± 0.02[a] | 1.40 ± 0.04[a] |
| 0–5 cm | Soil OC $\delta^{13}$C | −27.42 ± 0.37[a] | −27.22 ± 0.13[a] | −27.33 ± 0.10[a] |
| 5–15 cm | Total mg OC $g^{-1}$ soil | 15.38 ± 0.94[a] | 13.62 ± 0.45[a] | 15.42 ± 1.01[a] |
| 5–15 cm | Total mg N $g^{-1}$ soil | 1.77 ± 0.10[a] | 1.56 ± 0.04[a] | 1.70 ± 0.09[a] |
| 5–15 cm | Soil OC $\delta^{13}$C | −27.68 ± 0.15[a] | −27.50 ± 0.16[a] | −27.87 ± 0.12[a] |

**Fig. 2.** Mature maize plants in experimental mesocosms in a controlled greenhouse. Mesocosms were established as a randomized complete block design in three blocks and contained either Cambisol or Chernozem soils.

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) * 1000, \quad (1)$$

where R = $^{13}C/^{12}C$ of the sample or reference standard (PeeDee Belemnite for carbon). The precision of repeated analysis on laboratory standards for $\delta^{13}C$ was 0.12‰. All reported values are with ± standard error. Microbial biomass $\delta^{13}C$ values were not included in this study due to fractionation of $^{13}C$ during chloroform fumigation and soil extraction.

### 2.5. Data analysis

Microbial biomass C and N (mol per gram soil) was calculated according to Brookes et al. (1985) and Beck et al. (1997) as

$$[X]_{mic} = ([X]_{soil,F} - [X]_{soil,UF})/k, \quad (2)$$

where $[X]_{soil,F}$ is the mol of C or N per gram of chloroform treated soil; $[X]_{soil,UF}$ is the mol of C or N per gram of soil untreated with chloroform; and $k$ is the extractable portion of microbial biomass (0.45 and 0.54 for C and N, respectively).

Statistical analyses were performed using JMP 13 software (SAS, Cary, North Carolina, USA). Soil samples were treated as individual samples, without averaging by replicate or block. Plant samples from Grabenegg were analyzed similarly but plants from mesocosms were combined by treatment, as plant material was also combined per treatment and then applied as mulch. Effects of mulch on continuous variables, C and N concentration and $\delta^{13}C$ of soils and plants, were explored using standard least squares reports. Residuals and predicted values of these variables were plotted to check for assumption of linearity and homoscedasticity before use in linear models. Factors of interest in the Grabenegg field experiment included nominal variables, cropping system and mulching (nested within cropping system). Because mulch was not independent of cropping system, an interactive effect between these two factors was not explored. Block was also included as a random effect because SOC and total N concentration slightly varied by block at the initiation of the experimental site (Table 1). Factors of interest in the greenhouse mesocosm experiment included the nominal variables soil type and mulch (nested within site). Soil moisture content was not included as a random effect in either study as moisture did not significantly vary by cropping system or mulch in the Grabenegg field experiment and moisture was controlled in the mesocosm experiment. To test the degree to which SOC was influenced by mulch, cropping system and soil total N concentration as well as microbial biomass C and N in Grabenegg, we used a stepwise linear regression analysis. Soil total N concentration, moisture content and microbial biomass C and N were included as continuous variables and cropping system and mulching (nested within cropping system) were included as nominal variables.

## 3. Results

### 3.1. Field experiment

After experimental maintenance of field plots for five years in Grabenegg, only the topsoil at 0–5 cm depth with sole maize was significantly higher in SOC with mulching than soils without mulching and total N by 22% and 31%, respectively (Table 2, Fig. 3). All other soils, including sole vetch growth and vetch-maize rotation, did not exhibit differences in SOC and total N with mulching. Mulching did not significantly increase or reduce microbial C and N or soil moisture content at the time of soil harvest. Mulching also did not significantly enhance plant yields or C to N ratios (C:N) between 2012 and 2016.

**Table 2**
Analysis of SOC and total soil N as well as microbial biomass C and N of 0–5 cm and 5–15 cm Cambisol soils with vetch (V), maize (M) or vetch-maize rotation (VM) planting with (+) and without (−) mulch. Each value represents the mean of 3 replicates and standard errors. Significant differences due to mulch for each cropping system are noted for $p < 0.05$.

| Soil depth | Index | M− | M+ | V− | V+ | VM− | VM+ |
|---|---|---|---|---|---|---|---|
| 0–5 cm | Total mg OC $g^{-1}$ soil | 12.85 ± 1.09$^{b}$ | 15.73 ± 0.13$^{a}$ | 12.29 ± 0.32$^{b}$ | 12.91 ± 1.28$^{b}$ | 14.82 ± 1.34$^{ab}$ | 14.77 ± 0.53$^{ab}$ |
| 0–5 cm | Microbial mg C $g^{-1}$ soil | 0.19 ± 0.06$^{ab}$ | 0.32 ± 0.03$^{ab}$ | 0.20 ± 0.08$^{b}$ | 0.30 ± 0.09$^{ab}$ | 0.21 ± 0.06$^{ab}$ | 0.45 ± 0.06$^{a}$ |
| 0–5 cm | Total mg N $g^{-1}$ soil | 1.43 ± 0.10$^{c}$ | 1.87 ± 0.05$^{a}$ | 1.51 ± 0.04$^{bc}$ | 1.56 ± 0.16$^{abc}$ | 1.75 ± 0.13$^{ab}$ | 1.72 ± 0.06$^{abc}$ |
| 0–5 cm | Microbial mg N $g^{-1}$ soil | 0.03 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ | 0.02 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ | 0.03 ± 0.01$^{a}$ | 0.05 ± 0.01$^{a}$ |
| 5–15 cm | Total mg OC $g^{-1}$ soil | 12.79 ± 0.44$^{ab}$ | 14.90 ± 1.33$^{a}$ | 11.68 ± 0.69$^{b}$ | 11.68 ± 1.08$^{b}$ | 13.94 ± 0.69$^{ab}$ | 13.17 ± 0.54$^{ab}$ |
| 5–15 cm | Microbial mg C $g^{-1}$ soil | 0.20 ± 0.02$^{a}$ | 0.26 ± 0.02$^{a}$ | 0.29 ± 0.05$^{a}$ | 0.21 ± 0.05$^{a}$ | 0.19 ± 0.01$^{a}$ | 0.34 ± 0.05$^{a}$ |
| 5–15 cm | Total mg N $g^{-1}$ soil | 1.55 ± 0.04$^{ab}$ | 1.76 ± 0.10$^{a}$ | 1.47 ± 0.08$^{b}$ | 1.47 ± 0.13$^{b}$ | 1.68 ± 0.07$^{ab}$ | 1.60 ± 0.08$^{ab}$ |
| 5–15 cm | Microbial mg N $g^{-1}$ soil | 0.02 ± 0.01$^{a}$ | 0.02 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ | 0.03 ± 0.01$^{a}$ | 0.02 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ |

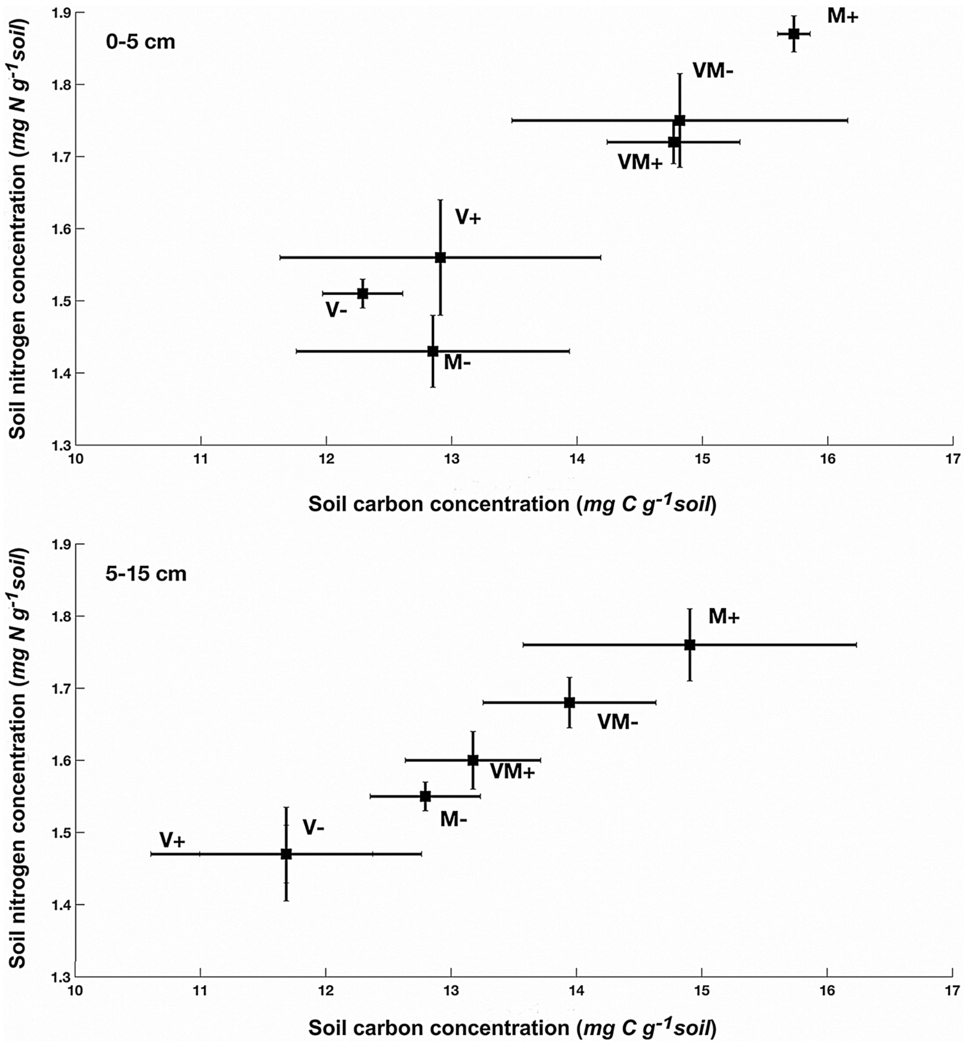


**Fig. 3.** SOC and total soil N content for 0–5 cm and 5–15 cm soils with sole vetch planting (V), sole maize planting (M) or vetch-maize rotation (VM) with (+) and without (−) mulching. Standard error bars are shown. In 5–15 cm soils, V− and V+ overlap and smaller error bars for N and SOC content are for V−.

Like mulch treatments, the type of cropping system did not influence soil moisture content at the time of harvest. Maize and vetch harvest varied by year ($p < 0.0001$, n = 95), with maize harvest ranging from 5.9 to 15.7 t $ha^{-1}$ (total aboveground biomass) and vetch harvest ranging from 0.4 to 3.4 t $ha^{-1}$ (total aboveground biomass) between years. Plant yields did not vary by monoculture or rotation cropping. Plant C:N varied significantly by year ($p < 0.0001$, n = 87) with average maize C:N ranging from 26.2 to 49.1 by year and average vetch C:N ranging from 12.8 to 20.3 by year.

$\delta^{13}C$ of SOC was affected by cropping system and mulching ($p < 0.0001$ and 0.0131, respectively), but not by depth down to 15 cm. In particular, sole vetch planting soils were more depleted in $^{13}C$ than vetch-maize rotation and sole maize planting with average $\delta^{13}C$ of soils with different crop systems ranging from an average of −27.6 to −26.1‰ (Fig. 4). $\delta^{13}C$ of soils with mulching were only slightly more negative than those without mulch by on average 0.3‰ or less in soils with sole vetch or maize planting. However, $\delta^{13}C$ of soils with vetch-maize rotation and mulching were less negative than those without mulch by on average 0.8‰ ($p = 0.0020$). Plant $\delta^{13}C$ was not significantly affected by mulching. Maize and vetch $\delta^{13}C$ did, however, also vary significantly by cropping system and year ($p < 0.0001$, n = 87 by both cropping system and year). Still, annual variation in $\delta^{13}C$ was small compared to the difference between these $C_3$ and $C_4$ plants, as averaged maize $\delta^{13}C$ ranged from -12.7 to - 13.6‰ by year and vetch $\delta^{13}C$ ranged from -28.8 to -30.6‰. To investigate the relationship between plant C:N and $\delta^{13}C$, as both varied by year and may be linked due to the effects of plant maturity at time of harvest, a linear regression analysis was performed. $\delta^{13}C$ explained a significant but small amount of the variance in maize and vetch C:N ($r^2 = 0.177$ and 0.490, $p = 0.0040$ and $< 0.0001$, $n = 45$ for both, for maize and vetch, respectively).

A stepwise linear regression was performed to determine controls on SOC. Cropping system, mulching, total soil N as well as soil moisture content at time of harvest, microbial biomass C and N were included as potential factors influencing SOC. In a model including total soil N and cropping system (adjusted $r^2 = 0.910$), N explained 97.2% of the variance and cropping system only explained 2.8% of the variance (Table 3). Amongst all soil samples from 0 to 5 cm and 5–15 cm depths, a strong linear relationship with an $r^2$ of 0.92 was also observed (Fig. 5).

In a separate comparison of increases in SOC and total soil N within experimental blocks after five years, rather than our previously reported comparisons between mulch and no mulch treatments, maize plots with mulching also consistently increased 0–5 cm SOC after five years by 2.03–3.41 mg C $g^{-1}$ soil $yr^{-1}$. Mulching did not increase SOC in any other cropping system after five years. Regardless of mulching treatment, vetch-maize rotation also consistently increased 0–5 cm SOC after five years by 0.48–3.41 mg C $g^{-1}$ soil $yr^{-1}$. No increases in SOC were observed at 5–15 cm after the five-year experiment. Total soil N concentration also consistently increased in 0–5 cm soil in maize plots with mulching after five years by 0.20–0.37 mg N $g^{-1}$ soil $yr^{-1}$ and all

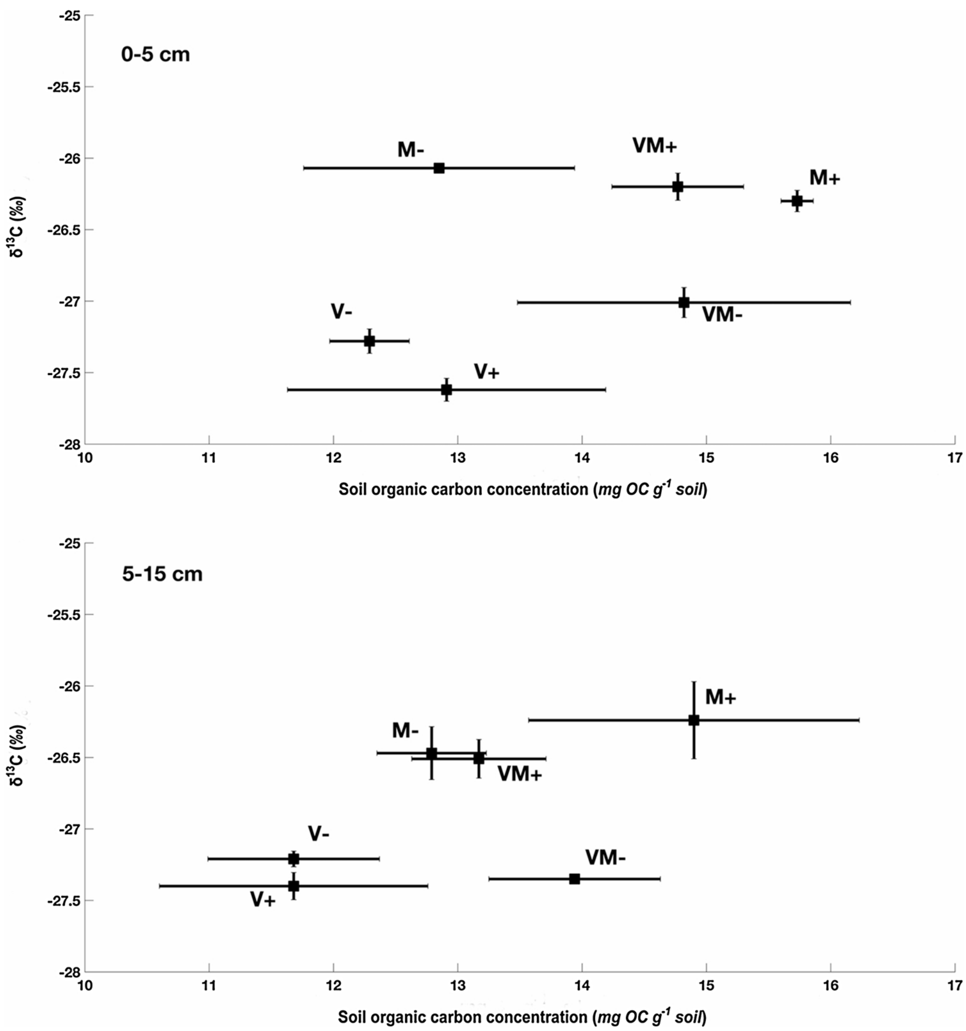


**Fig. 4.** SOC content and its $\delta^{13}$C for 0–5 cm and 5–15 cm soils with sole vetch planting (V), sole maize planting (M) or vetch-maize rotation (VM) with (+) and without (-) mulching. Standard error bars are shown.

**Table 3**
Stepwise linear regression model to evaluate influences over soil mg OC $g^{-1}$ soil. Significant influences included total soil N content and cropping systems (sole vetch planting [V], maize planting [M] and vetch-maize rotation [VM]). Adjusted $r^2$ was 0.910 ($n$ = 36, AICc = 64.97, $p$ = 0.025).

| Regression model parameter | Value ± SE | % Variance | $p$ value |
|---|---|---|---|
| Intercept | −0.20 ± 0.84 | | 0.8133 |
| Total mg N $g^{-1}$ soil | 8.40 ± 0.53 | 97.2 | < 0.0001 |
| Cropping system (V *vs.* M & VM) | −0.29 ± 0.11 | 2.8 | 0.0109 |

vetch-maize plots increased in total soil N regardless of mulching by 0.13–0.40 mg N $g^{-1}$ soil $yr^{-1}$. No other increases in total soil N concentration were observed after five years. Maize planting consistently increased $\delta^{13}$C of 0–5 cm soil after five years regardless of mulching treatment by 0.8–1.3‰. No trends in $\delta^{13}$C by vetch planting were observed after five years but vetch-maize planting with mulch consistently increased $\delta^{13}$C of 0–5 cm soil by 0.9–1.4‰.

### 3.2. Greenhouse mesocosm experiment

Similar to the field experiment, Cambisol soils in the greenhouse mesocosm experiment with soybean-maize planting rotation did not show an effect of mulching on SOC or total soil N availability or microbial C and N (Table 4). Also similar to the field study, the $\delta^{13}$C of greenhouse mesocosm Cambisol soils was less negative with mulching by 0.7‰ in the top 0–5 cm ($p$ = 0.0062). Mulching also did not improve SOC in the additional greenhouse mesocosm Chernozem soil, which was 144% higher in SOC and 103% higher in total soil N than the Cambisol at the beginning of the experiment, but N availability was higher by 13% in 0–5 cm soils with mulching. Similar to the greenhouse mesocosm and field Cambisol soils, the $\delta^{13}$C of Chernozem soils with mulching were higher by 0.5‰ in the top 0–5 cm than those without mulch ($p$ = 0.0197) and by 0.2‰ in the 5–15 cm Chernozem soil ($p$ = 0.0242).

As in the field study, mulching did not affect plant yields, $\delta^{13}$C or C:N. Plant yield varied by year ($p < 0.0001$, $n$ = 120), with maize yields (total aboveground biomass) ranging from 2.7 to 10.7 t $ha^{-1}$ and soybean yields (total aboveground biomass) ranging from 1.7 to 3.4 t $ha^{-1}$ between years. Plant $\delta^{13}$C also varied by year ($p < 0.0001$, $n$ = 24) but this variation was small compared to differences between the $C_3$ and $C_4$ plants and ranged from -13.5 to -14.5‰ for maize and -28.31 to -29.9‰ for soybeans. Plant C:N also varied by year ($p$ = 0.0120, $n$ = 24) and ranged from 21.5 to 33.1 for maize and 10.3 to 13.1 for soybeans. In contrast to the field study, a separate comparison of increases in SOC and total soil N after the four year experiment, rather than by comparison of mulch and no mulch treatments, revealed no consistent increases in SOC or total soil N concentration with the soybean-maize rotation. Average soil moisture to 15 cm of Cambisol and Chernozem soils the week leading up to soil harvest ranged from 18.8 to 20.0%

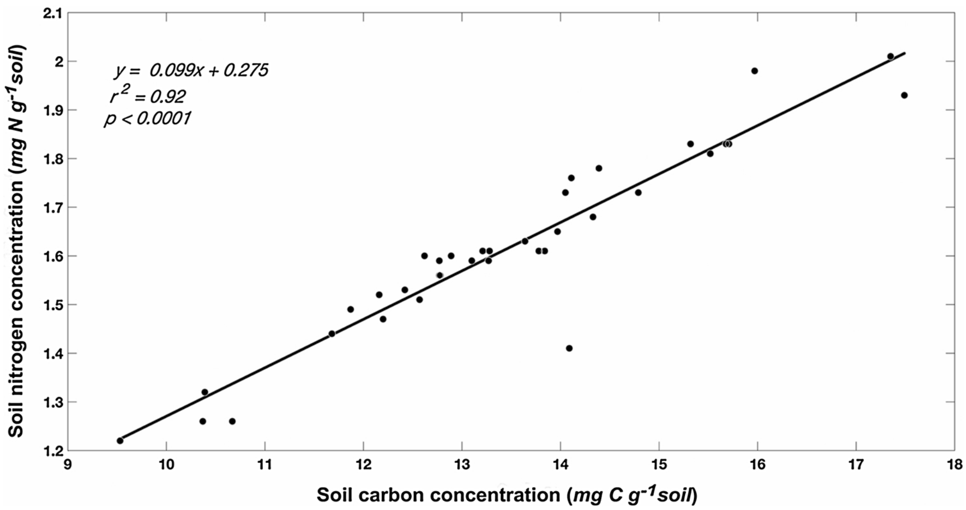


**Fig. 5.** SOC and total soil N content for all samples collected in 0–5 cm and 5–15 cm depths. Values for $r^2$ and $p$ are reported based on a non-parametric spearman's rank correlation coefficient used to evaluate the relationship behind SOC and total soil N content.

between soil types with and without mulch.

## 4. Discussion

This study demonstrates that the efficiency of crop residue mulch retention to improve SOC sequestration varies by cropping system and mulch type and that crop residues only improve SOC concentration in Austrian Cambisols with maize monocrops and not in cropping systems with legumes. Although we predicted that SOC and total soil N concentration would improve in all cropping systems with mulching and that SOC sequestration would be greater in legume-cereal rotations, only Cambisols with maize monocrops resulted in higher SOC and N with mulching as compared to no mulch retention. Furthermore, contrary to our prediction that mulching would have a greater effect on soils with relatively lower organic matter and higher potential to accumulate C, mulching did not improve SOC in the Cambisol or Chernozem soil types with soybean-maize rotation.

### *4.1. Field experiment*

Among field plots with sole maize and vetch monocrops and vetch-maize rotation, only maize monocrops exhibited a significant positive mulching effect on SOC as well as N after five years and in comparison to experimental soils without mulch. As is typical of soil C:N (Cleveland and Liptzin, 2007), total soil N concentration was tightly linked to SOC concentration and suggests that SOC levels limit nutrient availability and *vice versa*. Thus, we considered whether N fertilization used during maize production influenced the positive SOC response in maize plots to mulching, but this was refuted for two reasons. First, SOC and total soil N concentration in maize monocrop soils without mulch did not improve after the five year study despite also receiving fertilizer inputs and, secondly, plots with vetch-maize rotation and mulch did not have higher SOC concentration than plots without mulch despite also being supplied additional N fertilizer for maize production. We also considered whether different N inputs due to fertilizer as well as mulch C:N quality could have influenced SOC but sole maize monocrops with mulch had more SOC and total soil N even though vetch-maize rotation soils with mulch had the highest N inputs (due to the addition of N fertilizer, vetch mulch with low C:N and maize mulch with higher C:N) and vetch monocrops received mulch with a lower C:N. These results suggest that maize mulch, which decomposes more slowly (Manzoni et al., 2010), is responsible for immobilizing N in the top soil layer and for parallel SOC sequestration.

The relatively slower turnover of maize mulch compared to vetch is likely the mechanism for increased SOC sequestration in maize monocrops in our studied Cambisol in the Austrian setting. Although it has been proposed that maize crops and crop residues increase SOC sequestration due to the relatively greater productivity of this $C_4$ plant compared to $C_3$ plants (West and Post, 2002; Mathew et al., 2017), the positive response of SOC in our Cambisol to mulching in sole maize crops cannot be explained by higher production of crop residues and organic material because the amount of mulch applied to soils was controlled and the C concentration of maize and legume mulch was similar. Furthermore, the lack of response of vetch-maize rotation soils to mulch and the isolated positive response of SOC in maize monocrops to mulching suggest that the benefits of mulching are muted by legume production in the studied Cambisols. It has been proposed that legumes mute the benefits of mulching on SOC by causing a positive rhizosphere

**Table 4**
Analysis of SOC, total soil N and $\delta^{13}C$ as well as microbial C and N of mesocosm soils with low (L) or high (H) organic matter without (-) and with (+) mulch. Each value represents the mean of 3 replicates and standard errors. Significant differences in soils by soil type and mulch are shown for $p < 0.05$.

| Soil depth | Index | L− | L+ | H− | H+ |
|---|---|---|---|---|---|
| 0–5 cm | Total mg OC $g^{-1}$ soil | $10.82 \pm 0.12^{a}$ | $12.56 \pm 0.58^{a}$ | $24.66 \pm 1.55^{b}$ | $26.39 \pm 0.54^{b}$ |
| 0–5 cm | Microbial mg C $g^{-1}$ soil | $0.060 \pm 0.006^{a}$ | $0.063 \pm 0.004^{a}$ | $0.273 \pm 0.031^{b}$ | $0.154 \pm 0.023^{c}$ |
| 0–5 cm | Total mg N $g^{-1}$ soil | $1.38 \pm 0.04^{a}$ | $1.43 \pm 0.05^{a}$ | $2.26 \pm 0.04^{b}$ | $2.55 \pm 0.09^{c}$ |
| 0–5 cm | Microbial mg N $g^{-1}$ soil | $0.015 \pm 0.005^{a}$ | $0.015 \pm 0.002^{a}$ | $0.029 \pm 0.002^{b}$ | $0.026 \pm 0.006^{b}$ |
| 0–5 cm | SOC $\delta^{13}C$ | $-26.24 \pm 0.03^{ac}$ | $-25.59 \pm 0.23^{bd}$ | $-26.27 \pm 0.06^{ab}$ | $-25.76 \pm 0.06^{cd}$ |
| 5–15 cm | Total mg OC $g^{-1}$ soil | $10.83 \pm 0.58^{a}$ | $10.31 \pm 0.09^{a}$ | $24.05 \pm 0.36^{b}$ | $23.70 \pm 0.21^{b}$ |
| 5–15 cm | Microbial mg C $g^{-1}$ soil | $0.041 \pm 0.011^{a}$ | $0.040 \pm 0.004^{a}$ | $0.177 \pm 0.028^{b}$ | $0.147 \pm 0.022^{b}$ |
| 5–15 cm | Total mg N $g^{-1}$ soil | $1.28 \pm 0.04^{a}$ | $1.22 \pm 0.03^{a}$ | $2.33 \pm 0.05^{b}$ | $2.27 \pm 0.09^{b}$ |
| 5–15 cm | Microbial mg N $g^{-1}$ soil | $0.016 \pm 0.0003^{a}$ | $0.018 \pm 0.008^{a}$ | $0.020 \pm 0.004^{a}$ | $0.022 \pm 0.004^{a}$ |
| 5–15 cm | SOC $\delta^{13}C$ | $-26.52 \pm 0.05^{a}$ | $-26.39 \pm 0.04^{a}$ | $-26.27 \pm 0.05^{b}$ | $-26.06 \pm 0.06^{c}$ |

priming effect (Fu and Cheng, 2002) that stimulates decomposition of organic matter and loss of soil C. However, neither microbial C nor N improved with mulch and do not support this hypothesis and more detailed analyses of microbial activity and rhizosphere processes are necessary.

Isotopic analysis of SOC further suggests that maize mulch incorporates more carbon to topsoils than vetch mulch in our experiment. The less negative $\delta^{13}C$ in vetch-maize rotation soils with mulch than without mulch indicate that soil C pools received more mulch-derived C from relatively $^{13}C$ enriched maize mulch as opposed to relatively depleted $^{13}C$ vetch mulch (Barthès et al., 2004). This is contradictory to what we predicted and needs to be further investigated in maize mulch experiments, particularly those established in soils previously dominated by $C_3$ plants.

Crop yield, crop residue quality, plant $\delta^{13}C$ and soil moisture at the time of harvest could not be used to explain the effect of mulch on SOC concentration in maize monocrops as no differences in these factors by mulching were observed. Because plant yield and plant C:N varied by year, as it does in many systems, we recommend frequent evaluation of the effects of mulch on SOC sequestration. Variation in root production and quality of both root and mulch material can affect rates of decomposition and, ultimately, SOC sequestration (Manzoni et al., 2010). For example, mulch induced increases in SOC in maize plots may be muted in years when root production is large due to favorable climate. Furthermore, it is likely that differences in soil moisture by mulching did exist during the ongoing experiment (Mulumba and Lal, 2008), but this was not measured for the entire experiment and soil moisture content did not vary by mulching at the time of harvest. The mesocosm study discussed below specifically eliminates any potential role of soil moisture to investigate direct effects of mulch.

### 4.2. Greenhouse mesocosm experiment

By controlling soil moisture in a greenhouse environment, we were able to test the direct effects of mulch on SOC and eliminate any indirect effects that might occur due to increases in soil moisture with mulching (Erenstein, 2003). Like the field experiment, there was no effect of mulching on SOC and total soil N concentration in the same Cambisol with soybean-maize rotation after four years and $\delta^{13}C$ was less negative in treatments with mulch thus again indicating that there is relatively higher replacement of the old pool of $C_3$ plant-derived carbon by maize-derived carbon. These results suggest that the effects of mulch (particularly maize mulch) on SOC sequestration are direct effects and not due to an indirect effect on soil moisture.

In addition to testing the direct effects of mulching in Cambisols, we also tested these effects in a contrasting Chernozem with relatively higher organic matter. Similar to the Cambisol, SOC was not higher and $\delta^{13}C$ was less negative with mulching in Chernozem soils. However, in contrast to the Cambisol soils, total soil N was higher with mulching and, together with an observable positive trend in SOC with mulching at the top of all mesocosm soils, suggest that mulching would significantly increase C and N pools with slightly more time. This would support our initial prediction based on studies that have investigated the benefits of mulching (Spaccini et al., 2000; Erenstein, 2003; Smith *et al.*, 2008; Brandani et al., 2015).

## 5. Conclusions

Mulching is often prescribed as a sustainable agricultural method that improves soil C sequestration. However, as we demonstrate, the efficacy of mulching varies by cropping system and mulch type in Austrian Cambisol and Chernozem soils. Through our field study with Cambisol soils that are low in organic matter, we observed that only maize monocrops had higher SOC and total soil N with mulching and that the presence of a legume crop muted the benefits of mulching. Soil isotope analysis further revealed that maize mulch contributed to SOC improvement more than the legume crop. The controlled greenhouse study further confirmed these mulching direct effects in a legume-maize rotation system and revealed that even a Chernozem soil with relatively higher organic matter did not improve in SOC with mulching. In order to track the long-term effects of mulching and identify if SOC sequestration rate due to mulching eventually reaches a steady state due to saturation of SOC pools, continued effects of mulching in these experiments will be further investigated after a period of 10 yr after experimental initiation.

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