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Cover cropping in organic reduced tillage systems: Maximizing soil cover or plant above ground biomass input?

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Abstract

Cover crops are grown between two main crops to reduce periods of bare fallow. In highly diverse crop rotations, the lengths of break periods between two main crops vary highly over time and consequently the cover cropping management differs from year to year. Long-term field trials are thus of limited use because the same cover cropping approach only appears once in several years. This increases the need to better determine the immediate effects of different cover cropping strategies on soil properties. This study evaluated two cover cropping strategies and monitored the temporal development of several soil properties on six fields in Eastern Switzerland in the 9 months period between harvest of winter wheat and sowing of spring crops. The two tested strategies were (a) double cover cropping (DCC) where two cover crops mixtures were grown subsequently and shallowly (3 cm) incorporated into the topsoil and (b) permanent soil cover (PSC) with one grass-clover mixture, which was harvested and thus not incorporated into the soil. Soil samples at three different soil depths (0–5, 5–10 and 10–20 cm) were sampled four times in high spatial resolution and analysed using a combined approach of visible near infrared spectroscopy and conventional lab methods. Differences between the sampling times and field sites were stronger than effects of different treatments. For soil organic carbon (SOC), no significant difference was measured between treatments in 0–20 cm soil depth. Only when analysed per depth segment, the PSC treatment showed significantly higher SOC increase in 5–10 cm soil depth than the DCC treatment. This could be due to the longer soil cover and thereby associated longer root growth period in the PSC treatment, leading to higher below ground C inputs than in the DCC treatment. On the other hand, the DCC treatment showed generally higher increases in permanganate oxidizable carbon stocks (0–5 cm), microbial C (0–10 cm), microbial N (0–10 cm) and mineral N (0–10 cm) than the PSC treatment. We conclude that maximizing

cover crop above ground biomass input by planting two cover crops (DCC) benefitted soil microorganisms on most fields but was less beneficial on SOC than permanent soil cover (PSC) in 5–10 cm soil depth.

KEYWORDS

microbial biomass, regenerative agriculture, shallow incorporation, soil fertility, soil organic matter, soil spectroscopy, temperate climate

1 | INTRODUCTION

Soil fertility is crucial for sustainable crop production but is decreasing in arable soils across the world (Lal, 2015). Depletion in soil organic carbon (SOC) is an important driver of this process which has also been observed in Europe (Gubler et al., 2019). The beneficial effects of soil organic matter (SOM) lie in its dynamic nature where short-term formation and mineralization of organic matter influence nutrient availability and crop performance (Hacker et al., 2015; Janzen, 2006). Cover crops are an important element to promote SOM formation in a crop rotation (Jian et al., 2020; Kaye & Quemada, 2017; McClelland et al., 2021; Poeplau & Don, 2015), but region-specific limitations hamper their adoption in Europe (Heller et al., 2024). Cover crops, also referred to as catch crops or intercrops, are sown in the period between two main crops to avoid periods with bare soil. Additionally, cover crops can also be undersown in a main crop to increase the species richness on the field. The major goal of cover cropping is to improve nutrient cycling, avoid nutrient losses, increase SOC stocks, enhance microbial activity, increase soil cover and reduce erosion (Daryanto et al., 2018; Thorup-Kristensen et al., 2003).

While the overall benefits of cover crops are well documented, very little information is available on the effects of different cover cropping strategies on soil properties. Cover cropping strategies differ in terms of species diversity, incorporation method, biomass input and the frequency they are applied in a crop rotation. All these factors are relevant for both the decomposition and the accumulation of organic matter in soil. For example, SOM formation is more efficient when above ground residues were mixed with topsoil than just put on the soil surface (Mitchell et al., 2016, 2018; Sokol et al., 2019).

Several parameters have been suggested to evaluate the performance of cover crops. Since total SOC is a slowly reacting C pool, the analysis of labile C fractions to evaluate the effect of different agricultural management techniques has been recommended (Bongiorno et al., 2019; Wang et al., 2014). Among them, permanganate oxidizable carbon (POXC), also referred as active C, has been shown to be influenced by cover cropping (Jagadamma et al., 2019; Lucas & Weil, 2021). Another fast reacting and management sensitive C pool is soil microbial biomass carbon (Cmic), of which some studies have measured an increase due to cover cropping (Kim et al., 2020). This effect was more pronounced with species mixtures than with single species cover crops (Gentsch et al., 2020). Other studies showed that POXC and Cmic correlate with SOC and therefore suggested them as indicators for SOC development (Bongiorno et al., 2019; Lange, 2015). Besides soil C fractions, cover crops also influence the soil nitrogen (N) cycle, whereby some N fractions are more sensitive to cover cropping than others (Mohammed et al., 2020; Wang et al., 2007). Similar to SOC, total soil N is a slowly reacting N pool and cover crop research focuses mainly on the labile N pools such as mineral N (Nmin) and microbial N (Nmic). Cover crops use Nmin for their growth and can thereby prevent the leaching of some Nmin into deeper soil layers or into ground water (Tonitto et al., 2006). On the other hand, cover crops enhance the uptake of Nmin into the microbial biomass (immobilization) because microbial growth benefits from cover crop's labile C inputs (in't Zandt et al., 2018).

Two main mechanisms explain the beneficial effects of cover crops on soil C and N fractions. First, cover crops increase the organic matter input into the soil. Second, cover crops are used to suppress weed growth, which reduces the need for mechanical weed control and thereby prevents SOC mineralization (Singh et al., 2023). Traditionally, organic farming systems mainly rely on cover crops for increasing organic matter inputs whereas

Highlights

- Monitoring of two cover cropping strategies in high spatial and temporal resolution
- Permanent soil cover (PSC) strategy increased soil organic carbon in 5–10 cm depth
- Double cover cropping (DCC) increased soil microbial biomass on most fields
- Above ground biomass input in DCC strategy increased mineral N on most fields

conservation agriculture systems see the main benefit in the reduction of soil tillage. In both systems, cover crops are well established (Büchi et al., 2017; Hubbard, 2013; Welch et al., 2016). However, the combination of conservation tillage with organic farming remains challenging mainly because of increased weed pressure and reduced yields (Leifeld et al., 2009; Zikeli & Gruber, 2017). In conservation tillage systems, cover crops are often killed with herbicides, roller crimper or by frost periods whereas in organic systems cover crops are normally incorporated by inversion tillage (Alonso-Ayuso et al., 2020; Wayman et al., 2015).

New cover cropping approaches try to combine methods from both organic farming and reduced tillage by shallowly (3 cm) incorporating cover crop mixtures with a rotary tiller. The resulting plant-soil mixture serves as an energy source for the soil microbiome. Labile C inputs enhancing the soil microbiology are a key element for the stabilization of SOM (Cotrufo et al., 2013). Thereby, the microbial by-products and the microbial necromass can play a major role in SOM formation (Kallenbach, 2016; Miltner et al., 2012; Vidal et al., 2021). This shallow incorporation of cover crop mixtures is often used in 'regenerative agriculture' that has gained popularity in agricultural practice in recent years (Giller et al., 2021; Rhodes, 2017), yet, is still not clearly defined.

Most research on the effects of cover crops focuses on the comparison between a cover crop treatment and a bare soil control. However, in Switzerland long-term bare soil periods are not allowed (Swiss Ordinance 910.13, 2013) and cover cropping is widely applied (Heller et al., 2024). Also other European countries try to foster the adoption of cover crops (Kathage et al., 2022). The question on the type of cover cropping strategy and their effects on soil properties will thus become in future more important than whether or not to implement cover crops at all. In Swiss organic reduced tillage systems, two different types of cover cropping are commonly applied in the up to 9 months period between cereal harvest (end of July) and sowing of a next spring crop (April–May). The so-called 'double cover cropping' (DCC) aims to maximize fresh organic matter into the soil by sowing, growing and shallowly incorporating a summer cover crop mixture and a winter cover crop mixture subsequently. The DCC approach is expected to show beneficial effects on soil fertility parameters because it has a high above ground biomass input into the soil that is decomposing in interaction with the soil mineral phase. However, the double shallow incorporation requires shallow but intensive tillage that might increase SOM mineralization in the topsoil. Alternatively, the 'permanent soil cover' (PSC) aims for maximized soil cover and reduced soil tillage. This is achieved by a temporary ley where the above ground biomass can be harvested and used as forage. The same effect can also be achieved by undersowing a cover crop with grasses and clover in the cereal stand and use it as a temporary ley after the cereal harvest. In contrast to DCC, the PSC approach does not have any above ground biomass input into the soil but also no disturbance.

Given the increasing implementation of cover cropping, it becomes more and more relevant to evaluate the effects of these different strategies as management options on soil fertility. We thus monitored the immediate effects of the DCC and the PSC approach on soil C and N fractions at three different soil depths (0–5, 5–10 and 10–20 cm) over a period of 9 months in six fields in Switzerland. In highly diversified crop rotations, a long fallow period that is suitable for either the DCC or PSC cover cropping approach appears only once within several years. For this reason, the effects of these cover cropping approaches cannot be evaluated in experiments that span over several cropping seasons, as their immediate effects would be covered by any other crop or management effect. We thus took soil samples in high spatial and temporal resolution using a combination of near infrared spectroscopy and conventional lab methods to enable detection of small changes in the analysed parameters. This was done to achieve a better understanding of the effects of either maximizing cover crop biomass input (DCC) or soil cover (PSC) on soil fertility using cover crops. We formulated three hypotheses:

1. Given the short time period of the experiment, SOC and total N will not significantly differ between the two treatments.
2. The DCC treatment with above ground biomass input will show higher labile C and N (POXC and Nmin), compared to the PSC treatment with no such above ground biomass input.
3. The DCC treatment with above ground biomass input will promote the soil microbial biomass (Cmic and Nmic), compared to the PSC treatment with no such above ground biomass input.

2 | METHODS

2.1 | Study sites and experimental set-up

The trial was conducted on six agricultural fields in the canton of Thurgau, Switzerland (Table 1). All fields were at maximum 12 km apart from each other. In 2019, the mean temperature in the region was 10.8°C and total annual precipitation summed up to 815 mm, which was a bit warmer and drier than the long-term average (1991–2020) of 8.7°C and 853 mm. The trial comprised the period of 9 months between cereal harvest at the end of July and sowing of a cash crop in late spring (Figure 1). Before the onset of the

TABLE 1 Description of the trial sites.

Field	Elevation (m a. s. l.)	Trial area (ha)	Soil class (world reference base)	Soil texture (% of sand/silt/clay)	pH ($CaCl_2$)	Crop rotation (4 years before trial)	Last ploughing (year)	Fertilization
A	420	0.84	Eutric Cambisol	50/29/21 Sandy loam	7.18	2015: Temporary ley 2016: Celeriac 2017: Rye 2018: Potato 2019: Winter wheat	2012	None
B	420	0.67	Eutric Cambisol	44/35/20 Sandy loam	6.56	2015: Potato 2016: Dwarf beans 2017: Temporary ley 2018: Corn 2019: Rye	2016	Processed organic fertilizer (Bio-Enne, Timac Agro, Switzerland) N: 72 kg ha^{-1} C: 210 kg ha^{-1} Applied: 27.04.2020
C	600	0.44	Eutric Cambisol	27/35/38 Clay loam	7.19	2015: Sugar beet 2016: Winter wheat 2017: Temporary ley 2018: Temporary ley 2019: Winter wheat	2015	None
D	460	0.64	Eutric Cambisol	28/44/28 Clay loam	6.88	2015: Oat 2016: Spelt 2017: Field beans 2018: Red clover 2019: Winter wheat	2018	Chicken manure N: 112 kg ha^{-1} C: 740 kg ha^{-1} Applied: 01.04.2020
E	460	1.05	Eutric Cambisol	30/48/23 Sandy loam	6.6	2015: Spelt 2016: Dwarf bean and peas 2017: Winter wheat 2018: Linen 2019: Winter wheat	2018	None
F	380	0.3	Eutric Cambisol	39/43/18 Sandy loam	7.49	2015: Winter wheat 2016: Sugar beet 2017: Corn 2018: Potato 2019: Winter wheat	2017	None

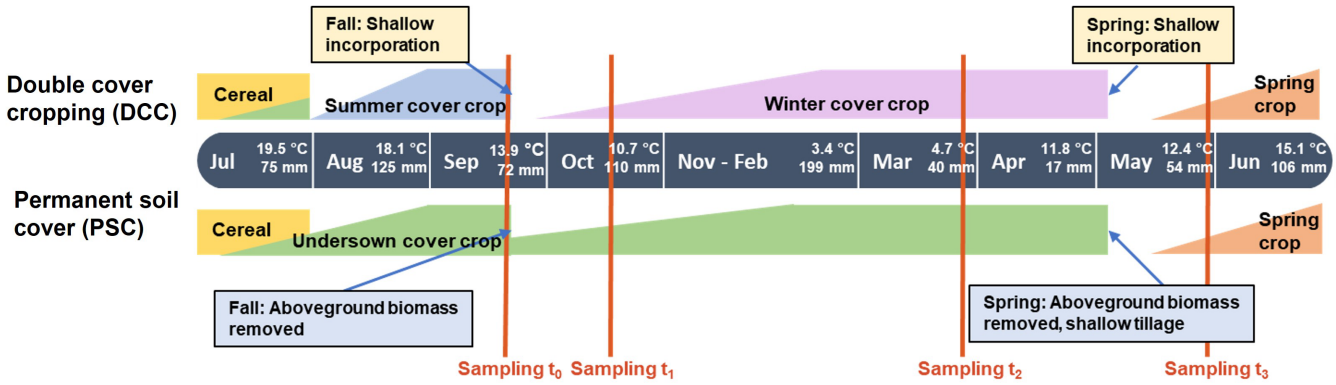

FIGURE 1 Timeline for the two treatments double cover cropping (DCC) and permanent soil cover (PSC). For every month the average temperature and total precipitation are indicated.

trial, every field was planted with winter cereal and an undersown cover crop called GreenCarbonFix that was purchased at Camena Samen (Germany) and contained six species: 55% perennial ryegrass (*Lolium perenne L.*), 25% crimson clover (*Trifolium incarnatum L.*), 5% white clover (*Trifolium repens L.*), 5% hop clover (*Medicago lupulina L.*), 5% bird's-foot trefoil (*Lotus corniculatus L.*) and 5% camelina (*Camelina sativa L.*). After the cereal harvest in July each field was divided into a PSC plot in the middle and two DCC plots on both sides. Plot sizes were between 1000 and 3500 m^2. Each plot comprised 13 GPS-referenced sampling points (circles with a radius of 1 m) that were homogeneously distributed across the plot in an unaligned design (Webster & Lark, 2013). The results of the two DCC plots (26 subplots) were combined and referred here as DCC plot. The unequal sample number for each treatment was accounted for in all statistical analyses (see Section 2.7). The management was conducted by the farmers and therefore we used a strip design and not a randomized block design which would have made the machine handling very complicated. In the DCC plots, two commercial cover crop mixtures were sown subsequently (Figure 1). The summer cover crop mixture (Dominanzgemenge; Camena Samen) was sown after cereal harvest (end of July) and comprised 12 species: 20% buck wheat (*Fagopyrum esculentum MOENCH*), 20% flax (*Linum usitatissimum L.*), 20% serradella (*Ornithopus sativus BROT.*), 8% corn (*Zea mays L.*), 7% sunflower (*Helianthus annuus L.*), 5% bristle oat (*Avena strigose SCHREB.*), 5% camlina (*camelina sativa L.*), 4% winter oilseed rape (*Brassica napus L.*), 4% white mustard (*sinapsis alba L.*), 3% deeptill radish (*Raphanus sativus var. oleiformis*), 2% sudan grass (*Sorghum sudanense STEUD.*), 2% lacy phacelia (*Phacelia tanacetifolia BENTH.*). After the shallow incorporation of the fall cover crop in September, a frost tolerant winter cover crop mixture (Wintergrün, Camena Samen) was sown that contained five species: 62% winter rye (*Secale cereale L.*), 26% Hungarian vetch (*Vicia pannonica CRANTZ.*), 10% crimson clover (*Trifolium incarnatum L.*), 1% winter oilseed rape (*Brassica napus L.*), 1% winter turnip rape (*Brassica rapa L.*). The winter cover crop was shallowly incorporated at the end of April or beginning of May. The shallow incorporation was done each time with a rotary tiller with right-angled knives that cut the plants 3 cm below the soil surface. The result was a plant soil mixture on the surface that was left on the soil for 10 days. After that the soil surface was again treated with a rotary tiller and the winter cover crop, respectively the spring cash crop, was sown. In the PSC plot the GreenCarbonFix mixture undersown in the cereal was kept and further on managed equal to a temporary ley. In fall, when the cover crop in the DCC plot was incorporated, the PSC plot was mowed and the above ground biomass was removed from the field. In spring, when the winter cover crop on the DCC plot was incorporated, the PSC plot was mowed again, and the stubbles were incorporated the same way as in the DCC plot using a rotary tiller. The exact dates and management details of the four sampling times are provided in Table S.1 and an overview about the used cover crop mixtures is provided in Table S.2. For the fields E and F, soil sampling had to be reduced to three time points because of management issues with the seedbed preparation in these two fields. Consequently, those two fields were ploughed in spring and therefore the soil sampling before the incorporation of spring cover crop (t_2) was the last sampling time on these two fields. All cover crops were grown without any fertilizer and under organic farming conditions. Yet, on fields B and D for the spring cash crop, an organic fertilizer was applied after cover crop incorporation between t_2 and t_3. Same amounts of fertilizers were applied on both treatment plots. The C and N inputs from fertilization can be seen in Table 1. Right before the cover crops were incorporated with a rotary tiller (DCC in fall, DCC and PSC in

spring) 100 L ha^{-1} commercially purchased effective microorganisms (EM; Rottelenker, EM Schweiz AG, Switzerland) were sprayed on the cover crops. The objective of this measure is to improve the decomposition process and reduce C and N losses (Oberholzer, Herrmann, et al., 2024). This practice is commonly used by farmers in the region when they shallowly incorporate a cover crop and was therefore part of both cover cropping systems.

2.2 | Plant biomass sampling

In the DCC plots cover crop biomass was cut right before cover crop incorporation in a square of 50 × 50 cm with seven replications per field and subsequently dried at 65°C for 48 h to determine the dry weight. The sampling replication with the median weight was ground and analysed for C and N content by dry combustion (vario MICRO tube, Elementar, Germany), separately for each field. The concentrations of plant C and N were multiplied by the dry matter weight to obtain the cover crop C and N input.

2.3 | Soil sampling and sample treatment

Soil sampling was done before incorporation of the fall cover crop (t_0, September), about 4 weeks after the shallow incorporation (t_1, October), in early spring (t_2, March) and about 4 weeks after the incorporation of the spring cover crop (t_3, May; Table S.1 in the Supplementary Material). At every sampling time, three batches of soil samples were obtained for different analyses. Batch one to determine SOC, POXC and total N was sampled by taking five samples per sampling point using an auger (0–20 cm, 2 cm diameter) and subsequently separated per depth segment of 0–5, 5–10 and 10–20 cm. The GPS reference for each sampling point was done using a dGPS device (Geo7X, Trimble, USA) with an approximate measurement accuracy of 10 cm, allowing for point specific monitoring of soil properties over time. In total, six fields with each three plots (two DCC, one PSC), each with 13 sampling points, were sampled in three depths at four (field A, B, C and D) respectively three (field E and F) sampling times which resulted in a total number of 2574 soil samples. These samples were dried for 72 h or constant weight at 40°C and sieved to 2 mm. Batch two to determine Cmic, Nmic and Nmin was obtained by randomly sampling 15 subsamples in 0–10 cm depth in four replicates per plot and sampling time ($n = 264$). Samples were stored at 4°C and sieved to 2 mm before the analysis of Cmic and Nmic. Thereof a part of sieved soil was frozen at −20°C for the analysis of Nmin. Batch three to determine soil bulk density and soil water content was obtained by sampling three undisturbed soil cores per plot and sampling time from 0 to 20 cm with 5 cm diameter that were taken with an impact probe (HumaxTube®, Switzerland). These cores were cut into 5 cm segments, weighed and dried at 105°C for at least 48 h to assess soil bulk density and water content for each 5 cm layer ($n = 792$).

2.4 | Spectral measurement and modelling

All 2574 samples of batch one were measured with a vis–NIR spectrometer (350–2500 nm, ASD FieldSpec 4 Hi-Res, Malvern Panalytical, USA) in five replicates using a contact probe in a dark room. We treated the samples from each field as one individual dataset ($n = 468$ for fields A, B, C and D and $n = 351$ for fields E and F) for the spectral modelling. For every field 15% of the samples were selected as reference samples for wet chemistry analysis based on a Kennard-Stones algorithm that uses the principal component scores to select a representative subset of a given dataset (Wadoux, 2021). Therein, sampling times and soil depth were similarly represented. For each parameter (SOC, POXC and total N) and for every field a spectral model was calibrated with the reference samples to predict the values for the other samples. For every spectral model we selected the optimal preprocessing technique and applied a partial least square regression (PSLR; Wold et al., 1983). A five times repeated fivefold cross-validation approach was used to calibrate for a spectral model for each field and soil property. We evaluated the model performance using the three model performance parameters, coefficient of determination (R^2), root mean standard error (RMSE) and the ratio of performance to deviation (RPD) which is the ratio of standard deviation of the measured reference values to RMSE. According to Chang et al. (2001) and Zhang et al. (2018) we considered an RPD above 3 as excellent, above 2 as accurate, above 1.4 as approximate and below 1.4 as poor model performance. The RMSE has always the unit of the measured parameter and therefore does not allow a generalized evaluation scheme. The executed preprocessing steps and the accuracy of the final chosen model for SOC, POXC and total N can be found in Table S.3 in the Supplementary Material. Spectral models for SOC and POXC on fields A and F showed an approximative performance while all other models showed an accurate or even excellent performance. The slightly lower model performance of fields A and F can probably be explained by their higher carbonate content (see Oberholzer, Summerauer, et al. (2024)). The RMSE

ranged between 1.07 and 2.43 g kg^{-1} for SOC, 0.03 and 0.05 g kg^{-1} for POXC and between 0.09 and 0.14 for total N. These achieved RMSE from spectral models were relatively close to the lab measurement errors that were 1.01 ± 0.40 g kg^{-1} for SOC, 0.02 ± 0.01 g kg^{-1} for POXC and 0.07 ± 0.02 g kg^{-1} for total N (Oberholzer, Summerauer, et al., 2024).

2.5 | Chemical soil analyses

For the reference samples of batch one ($n = 386 \triangleq 15\%$ of all samples) concentrations of total C and total N were determined by dry combustion (vario MICRO tube, Elementar, Germany). Inorganic C was determined through dissolution of carbonate in 10% HCl-solution and measurement of the volume of the evolved CO_2, and SOC as the difference between total C and inorganic C. POXC was measured based on the protocol of Weil et al. (2003) with the modifications of Lucas and Weil (2012), where 2.5 g instead of 5 g soil were used to make sure that enough reactant (0.2 M $KMnO_4$) is available (Culman et al., 2012; Lucas & Weil, 2021).

Cmic and Nmic were measured based on the protocol of Vance et al. (1987) with some adaptations: We weighed moist soil equal to 10 g dry matter and used 40 mL of 0.5 M K_2SO_4. After the extraction, dissolved C and N were measured with a TOC-analyser (DIMATOC® 2100, DIMATEC Analysetechnik GmbH, Germany). We did not use any conversion factor and report Cmic and Nmic as chloroform labile C and N. For the measurement of Nmin as the sum of nitrate and ammonium, 4 g of soil were extracted with 40 mL 1 M KCl. Nitrate was determined by using vanadium (III) as a reductant according to the Protocol of Garcia-Robledo et al. (2014). Nitrate content in the solution was colorimetrically determined by measuring the absorbance at 540 nm with a Spectrophotometer (UV-1800, Shimadzu Corporation, Japan). Ammonium was determined as described in Rhine et al. (1998) with salicylate as a reactant. The ammonium absorbance was measured at 650 nm with the same spectrophotometer.

2.6 | Calculation of soil organic carbon, permanganate oxidizable carbon and total N stocks

Due to seasonal and management induced changes in soil bulk density over the nine-month period of the trial, we used an equivalent soil mass (ESM) approach to calculate stocks and stock changes of SOC, POXC and total N. The concept of ESM was introduced by Ellert and Bettany (1995) and evaluated by Lee et al. (2009). When the soil bulk density varies over time and between treatments, stocks of a fixed depth (FD) contain different soil masses, which makes a comparison between them uneven. The ESM approach uses a reference soil mass that is used for a correction to obtain stocks of same soil masses among all treatments and sampling times. We used here the minimum ESM approach (Lee et al., 2009) and used the sampling time with the lowest bulk density to set the minimum reference soil mass. The SOC, POXC and total N stocks of the other sampling times were accordingly adjusted to an equivalent soil mass. For every soil layer (i) the FD stock was calculated as:

$$C_{i,fixed} = conc_i * M_i$$

where $conc_i$ is the concentration and M_i the dry soil mass of the corresponding layer. Then for every soil layer the surplus soil mass ($M_{i,add}$) was calculated:

$$M_{i,add} = M_i - M_{i,equiv}$$

where $M_{i,equiv}$ is the equivalent or reference soil mass of the corresponding layer. The stocks of the first soil layer (0–5 cm) were obtained by subtracting the surplus soil mass times the concentration in 0–5 cm:

$$C_{0-5} = C_{0-5,fixed} - M_{0-5,add} * conc_{0-5}$$

For the 5–10 cm layer ESM stocks were obtained by adding the surplus stock of the 0–5 cm layer and deducting the surplus soil masses from 0–5 and 5–10 cm times the concentration of the 5–10 cm layer:

$$C_{5-10} = C_{5-10,fixed} + M_{0-5,add} * conc_{0-5} - (M_{0-5,add} + M_{5-10,add}) * conc_{5-10}$$

Accordingly, the calculation was also done for the 10–20 cm layer. At the end there remains a soil mass that is unaccounted and must be dropped to obtain an ESM.

We calculated stocks based on the ESM approach for SOC, POXC and total N. We did not calculate stocks for Cmic, Nmin and Nmic because we measured them only in one depth (0–10 cm).

2.7 | Data evaluation and statistics

2.7.1 | Field specific evaluation

The objective of this study was to assess the influence of two cover cropping strategies on soil parameters. We thus do not focus much on absolute values but rather on the changes of these values over time. For SOC, POXC and

total N, we subtracted the measured values of sampling t_0 from the values of sampling t_1, t_2 and t_3 to obtain a stock change for every GPS-referenced sampling point and sampling time t_1, t_2 and t_3. Since samples for Cmic, Nmin and Nmic were not GPS referenced, we subtracted the mean of sampling t_0 from concentration values of samplings t_1, t_2 and t_3. These changes relative to sampling t_0 showed for all data a normal distribution or could be transformed to fulfil the requirement of normality with log(x), sqrt(x) or 1/x. Since we had an unequal sample size, we used the Levene's-test to check for equal variances. To detect significant differences in changes between treatments a Welch two-sample *t*-test was applied for every field and sampling time separately.

To test the changes over time within one treatment we applied a multiple pairwise comparison using a paired *t*-test for the GPS referenced samples (SOC, POXC, total N). When the different sampling times of the samples of batch two (Cmic, Nmin and Nmic) were combined per field, the data often could not be transformed to a normal distribution. For these samples we therefore used the non-parametric Kruskal–Wallis test followed by a Dunn's post hoc test to detect significant changes over time within one treatment. For both, the multiple *t*-test as well as the Dunn's test, we used the Holm method to correct for multiple pairwise comparisons.

2.7.2 | Statistics across fields

To test the treatment influence in different soil depths, we took the changes in SOC, POXC and total N between t_0 and t_3 for field A, B, C and D and applied a general mixed model with treatment and soil depth as fixed factors and field as random factor.

We related the changes between t_0 and t_1 to the fall cover crop input and the changes between t_2 and t_3 to the spring cover crop input to analyse the relationship between C inputs and changes in SOC, POXC and Cmic as well as N inputs and changes in total N, Nmin and Nmic. For this analysis we only considered the data from the DCC plots since the PSC plots did not have any above ground input. For field C and D, we added the fertilizer C and N input to the spring cover crop input.

All analyses were performed in R version 4.0.3 (R Core Team, 2020). The spectral datasets were analysed using the R-package simplerspec (Baumann, 2019) in combination with the packages prospectr (Stevens & Ramirez-Lopez, 2020) and caret (Kuhn, 2020). In the figures and in the text means and standard errors are presented.

3 | RESULTS

3.1 | Cover crop performance

Cover crop above ground biomass showed large differences between the six fields in the DCC treatment. In Figures 2 and 3, fields are therefore ordered according to total cover crop biomass in the DCC treatment (fall and spring) whereby field A had the highest (716 g m^{-2}) and field F the lowest (102 g m^{-2}) cover crop biomass produced in the entire duration of the trial. The cover crop C content ranged between 38% and 42% and the N content between 1.8% and 3.1%. All figures that show changes in the selected parameters also indicate the cover crop C or N inputs for each field.

3.2 | Changes in soil C fractions (SOC, POXC, Cmic)

Soil organic carbon stocks ranged from 4.2 ± 0.1 (Field E) to 8.2 ± 0.2 kg m^{-2} (Field F) at t_0 and on each field, the changes over time in 0–20 cm soil depth were quite similar between the DCC and PSC treatment (Figure 2a). The only significant difference between treatments was observed on field F for t_1 where the PSC treatment showed significantly higher increases in SOC stocks than the DCC treatment. On every field we determined significant differences in SOC stocks over time in at least one treatment. At the end of the nine-month trial, the maximum increase in SOC stocks over time in 0–20 cm soil depth was measured on the PSC plot of field A with +0.46 ± 0.06 kg m^{-2}. The maximum decrease in SOC stocks was measured on the DCC plot on field E at t_1 (−0.38 ± 0.05 g m^{-2}). In relative terms, SOC stocks changed between −8.5 ± 1.1% (DCC field E, t_1) and + 8.3 ± 1.0% (PSC field A, t_2) over the monitoring period of 9 months in 0–20 cm soil depth.

At the start of the experiment (t_0), POXC stocks ranged between 181 ± 8 g m^{-2} (Field E) and 225 ± 8 g m^{-2} (Field C) and only a few differences between PSC and DCC treatment were measured over time (Figure 2b). The DCC treatment exceeded the PSC treatment significantly on field C at t_2 and on field D at t_1 (Figure 2b). On the other hand, the PSC treatment on field F showed significantly higher changes in POXC stocks than the DCC treatment at t_1. On all fields, the POXC stocks in the DCC treatment were significantly higher at the last sampling time than at t_0. For the PSC treatment, only on field B, the POXC did not significantly increase during the trial while all other fields showed significantly higher POXC stocks at the last sampling time compared to t_0. The maximum significant increase in POXC stocks over time was +18.9 ± 2.1 g m^{-2} (PSC field F, t_2), which corresponds to a relative increase of +10.4 ± 1.2%.

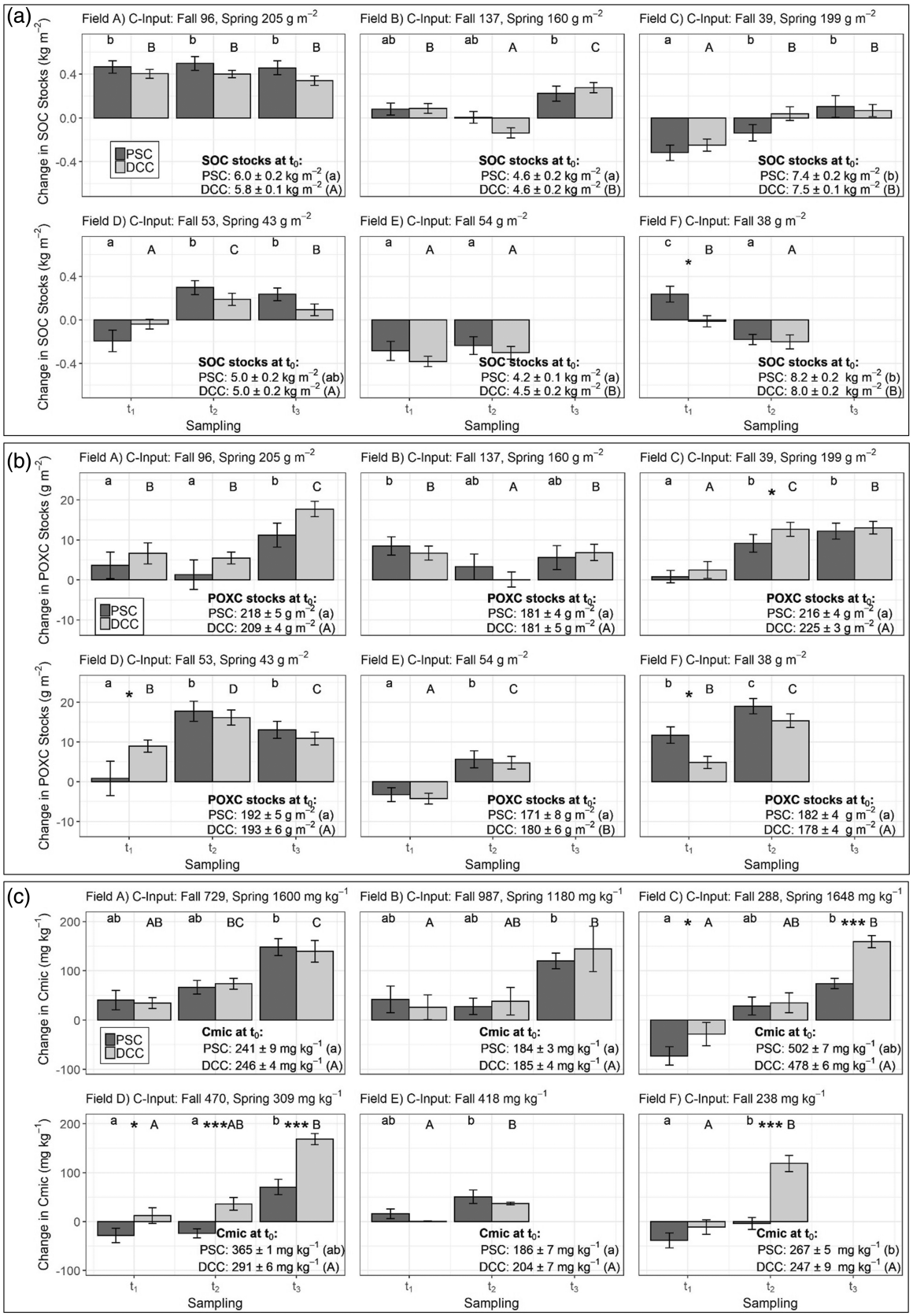

FIGURE 2 Legend on next page.

Microbial C ranged from 184 ± 3 mg kg^{-1} (Field B) to 502 ± 7 mg kg^{-1} at t_0 and increased over time reaching highest values in all treatments at the last sampling time (Figure 2c). On fields C, D and F, microbial C was at least at one sampling time significantly higher in the DCC than in PSC treatment. At the end of the trial on field C, D and F the changes in Cmic in the DCC plots exceeded the PSC plots significantly by 85 ± 23, 97 ± 27 and 123 ± 29 mg kg^{-1} which corresponds to a relative increase of +17.8 ± 4.5, +33.6 ± 9.3 and + 49.6 ± 11.5% compared to t_0.

3.3 | Changes in soil N fractions (total N, Nmin and Nmic)

Soil N stocks ranged from 496 ± 19 g m^{-2} (Field E) to 734 ± 6 g m^{-2} (Field C) at t_0 and the development of total N stocks over time was very distinct on the different fields and did not show a clear pattern. Only at two time points significant differences in changes of total N stocks (0–20 cm) between the two treatments were observed (t_1 on field D and F; Figure 3a). Compared to t_0, total N stocks varied between −7.6 ± 1.1% (PSC field E, t_2) and + 7.3 ± 1.0% (PSC field F, t_1).

Mineral N ranged from 55 ± 7 mg kg^{-1} to 112 ± 5 mg kg^{-1} at t_0 and showed on most fields a higher increase in the DCC treatment. On fields A, B, D and F we observed at least at one sampling time significantly higher Nmin changes in the DCC treatment with highest differences in spring (t_3) where the Nmin changes in the DCC plots of fields A, B and D exceeded the changes in the PSC plots by +19 ± 10, +11 ± 9 and + 18 ± 6 mg kg^{-1} (Figure 3b). On all fields Nmin decreased from t_0 to t_2 after winter between −21 ± 17% (PSC plot field C) and − 77 ± 10% (DCC plot field E) compared to t_0. The ratio between nitrate-N and ammonium-N did not show a treatment effect but varied substantially over time and was for all fields highest in fall at t_0 or t_1 (between 9 and 15) and lowest in spring at t_2 or t_3 (between 0.5 and 6; Figure S.1 in the Supplementary Material).

Similar to Nmin, Nmic showed similarly large differences between treatments at several time points on all fields except field E (Figure 3c). Highest differences were measured at t_3 where changes in the DCC plots significantly exceeded the changes in the PSC plot on field B, C and D by +25 ± 15, +29 ± 7 and + 25 ± 8 mg kg^{-1}. This corresponds to percental increases of +63 ± 29, +102 ± 12 and + 97 ± 8% in Nmic compared to t_0 for fields B, C and D, respectively.

3.4 | Changes in SOC, POXC and total N in different soil depths

During the experimental period of 9 months, SOC, POXC and total N generally showed an increasing trend, yet depth-specific differences (Figure 4, fields A-D only). In particular, the 5–10 cm depth segment always showed the highest increases, compared to the 0–5 cm and the 10–20 cm depth segments (Figure 4). In 5–10 cm soil depth, the PSC treatment showed significantly higher increases in SOC (but not POXC or total N) than the DCC treatment ($p = 0.026$). On the other hand, in depth 0–5 cm the DCC treatment showed significantly higher increases in POXC stocks ($p = 0.037$) compared to the PSC treatment. The absolute stocks for SOC, POXC and total N per depth segment and sampling time can be seen in Table S4 in the Supplementary Material. The same analysis applied on concentrations instead of stocks obtained the same results (see Figure S.2 in the Supplementary Material).

3.5 | Relationship between C and N input by double cover cropping and soil C and N fractions

There was a significant linear relationship between above ground cover crop plus fertilizer C or N input by the two cover crop incorporations in the DCC treatment and changes in Cmic or Nmic (Figure 5). Parameters SOC, POXC, total N and Nmin did not show a significant relationship with above ground C or N inputs (data not shown).

4 | DISCUSSION

Cover crop growth on the six fields showed a high variability and reflects the difficulties to predict nitrogen dynamics under organic farming conditions with no mineral N fertilization. All cover crops were grown without

FIGURE 2 Changes in soil organic C stocks (SOC, 0–20 cm, a), permanganate oxidizable C stocks (POXC, 0–20 cm, b) and microbial biomass C (Cmic, 0–10 cm, c) over time relative to sampling t_0, which is listed for each field in the subplots. For every field A–F, the above ground cover crop C input in the double cover cropping (DCC) treatment is given in the title. Within each field, significant differences between treatments were tested with a *t*-test and are indicated with the codes: *** < 0.001, ** <0.01, * < 0.05. Significant changes over time within each treatment are indicated with letters for both treatments separately and were tested with a paired *t*-test for SOC and POXC and with a Kruskal–Wallis-test for Cmic. Error bars represent standard errors.

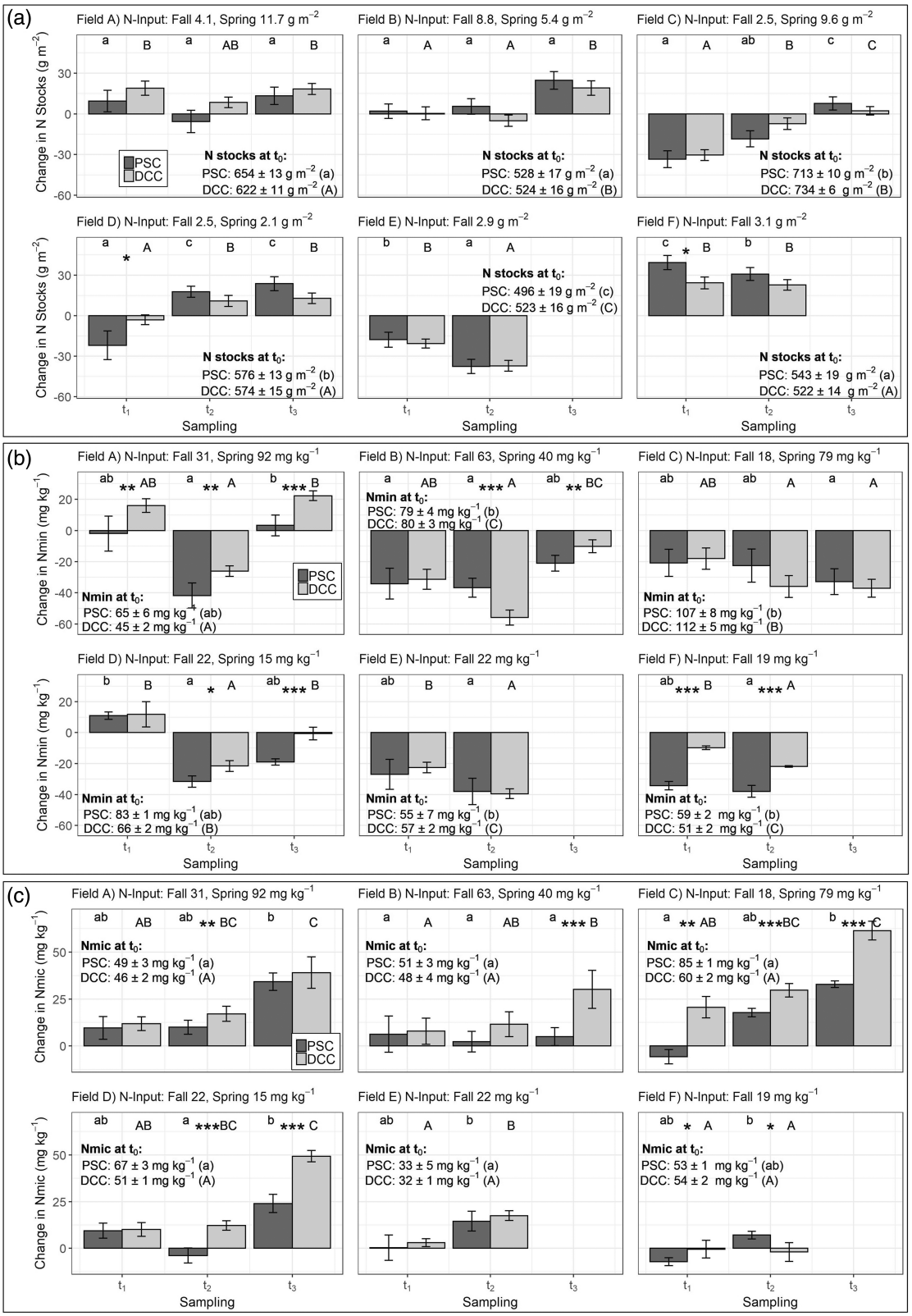

FIGURE 3 Changes in total N stocks (0–20 cm, a), mineral N (Nmin, 0–10 cm, b) and microbial biomass N (Nmic, 0–10 cm, c) over time relative to sampling t_0, which is listed for each field in the subplots. For every field A–F, the above ground cover crop N input in the double cover cropping (DCC) plot is given in the title. Within each field, significant differences between treatments were tested with a *t*-test and are indicated with the codes: *** < 0.001, ** <0.01, * < 0.05. Significant changes over time within each treatment are indicated with letters for both treatments separately and were tested with a paired *t*-test for total N and with a Kruskal–Wallis-test for Nmin and Nmic. Error bars represent standard errors.

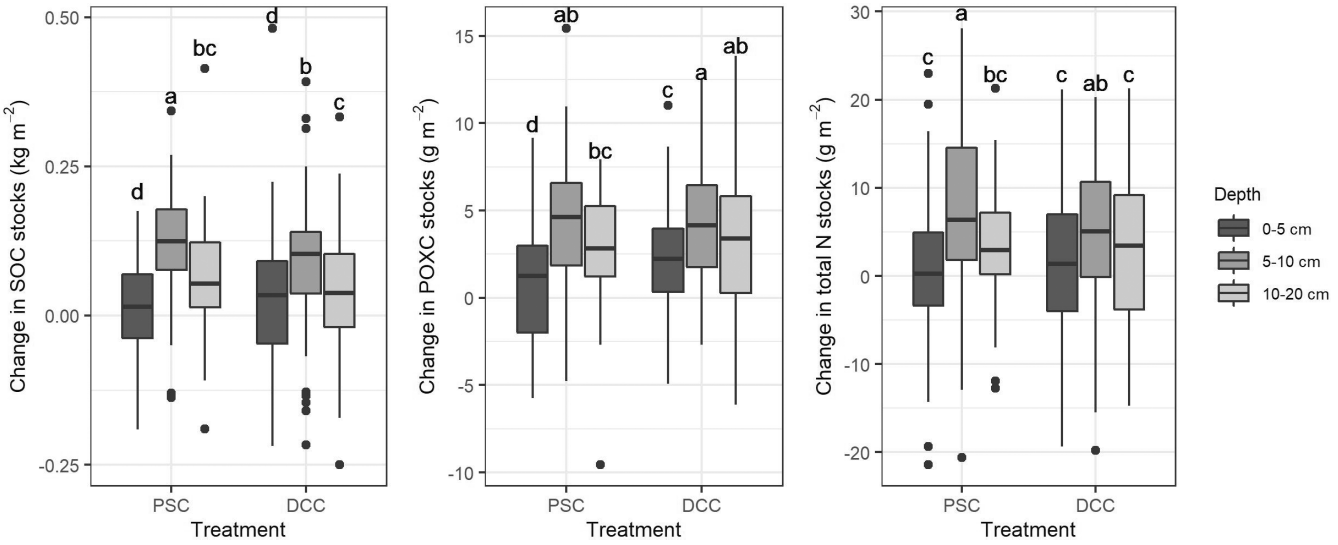

FIGURE 4 Boxplot of changes in soil organic C (SOC) stocks, permanganate oxidizable C (POX) stocks and total N stocks per depth and treatment between sampling t_0 and t_3 (only fields A–D). The values from segment 10–20 cm were divided by 2 to reach equal layer thickness. Different letters indicate significant differences between depth: treatment combinations according to the mixed linear model with fixed factors treatment and depth and random factor field.

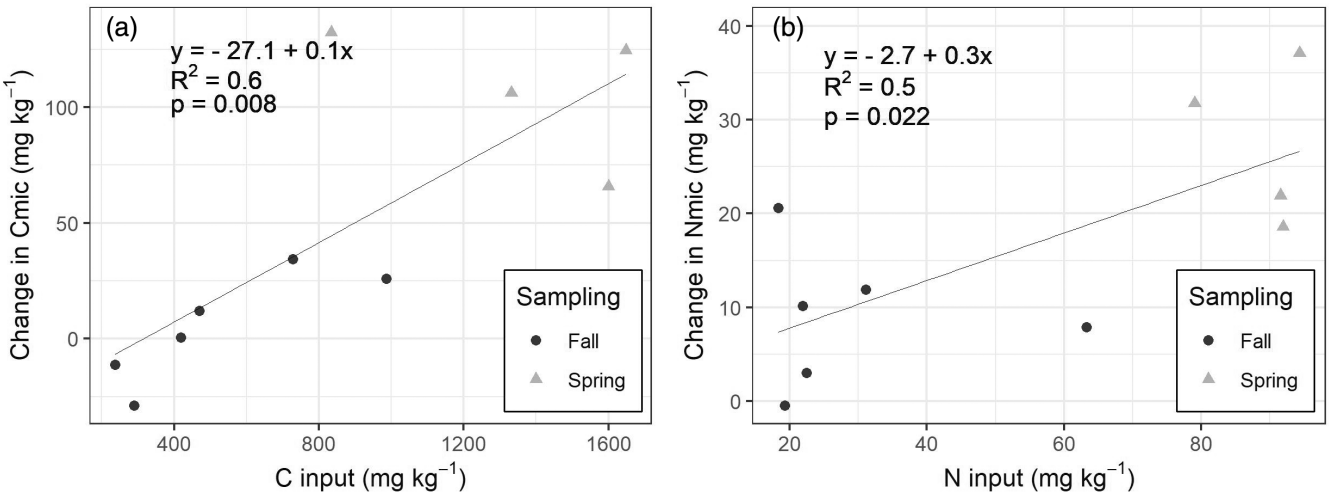

FIGURE 5 Correlation of organic C and N inputs (cover crop + fertilization) with changes in microbial biomass C (Cmic, a) and N (Nmic, b) in the double cover cropping treatment. Changes are calculated for the cover crop incorporation in fall (between sampling t_0 and t_1) and in spring (between sampling t_2 and t_3).

any starter fertilization which resulted in poor cover crop growth on fields E and F with lowest initial Nmin concentration (Figure 3c). Despite the variability, the following general trends were observed between and within the two cover cropping strategies.

4.1 | High short-term temporal variability

We observed significant differences between the two cover cropping strategies in Cmic, Nmin and Nmic at multiple time points, but only at very rare occasions in SOC, POXC and total N in 0–20 cm soil depth (Figures 2 and 3). Due to the high spatial and temporal variability, SOC, POXC and total N did not show consistent effects that could be attributed to cover crop management or above ground biomass input. However, we determined a few significant but non-consistent changes over time for SOC and total N. Changes in SOC stocks in 0–20 cm soil depth ranged between -0.38 ± 0.05 kg m^{-2} and $+0.46 \pm 0.06$ kg m^{-2} in both treatments established during the 9 months cover cropping period. This latter number is much larger than the estimated annual C sequestration potential of cover

cropping of 0.02 to 0.06 kg m^{-2} yr^{-1} in the latest literature (Jian et al., 2020; McClelland et al., 2021; Poeplau & Don, 2015). The difference may be explained by the fact that we looked here into the immediate changes in SOC that is induced by different cover cropping methods, while the cited meta-analyses only considered SOC changes from longer-term trials. Therefore, short-term changes should not be used to deduce long-term C-sequestration rates. Nevertheless, the relative changes in SOC stocks between t_0 and other sampling times ranging between $-8.5 \pm 1.1\%$ and $8.3 \pm 1.0\%$ agree with a study reporting SOC to vary up to 15% around the annual mean (Wuest, 2014). The temporal variability of total N (between $-7.6 \pm 1.1\%$ and $+ 7.3 \pm 1.0\%$) was very similar to the one of SOC. This is plausible, because both parameters are strongly connected with soil organic matter dynamics. We can confirm our first hypothesis because for the full sampled soil depth (0–20 cm), we did not measure consistent differences between the two treatments. On the other hand, POXC showed a consistent and significant increase in most fields and time points and both treatments over time (Figure 2b). The maximum changes in POXC stocks between two sampling dates were $+18.9 \pm 2.1$ g m^{-2} which corresponds to a concentration change of $+72.7 \pm 8.1$ mg kg^{-1} (assumed bulk density = 1.3 g cm^{-3}) were in the same range as the maximum changes of POXC concentrations in Lucas and Weil (2021) after a two-year cover cropping period. However, in a soil depth of 0–20 cm we did not observe consistent treatment effects on POXC, which does not confirm our second hypothesis of higher POXC stocks in the DCC treatment due to above ground biomass input.

4.2 | Relating changes in soil C fractions with C input

The maximum above ground C input of both cover crops in the DCC treatment of around 300 g m^{-2} (field A) was in the same range as the maximum changes in SOC stocks and around 15 times higher than the maximal changes in POXC stocks (Figure 2a,b). We did not find any relationship between above ground biomass C input and SOC or POXC stock changes. This suggests that most C input by incorporated cover crops was quickly used by soil microorganisms as also indicated by the strong relationship between C inputs of cover crop biomass and changes in Cmic (Figure 5). The consistent increase in POXC stocks with cover cropping was also observed by Burke et al. (2019) and was probably related to active cover crop root growth and not to above ground biomass input.

POXC is often seen as a sensitive indicator for agricultural management and changes in POXC are sometimes considered as an indicator for changes in SOC (Bongiorno et al., 2019; Jagadamma et al., 2019). We found significant linear relationships between SOC and POXC concentrations ($0.23 \leq R^2 \leq 0.85$, $p < 0.001$) on every field (Figure 6a) and for fields B, C, D and E also a significant positive relationship between changes in POXC and SOC stocks ($0.11 \leq R^2 \leq 0.59$, $p < 0.001$; Figure 6b). However, the relationship between changes in POXC and SOC stocks is relatively weak indicating that in the short-term these two C fractions can react quite independent from one another.

4.3 | Differences between treatments in different soil depths

Taking the fields with four sampling times (A–D) together we found significantly higher changes in SOC, POXC in 5–10 cm soil depth compared to 0–5 cm soil depth in both treatments (Figure 4). We see three potential mechanisms that might explain why the highest increase in SOC was measured for both treatments in 5–10 cm and not in 0–5 cm depth. First, the 0–5 cm soil depth was also the layer that was intensively tilled in fall and spring for the DCC and only in spring for the PSC treatment. Despite the shallow tillage depth of around 3 cm, one must be aware that a rotary tiller is a very intensive tillage method since it cuts the cover crop plants below ground with a speed of around 500 revolutions per minute and therefore potentially broke soil aggregates (Li et al., 2023) which could have led to C loss due to increased microbial respiration. This intensive tillage in the 0–5 cm soil depth was likely a main driver why, despite higher organic matter input, lower accumulation rates of SOC and POXC were observed than in the below layer of 5–10 cm soil depth. A second explanation might be that the lower C saturation in the 5–10 cm layer fostered C accumulation more compared to the top 0–5 cm where SOC concentrations were already higher. Thirdly, and in relation to that, dissolved organic C (DOC) might have leached from the topsoil and absorbed in the 5–10 cm layer.

Looking at each depth segment separately, we also found significant treatment effects for SOC and POXC: in the PSC treatment, we found significant higher increases in SOC stocks in 5–10 cm depth but significantly lower increases in POXC in 0–5 cm depth compared to the DCC treatment. The higher increase of SOC in the PSC treatment in 5–10 cm soil depth can be explained by possibly higher below ground C inputs in the PSC compared to the DCC plot. Literature values demonstrated that the cover crop species in the PSC treatment (mainly perennial ryegrass and clovers) had higher root/shoot ratio than most other cover crop species that were present in the mixtures of the DCC treatment (Hu et al., 2018).

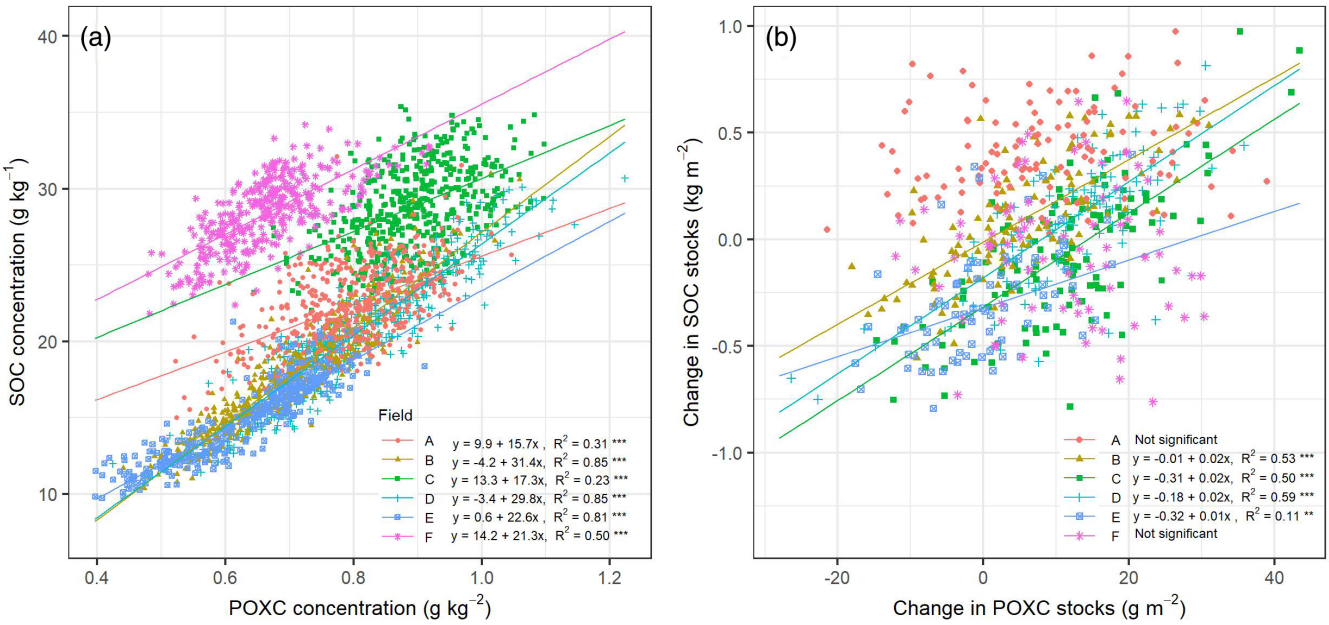

FIGURE 6 (a) Scatter plot of soil organic C (SOC, 0–20 cm) and permanganate oxidizable C (POXC, 0–20 cm) with a regression for each field. (b) Scatter plot of changes in SOC and POXC stocks with a regression for each field (if significant). Significant codes: *** < 0.001, ** <0.01, * < 0.05.

Additionally, since there was only one cover crop mixture sown in the PSC treatment the root system had a longer time to develop than in the DCC treatment and root biomass is considered to be more important than above ground biomass for stabilizing soil organic matter (Balesdent et al., 2017; Ghafoor et al., 2017). The significantly higher increase in POXC in 0–5 cm depth in the DCC compared to the PSC treatment is probably related to the increase in microbial biomass. As can be seen in Figure 7, Cmic is stronger related to POXC than SOC and we think that the significant treatment difference in POXC in the topsoil (0–5 cm) is related to the stronger increase in microbial biomass and potentially microbial necromass in the DCC treatment.

4.4 | Microbial C

In general, among the analysed C-fractions, Cmic was most sensitive to the two treatments (Figure 2c). On three fields (C–F) we found significantly higher changes in Cmic for the DCC than the PSC treatment, which confirms our third hypothesis that the above ground biomass input increases Cmic. Above ground plant C input was linearly correlated with Cmic (Figure 5a) suggesting that the incorporated plant material had a large effect on Cmic ($R^2 = 0.6$). Besides cover crop C input, also tillage could have triggered soil microbial activity. In fall at t_1 when the DCC plots were shallowly tilled and the PSC plot were not, we observed significantly higher increases in Cmic on fields C and D in the DCC treatment compared to the PSC treatment. These differences between treatments on field C and D became even more pronounced in spring (t_3) when both plots were tilled the same way. Increases in Cmic in spring might be explained by the combination of labile C-inputs and rising temperatures. Other studies also saw an increase in Cmic of 27% to 40% due to cover cropping which is in the range of our results (18% to 50%; Kim et al., 2020; Muhammad et al., 2021). Unlike the cited literature, we found these increases in microbial biomass in a single cover cropping period which suggests that bringing labile organic material directly into the biologically most active soil layer (shallow incorporation) led to an immediate response of microorganisms. However, on fields A and B, we did not see a significant difference in changes in Cmic between the DCC and the PSC treatment even though these fields had highest amount of above ground cover crop input. The beneficial effects of the DCC treatment on Cmic could in the long-term lead to an increase in SOC and POXC stocks since labile organic matter input leads to microbial products that form a big part of stable soil organic matter as it is proposed by the 'microbial efficiency-matrix stabilization (MEMS) framework' (Cotrufo et al., 2013; Robertson et al., 2019).

4.5 | Mineral and microbial N

The two labile N fractions, Nmin and Nmic showed more pronounced treatment effects than Cmic (Figure 3). For both parameters we measured on four fields higher

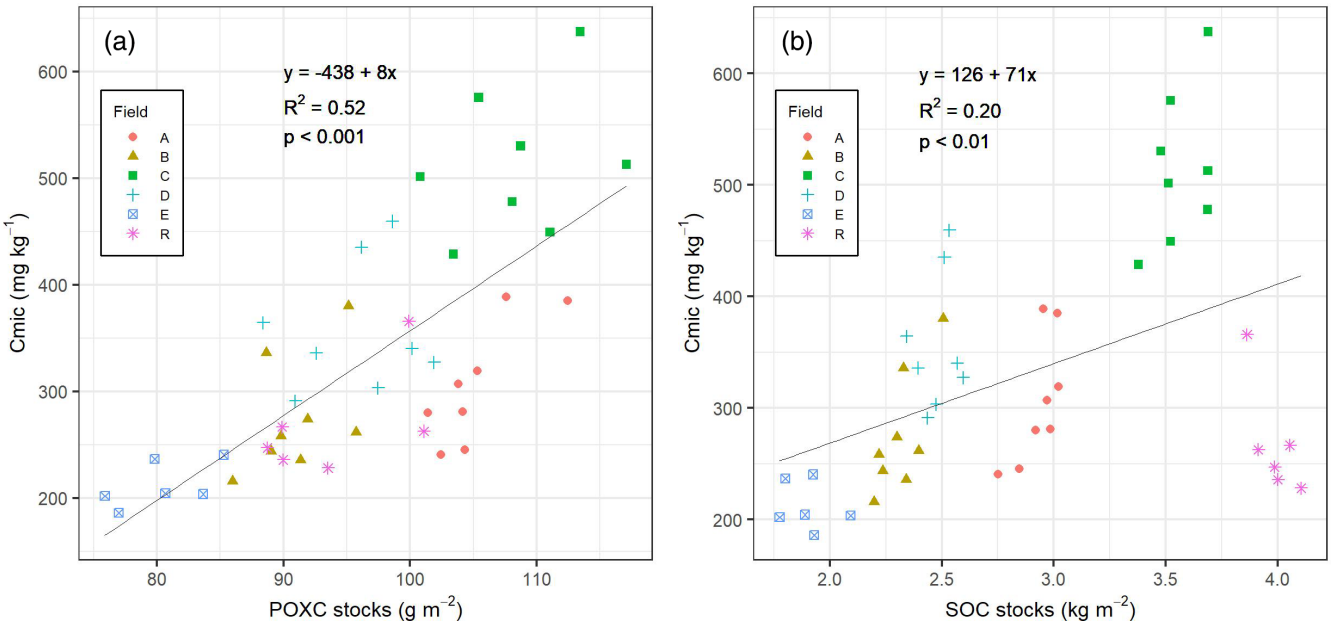

FIGURE 7 (a) Scatter plot of microbial biomass C (Cmic, 0–10 cm) and permanganate oxidizable C stocks (POXC, 0–10 cm, a) and soil organic carbon stocks (SOC, 0–10 cm, b) with a regression for all fields together.

increases in the DCC than in the PSC plot which confirms our third hypothesis. Despite their similar treatment effects, Nmin and Nmic showed an opposite development over time. Nmin decreased on most fields whereas Nmic increased on most fields. The decrease in Nmin by 21% to 77% was in a similar range as in other cover crop studies (Kramberger et al., 2009; Mohammed et al., 2020; Zhou et al., 2020) and can be explained by four possible mechanisms: uptake through growing plants, leaching into deeper soil layer or ground water, microbial immobilization, and gaseous N losses. We found on all fields, irrespective of the treatment, lowest Nmin values at sampling time t_3 after winter and since plant growth and microbial activity is low during winter, it is very probable that despite the cover crops some N was lost through leaching. This assumption is supported by the decreased ratio between nitrate-N and ammonium-N after winter since mainly nitrate is susceptible to leaching (Figure S.1 in the Supplementary Material). The increase in Nmic can be explained by cover crop N input and immobilization of already present Nmin in the soil. Though, we did not find a quantitative relationship between the decrease in Nmin and the increase in Nmic. During cover crop biomass decomposition, gaseous N losses (N_2O) may play a crucial role (Baggs et al., 2000; Carter et al., 2014), but are quantity-wise often in much lower ranges (Skinner et al., 2019). However, since we observed higher changes in Nmin and Nmic in the DCC plot compared to the PSC plot, we assume that at least some of the above ground plant biomass N stayed within the plant-soil-microbial system. Since Nmic is not available for plants, one cannot expect an immediate fertilization effect (Kramberger et al., 2009; Nevins et al., 2021), moreover the crop might not meet its N demand (Thorup-Kristensen et al., 2003). Research dealing with the benefits of cover cropping on N management mainly focuses on Nmin (White et al., 2017), while the dynamics of immobilized N (Nmic) in cover cropping systems is still understudied. Late incorporation time, as in this study, favours immobilization over mineralization of cover crop N input (Andersen & Jensen, 2001; Wyland et al., 1995) but we cannot make any assumption if and when this immobilized N may become plant available for the following crop.

5 | SUMMARY AND CONCLUSION

The widespread implementation of different cover cropping strategies requires information on their effects on soil organic matter dynamics for optimal management decisions. By assessing these dynamics in close spatial and temporal resolution for two cover cropping strategies during a nine-month period, we saw a high variability over time and between the six experimental sites. For SOC, total N and POXC we did not observe clear differences between strategies in 0–20 soil depth. When considering different soil depth segments, we observed that the strategy of PSC had significantly higher increases in SOC in 5–10 cm soil depth. The strategy of DCC showed instead significantly higher increases in POXC stocks in

0–5 cm soil depth. For the labile fractions Nmin, Cmic and Nmic, we observed generally higher increases in the DCC treatment, but these effects have not been observed consistently on all experimental fields.

We therefore conclude that the above ground biomass input in the DCC strategy was more beneficial for soil microbiology and Nmin, but the PSC strategy was more beneficial for short-term changes in SOC stocks. We hypothesize that the longer soil cover in the PSC treatment was accompanied by increased root growth and therefore higher below ground C inputs which seemed to be more important for SOC stocks than above ground biomass input. To find explanations for the effects of different cover cropping systems on SOC dynamics in more depth we therefore highly recommend to also measure below ground C inputs.

AUTHOR CONTRIBUTIONS

Simon Oberholzer: Writing – original draft; conceptualization; investigation; methodology; validation; visualization; writing – review and editing; software. **Klaus A. Jarosch:** Conceptualization; writing – review and editing; visualization. **Nadine Harder:** Investigation; methodology. **Markus Steffens:** Conceptualization; writing – review and editing; supervision. **Chinwe Ifejika Speranza:** Conceptualization; writing – review and editing; funding acquisition; supervision.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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