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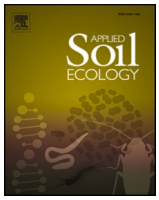

No effect on biological or chemical soil properties when amended with effective microorganisms for improved cover crop decomposition

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ABSTRACT

The implementation of cover crops into a crop rotation can contribute to a more sustainable soil management. For the improved decomposition of cover crop residues, the commercial inoculant Effective Microorganisms® (EM) is increasingly applied. Despite its extensive application, comprehensive studies on the effect of EM application on soil processes are lacking, since rarely a clean differentiation between an EM-effect (induced by living EM directly) or a substrate effect (induced by the accompanying EM substrate) is made. To determine the potential effects of EM application after cover crop integration to soil we conducted a lab incubation experiment under spring-like conditions in temperate climates and applied EM either on bare soil or on cover crops prior to soil incorporation at recommended and 100 times the recommended doses. Control groups included treatments with no EM addition and a sterilized EM solution applied at 100 times the recommended dose. Over a monitoring period of 28 days, the application of EM at the recommended dose showed no consistent effect on soil respiration, microbial bound carbon or nitrogen, soil pH, permanganate oxidizable carbon or water extractable nutrients and trace elements. Any observed effects in the treatment that received 100 times the recommended dose was attributed to the substrate introduced with the EM solution rather than the living EM themselves. Amplicon sequencing showed that certain EM taxa could be detected in soil at low abundance after EM application, but only when EM were applied at 100 times the recommended dose. We conclude that the application of EM did not produce a discernible effect on soil biological or chemical properties, nor did it influence the decomposition process of the cover crop.

1. Introduction

Sustainable agroecosystems aim to maintain a high level of soil fertility to minimize the external inputs. For that, periods of bare soil should be avoided because they lead to nutrient losses, soil erosion and loss in soil organic matter, leading to a decrease of soil fertility (Daryanto et al., 2018). Cover crops bridge the break time between two main crops and are therefore a key element in soil fertility and nutrient management (Thorup-Kristensen et al., 2003). However, particularly in organic farming systems with reduced tillage, where cover crops are shallowly incorporated or left on the soil surface, the management of cover crops faces major challenges. A fast decomposition of the incorporated cover crop residues is crucial for a good seedbed preparation (Gollner et al., 2020; Vincent-Caboud et al., 2017). Yet, when environmental conditions are cold and wet, as it often happens during spring in temperate climates, the cover crop material on the soil surfaces often does not decompose properly but becomes slimy and malodorous. This largely affects the seedbed preparation and the growth of the subsequent crop. Ideally, most cover crop material should be decomposed to smaller pieces within 10 days so that residues do not disturb the sowing of the subsequent cash crop.

One increasingly used approach to accelerate the decomposition process of freshly incorporated cover crop material is the use of microbial inoculants. The most applied microbial inoculant with this purpose

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is Effective Microorganisms® (EM), a product that was developed in the 1980s and was trademarked by EMRO Japan (2023). The commercial EM product consists of a mix of up to 80 naturally occurring aerobic and anaerobic microorganisms which coexist in a liquid culture (Higa, 1991). The exact composition is not made available by the producer, but previous analyses have shown that EM solutions are mainly dominated by lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus casei, Streptococcus lactis*) and yeasts (*Saccharomyces cerevisiae, Candida utilis*) with smaller numbers of photosynthetic bacteria (*Rhodopseudomonas palustris, Rhodobacter sphaeroides*), actinomycetes (*Streptomyces albus, Streptomyces griseus*) and fermenting fungi (*Aspergillus oryzae*; Ahn et al., 2014; Xu, 2000). Similar to other plant growth promoting rhizobacteria (PGPR), EM are applied to alter the soil microbial community towards more favorable growing conditions for the crop (Gouda et al., 2018). In practice the expectations of EM application are, among others, enhanced soil fertility, higher crop yield and quality, higher nutrient use efficiency of organic fertilizers and amendments, improved soil physical characteristics, and better pathogen control (Balogun et al., 2016; Olle and Williams, 2013). In the specific application of EM on cover crops before shallow incorporation, farmers expect to accelerate the decomposition process, improve nutrient cycling and soil organic matter formation (EM Schweiz, 2023).

The suggested mechanisms how EM might influence the decomposition of cover crop biomass or other organic matter in soil are derived from analogies of food preservation and processing of kitchen wastes through anaerobic fermentation widely practiced in Asia. For anaerobic fermentation, it is of most importance that fermenting microorganisms are dominant over putrefactive bacteria that might damage the product and lead to malodorous and potentially harmful metabolites (Rhee et al., 2011; Wang et al., 2001). Putrefaction is associated with the emission of ammonia, methane and nitrogen (N) oxides and occurs under at least partly anoxic conditions. Effective microorganisms are supposed to avoid putrefaction in periods or locations of low oxygen availability and shift the metabolic pathways towards fermentation and stabilization of organic matter (Higa and Parr, 1994). Most arable soils are mainly under oxic conditions, but anoxic microsites are always present in well aerated soils as well (Keiluweit et al., 2018; Keiluweit et al., 2017; Lacroix et al., 2022). Accordingly, EM is proclaimed to benefit the decomposition of organic matter even in well aerated soil with rather oxic conditions (Hu et al., 2018; Javaid, 2011). Lactic acid bacteria and yeasts, the dominant groups in the EM consortia, are facultative anaerobic, meaning that they can survive in an environment with oxygen and are therefore also found in natural soils (Lamont et al., 2017). The application of EM for enhanced organic matter decomposition relies thus on the assumptions that first the inoculated EM can establish themselves in the soil system and second that they play a dominant role in the decomposition process.

Up to now only a very limited number of studies surveyed the effect of EM application on critical soil properties such as soil respiration (Fatunbi and Ncube, 2009; Schenck zu Schweinsberg-Mickan and Müller, 2009; Valarini et al., 2003) or nutrient availability (Hu et al., 2018; Jusoh et al., 2013; Van Fan et al., 2018; Zhong et al., 2018). Other studies suggest, but did not demonstrate that the enhanced decomposition of organic matter via EM application could also lead to an increased availability of micronutrients (Daur, 2016), or a reduction of potential toxic trace elements (PTTEs), (Zhou et al., 2020). Unfortunately, many of the studies that tested EM failed in differentiating between i) the EM-effect (an effect that is induced by the actual living EM in the inoculant) and ii) the substrate effect (an effect that is induced by the nutrients, carbon sources and other compounds that is provided in combination with the EM inoculant solution). By not differentiating between these two effects, it is easy to reach misleading conclusions, yet, the inclusion of these critical controls quickly increases the number of necessary samples.

Given the large discrepancy between expectations and actual scientific evidence on the actual effects of EM we conducted a lab incubation study to rigorously differentiate between EM induced effects and substrate induced effects on soil properties during cover crop decomposition. The question whether or not the decomposition of freshly incorporated cover crops can be positively influenced by EM application has a particular relevance, since the lack of adequate alternatives was identified as a major challenge that hampers the practice of cover cropping in organic reduced tillage systems (Vincent-Caboud et al., 2017). To obtain a mechanistical understanding on the potential effectiveness of EM application on soil processes we conducted a soil incubation experiment mimicking spring-like field conditions in temperate climates. Soils were incubated alone or in combination with cover crop plant material and amended with typical or 100 times the typical application dose. As control treatments, we sterilized the EM solution prior to application to rigorously differentiate between an EM effect and a substrate effect. We followed several soil biological and biochemical soil properties over the course of 28 days to determine any immediate or mid-term effect of EM application to soil properties.

2. Methods

2.1. Sampling and preparation of soil and cover crop biomass

The soil and the cover crop biomass were sampled from an agricultural field situated in the temperate climate zone in Diessenhofen, Canton Thurgau, Switzerland at 414 m elevation. The farmer has been practicing the shallow incorporation of cover crops with the application of EM in the last five years and reported positive experiences with respect to soil structure and crop yields. The sampling was conducted on 5 May 2020 when the cover crop was well established and about to be shallowly incorporated. Approximately 200 soil cores (0–10 cm) were taken randomly with an auger (2.5 cm diameter) on the field of 1.3 ha size. The cover crop aboveground biomass was cut in a representative 50 × 50 cm square on the same day. The sown cover crop was purchased (Wintergrün, Camena Samen, Germany) and contained 5 frost tolerant 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.*). In our plant sample we only collected winter rye, hungarian vetch, and crimson clover. The harvested and dried cover crop had a carbon (C) concentration of 42.2 % and a C/N ratio of 17.7.

The sampled soil (approx. 15 kg) was air dried at room temperature for three days before it could be sieved (2 mm). Remaining larger pieces of organic material were removed manually. The collected cover crop biomass was placed in the drying oven at 40 °C for a week and then cut into small pieces.

2.2. Effective microorganisms®

For this experiment we applied a commercial EM product called Rottelenker (EM Schweiz, Switzerland) that was specifically developed to support the shallow incorporation of cover crops. The liquid was purchased five days before the application to ensure original product quality. EM Rottelenker is recommended for application when temperatures rise >8 °C in a quantity of 100 L ha^{-1} and to be diluted with an amount of water that suits a proper and even application (EM Schweiz, 2023). In practice, that means a dilution factor between 1 and 10 depending on the application technique. To distinguish between the effects of living EM and a pure-substrate effect, we ran a treatment with sterilized EM. For the sterilized treatments, EM solution was taken from the original container one day before the start of the incubation and was autoclaved twice at 121 °C for 20 min within 24 h. To test both, the living status of the purchased EM solution as well as the sterilization, we ran a colony forming unit analysis (CFU). For this, original and sterilized EM liquid were plated on Trypticase Soy Broth (TSB) media and TSB media amended with the fungicide cycloheximide, respectively, within 24 h of the launch of the incubation experiment. The dilution rows were done in five steps from 1 to 10^{-5} with five replicates per sample and then

the plates were incubated at room temperature for three days. The CFU analysis showed that living microorganisms were present in the purchased solution and that no living microorganism was present in the sterilized EM solution on either of the two TSB media (for details see Fig. S1 in the supplementary material).

2.3. Experimental design

We conducted the 28-day soil incubation experiment with the two factors cover crop and EM-level. We chose the time span of 28 days to capture the time between shallow cover crop incorporation and sowing of spring crop (around 10 days) as well as the start of the spring crop. We tested four different levels of EM- application: no EM (EM0), EM as recommended in agricultural praxis (100 L ha^{-1}; EM1), 100 times higher quantity (EM100), and 100 times higher quantity of sterilized EM (EM100st). In a fully orthogonal design, these four EM-levels were combined with the factor cover crop resulting in four treatments with cover crop input (CC-EM0, CC-EM1, CC-EM100, CC-EM100st) and four treatments with no cover crop input and only EM application (NCC-EM0, NCC-EM1, NCC-EM100, NCC-EM100st; Fig. 1). We imitated the process in the field with a cover crop aboveground biomass of 5 t ha^{-1} and a topsoil (0–3 cm) bulk density of 1.3 g cm^{-3}, which corresponds to a cover crop biomass input of 12.82 g dry matter per kg of soil. The EM application of 100 L ha^{-1} corresponds to 0.256 mL per kg of soil for the level EM1 and accordingly 25.6 mL per kg soil for the EM100 level (Table 2).

2.4. Soil incubation

Three days after soil sampling, the air-dried and sieved soil was slightly rewetted to a gravimetric water content (GWC) of 0.16 g H_2O g^{-1} soil and then preincubated seven days before the start of the experiment to re-establish basal respiration. Pre-incubation was conducted at 16 °C and 80 % air humidity to prevent a peak of microbial respiration induced by the soil sieving before the onset of the experiment.

The eight soil treatments were prepared on the start day of the incubation (day 0). The pre-incubated soil was brought to a GWC of approximately 0.2 g H_2O g^{-1} soil by gently spraying Milli-Q water on top whilst constantly mixing the soil by hand wearing plastic gloves to avoid any contamination. After that, the moist soil was separated into sealable 3 L plastic bags. The different levels of EM and cover crop biomass were added whereby the EM0-level received the same amount of water. Where cover crop biomass was added, the liquid was carefully sprinkled onto the plant material before being added to the soil to imitate the incorporation of cover crops as practiced in the field. Each bag was then sealed, and the content carefully mixed by hand for multiple minutes until a homogenous mixture was achieved and then transferred to plastic beakers for the incubation experiment. The incubation was conducted at 12 °C with 80 % air humidity. The final GWC of the incubated soil was 0.23 g H_2O g^{-1} soil which corresponded to 64 % of the maximum water holding capacity of the soil. Mixed soil samples were split into three different groups for soil respiration (separate glass jars), POXC (separate corning tubes) while for all other analyses, 75 g of moist soil were placed in plastic beaker with four replicates per time point (3) and treatment (8) and covered with a paper tissue to allow gas exchange but to avoid water loss within the incubator. These were opened on the respective sampling date and the soil was split into the different volumes and beakers for further analysis. Throughout the incubation period no signs of dried aggregates on the soil surface could be visually detected. An overview of the timeline and measurement intervals can be found in Table S1 in the supplementary material.

2.5. Assessment of biological soil parameters

Soil respiration was measured according to the protocol of (Alef, 1995). In brief, two small plastic cups were placed into 1 L sealable glasses, where 1 cup would hold 40 g of dry soil equivalent and the other cup 10 mL of 0.2 M NaOH to trap produced CO_2. We used 36 sealable glass jars (8 treatments * 4 replicates +4 blanks) and 13 time points resulting in 478 measurements. At every measurement time point the jar was opened and about 4 mL (in excess) of 1 M $BaCl_2$ and few drops of phenolphthalein were added to the NaOH solution and then trapped CO_2 was determined by a titration with 0.1 M HCl. Each mole of dissolved CO_2 led to the production of 2 mol of H^+ which neutralize 2 mol of OH^- according to formula 1:

$$CO_2[\text{mmol}] \text{ trapped in NaOH} = 0.5*\left(HCl_{blank}[\text{ml}]–HCl_{sample}[\text{ml}]\right)*M_{HCl} \quad (1)$$

Microbial C (Cmic) and N (Nmic) were measured according to the protocol of (Vance et al., 1987) with some adaptions. We weighed moist soil equal to 10 g dry matter and used 40 mL of 0.5 M K_2SO4 for the extraction. The dissolved C and N in the extracts were measured with a TOC-analyzer (DIMATOC® 2100, DIMATEC Analysetechnik GmbH, Germany). We report Cmic and Nmic as chloroform labile C and N did not use any conversion factor to account for incomplete extraction efficiency.

The analysis of the microbial community in the EM solution and incubated soil was performed on treatments with cover crop addition (CC-EM0, CC-EM1, CC-EM100, CC-EM100st) at day seven of the experiment. For that, DNA was extracted from pure EM solution and approximately 0.45 g soil sample using the “NucleoSpin® 96 Soil” kit (Macherey- Nagel, Düren, Germany) with lysis buffer SL2 and enhancer SX following the manufacturer’s instruction. Extracted DNA was quantified fluorometrically with the plate reader Infinite M Nano+ (Tecan, Maennedorf, Switzerland) and the Qubit dsDNA HS Assay Kit (Invitrogen by Thermo Fisher Scientific, Waltham, USA). The bacterial community was characterized using 16S rRNA amplicon sequencing using a similar protocol as Lori et al. (2022). Briefly, primers 314F and 806R (Frey et al., 2016) were used for the first PCR with Kapa Sybr fast qPCR kit Master Mix (Kapa Biosystems, Wilmington, USA) and 200 nM of each primer. Samples were used either undiluted, 1:5, 1:10 or 1:50, depending on their concentration. The cycling program consisted of 3 min initial denaturation at 95 °C, 38 cycles of 20 s denaturation at 95 °C, 20 s annealing at 58 °C and 40 s elongation at 72 °C followed by 10 min final elongation. Amplicons were purified with homemade magnetic bead solution (SpeedBead Magnetic Carboxylate Modified Particles, GE Healthcare) and visualized on agarose gel for validation. The second PCR to barcode the samples and MiSeq sequencing were performed at the Genome Quebec Innovation Center (Montreal, Canada).

The fungal community was characterized using ITS amplicon sequencing with PacBio following Bodenhausen et al. (2019). M13-tagged primers ITS1F and ITS4 were used for the first PCR with HiFi

Experimental Design (2 Factors)		**EM-Level (4 levels)**			
		EM0	EM1	EM100	EM100st
Cover Crop (2 levels)	Cover Crop	CC-EM0	CC-EM1	CC-EM100	CC-EM100st
	No Cover Crop	NCC-EM0	NCC-EM1	NCC-EM100	NCC-EM100st

Fig. 1. Experimental design with four EM levels and two cover crop levels.

HotStart Ready Mix (Kapa Biosystems, Roche, Basel, Switzerland) and 300 nM of each primer. The first cycling program consisted of 3 min initial denaturation at 95 °C, 25 cycles of 20 s denaturation at 98 °C, 20 s annealing at 60 °C and 60 s elongation at 72 °C followed by 5 min final elongation. 3 ul of the first PCR was used as template for the second PCR reaction with M13-tagged barcodes. The second cycling program was similar as above except after the first two cycles, the annealing temperature was increased to 65 °C and the total number of cycles was 22. After cleaning-up with homemade magnetic bead solution, PCR products were quantified NanoQuant (Tecan, Maennedorf, Switzerland) and pooled in equimolar fashion. Negative controls were included and sequenced with the other samples. The library was sequenced with Pacbio at the Next Sequencing Platform of the University of Bern on a Sequel II instrument according to their standard protocols. Raw sequences were deposited at NCBI Short Read Archive (PRJNA1026363).

MiSeq reads were demultiplexed by the sequencing facility. The bioinformatics analysis of MiSeq data was performed on Scientifc Computer Cluster Euler at the ETH Zurich. Briefly, USEARCH v11.0.667 (Edgar, 2013) was used to merge the reads and remove primer sequences. PRINSEQ-lite 0.20.4 was used to filter for quality (Schmieder and Edwards, 2011). After chimeral removal with UPARSE (Edgar, 2013), reads were clustered into zero radius operational taxonomic units (ZOTU) with UNOISE3 (Edgar, 2016). ZOTU were further clustered at 97 % similarity with UPARSE (Edgar, 2013). Finally, taxonomy was assigned with SINTAX v11.0.667 (Robert, 2016) and the SILVA database, SILVA138_RESCRIPt.fasta (Quast et al., 2013). The bioinformatics analysis of PacBio data was similar except that lima 2.7.1 (https://lima.how) was used for demultiplexing and the taxonomy assignment was with the UNITE database, UNITE_v83_AllEukaryotes_10.05.2021.fasta (Abarenkov et al., 2010).

Relative shares of OTUs from the pure EM solution with >50 counts served as target EM taxa and were traced during the soil incubation.

2.6. Assessment of chemical parameters

To measure the dynamics of easily oxidizable carbon, we determined permanganate oxidizable carbon (POXC) at several time points of the incubation. For that, 5 g of moist soil were put in 50 mL corning tubes covered with a paper tissue to allow for gas exchange but to avoid water loss within the incubator. Four replicates per treatment and sampling time point were prepared (n = 4*8*8 = 256) and when the sampling date arrived, they were covered with a lid and frozen until analysis. Afterwards, POXC was then measured in one run according to the protocol of Weil et al. (2003) with 0.2 M $KMnO_4$ as reactant and absorption measurement at 550 nm with a Spectrophotometer (UV-1800, Shimadzu corporation, Japan).

Water-soluble ions were measured by extracting soil equivalent to 8 g dry soil from the collective beakers with 40 mL of Milli-Q water for one hour on day 0, 7, 14 and 28. These samples were centrifuged (3000 rpm for 15 min) and 5 mL of the supernatant was syringe filtered (hydrophilic, 0.45 µm) and stored at 5 °C. Ion chromatography (IC) was performed in one run two weeks after the end of the incubation on a Dionex Aquion™ (Thermo Fisher Scientific Inc., Waltham, USA) to measure the concentrations of the anions fluoride (F^-), chloride (Cl^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) as well as the cations sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}).

For the analysis of water-soluble elements, 25 mL of the same supernatant as for the water-soluble ions-measurements were used. To remove dispersed clay particles in the liquid, 1 mL of 1 M $MgCl_2$ was added, and then the samples were vigorously shaken and centrifuged (3000 rpm for 15 min). From this solution 9.8 mL were filtered (hydrophilic, 0.45 µm) and then mixed with 0.2 mL nitric acid (HNO_3, 69 %) resulting in 10 mL samples containing 1 % HNO_3. These samples were then stored at 5 °C and analyzed in one common run one month after the end of the incubation experiment on a 7700× ICP-MS from Agilent Technologies (Santa Clara, USA) measuring the concentrations of arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), silver (Ag), aluminum (Al), phosphorus (P), vanadium (V), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and uranium (U).

To characterize the soil, the cover crop biomass and the EM solution for elemental composition we conducted a total multielement analysis in triplicates. For that, 0.2 g of soil, 0.2 g of cover crop biomass and 0.2 mL of a 121-times diluted original EM-solution were mixed with 8 mL of 69 % HNO3 and 2 mL 37 % H2O2 and then digested in a CEM MARS 6 microwave (stage 1: 10 min at 120 °C, stage 2: 40 min at 170 °C). After, the cooled down samples were brought to 50 mL volume with Milli-Q, centrifuged (2500 rpm for 5 min) and analyzed with the above mentioned ICP-MS. The turbidity of the EM solution did not allow for an analysis of containing ions via IC analysis.

2.7. Statistics

Fungal and bacterial richness and Shannon diversity were assessed on the base of rarefied read counts using the vegan R package (Oksanen et al., 2019). Additionally, differences between the fungal and bacterial community composition were tested with a PERMANOVA with 10^4 permutations based on Bray-Curties dissimilarity matrices. All other parameters mostly fulfilled or just showed minor deviations from the requirements of normality (Shapiro-Wilk test) and homoscedasticity (Levenes' test). Therefore, we decided to use parametric tests. We tested a multiplicative analysis of variance (ANOVA) with the factors cover crop and EM-level for soil respiration, microbial biomass, pH, POXC and water extractable ions and elements. We used Tukey HSD as a post hoc test to evaluate significant differences between different EM-levels or different treatments. Only for the cumulative respiration, which was very different between treatments with and without cover crop addition, we used a separate Tukey-HSD test for the CC and NCC treatments. For the other response variables, if the ANOVA did not show a significant interaction, we only discuss the main effects of EM-level. Otherwise, if the interaction effect was significant, we discuss only the comparisons between CC-EM0 and CC-EM1, NCC-EM0 and NCC-EM1, CC-EM100 and CC-EM100st as well as NCC-EM100 and NCC-EM100st, because all other possible 24 comparisons were not of practical relevance. All analysis were performed in R version 4.2.2 (R Core Team, 2020).

3. Results

3.1. Soil respiration

The incubation experiment started with a basal respiration rate of 20.2 ± 0.3 mg C kg^{-1} d^{-1} (day 0), which was maintained at a similar level for the course of the soil incubation in the NCC-EM0 and NCC-EM1 treatments (Fig. 2a). Addition of living or sterilized EM in high dose on bare soil (NCC-EM100 and NCC-EM100st) caused an increase in soil respiration of up to 159 ± 10 mg C kg^{-1} d^{-1} on day 1 and basal soil respiration was reached again latest by day four. The addition of cover crop biomass clearly had the strongest effect on soil respiration, peaking at 647 ± 8 mg C kg^{-1} d^{-1} for CC-EM100st at day 1. After that, soil respiration rates continuously decreased, but CC treatments did not reach basal soil respiration rates during the whole incubation period. Differences between the different EM-levels mainly occurred during the first 4 days. During the 28-day incubation the cumulated respiration summed up between 0.42 ± 0.01 and 0.7 ± 0.04 g C kg^{-1} for the NCC treatments and between 4.09 ± 0.9 and 4.57 ± 0.08 g C kg^{-1} for the CC treatments (Fig. 2b and c). The addition of cover crop biomass as well as the addition of EM in high dose (Fig. 2d) increased the cumulated respired C. However, we did not see any effect on cumulated respired C by the combination of cover crops with any level of EM application (no significant interaction in the multiplicative ANOVA between factors cover crop and EM-level, p-value = 0.13, see Table S2 in the supplementary material). The differences in cumulated respired C among the EM-levels were more pronounced in the NCC (Fig. 2b) than in the CC

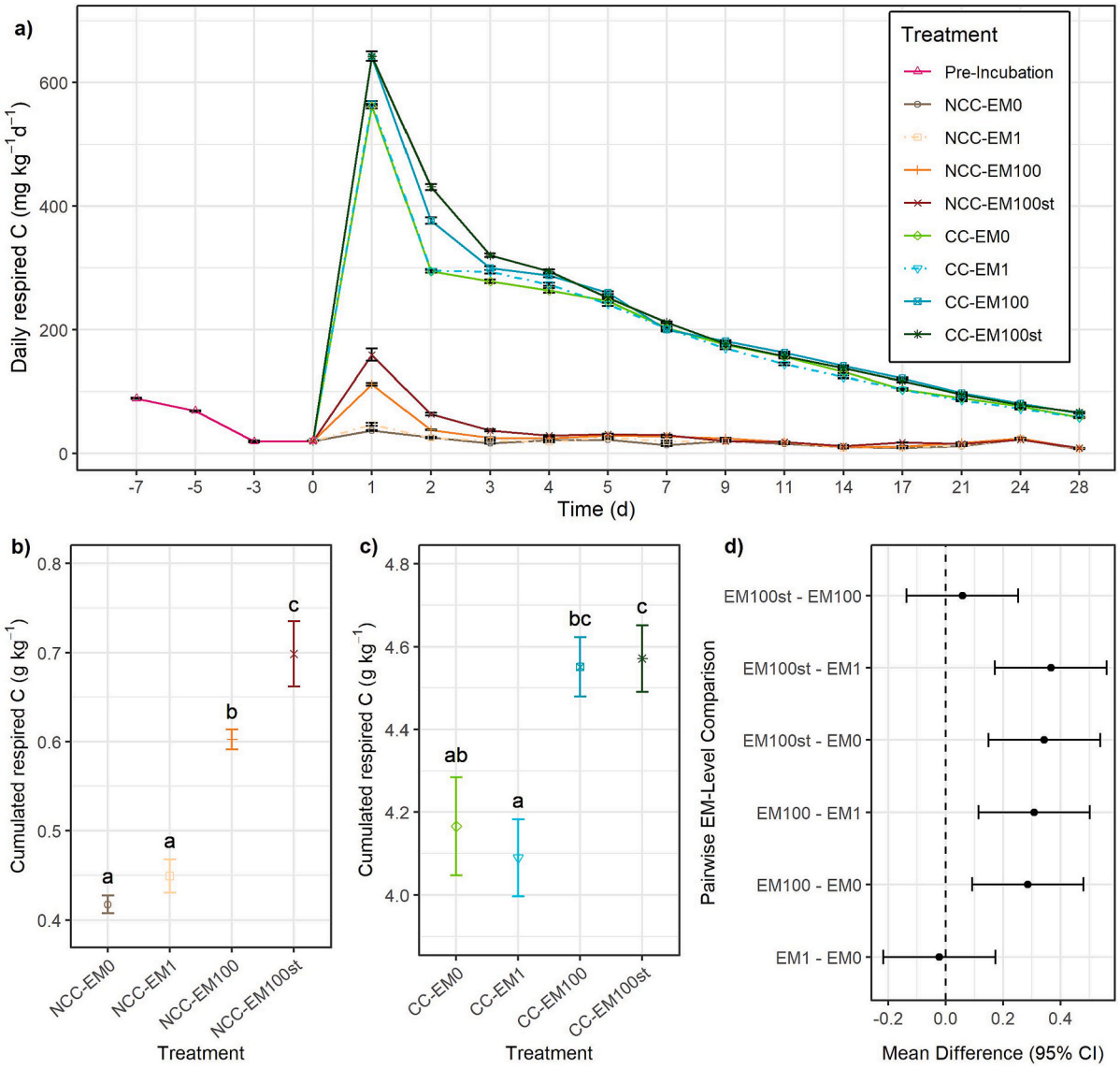

Fig. 2. a) Daily respiration rates. The value of CC-EM100 at day 1 is based only on one replicate, since for the other three replicates the NaOH trap was already completely saturated, suggesting even higher overall respiration rates in this treatment. b) Cumulated mean soil respiration after 28 days of incubation for treatments without cover crop addition. c) Cumulated mean soil respiration after 28 days of incubation for treatments with cover crop addition. d) Tukey's mean difference with 95 % confidence interval for the factor EM-level in the two-way ANOVA with cumulated respiration at the end of the incubation (day 28) as response variable. The panels a, b and c show the mean of four replicates with error bars indicating the standard error.

treatments (Fig. 2c). Pairwise comparison within the NCC treatments also showed that the cumulative respiration was significantly higher for NCC-EM100st than for NCC-EM100 (Fig. 2b). Addition of EM at recommended dose had no effect on soil respiration as we did not find any significant difference between the EM1 and the EM0 level.

3.2. Microbial biomass

At the start of the incubation experiment (day 0), soil microbial biomass contained 342 ± 5 mg C kg^{-1} and 67 ± 1 mg N kg^{-1} (Fig. 3a). In the NCC treatments, there were only minor changes in microbial C and N over time, with most times highest values in the NCC-EM100st treatment (366 ± 14 mg C kg^{-1} and 68 ± 2 mg N kg^{-1}) followed by the NCC-EM100 treatment. In contrast, microbial C and N almost doubled in all CC treatments with highest values in the CC-EM100st treatment (810 ± 34 mg C kg^{-1} and 134 ± 6 mg N kg^{-1}) followed by the CC-EM100 treatment. There was no significant interaction at any day between the two factors cover crop and EM-level for the response variables Cmic and Nmic (lowest p-value for the interaction term was $p = 0.33$ for Nmic at day 28). Independent of cover crop input, application of EM in high dose led to slightly higher Cmic and Nmic but only the EM100st level showed on some days significantly higher Cmic and Nmic than the EM1 or EM0 level (Fig. 3b). Independent of cover crop addition, no effect of the addition of EM at the recommended dose existed as there was no significant difference between the EM1 and EM0 level.

3.3. Identifying and tracing EM taxa

Taxonomic identification of bacterial and fungal taxa within the applied EM solution showed domination of fungal taxa by OTU5, which made up >90 % of fungal OTUs and was assigned to the Order of *Saccharomycetales*. Other identified fungal taxa within the EM solution include OTUs assigned to the orders of *Mortierellales*, *Filobasidiales* and *Hypocreales* but they comprise only a small fraction of the inoculated fungal community (Table 3). Bactria taxa on the EM solution were dominated by OTUs assigned to the genus of *Lactobacillus*. Five different

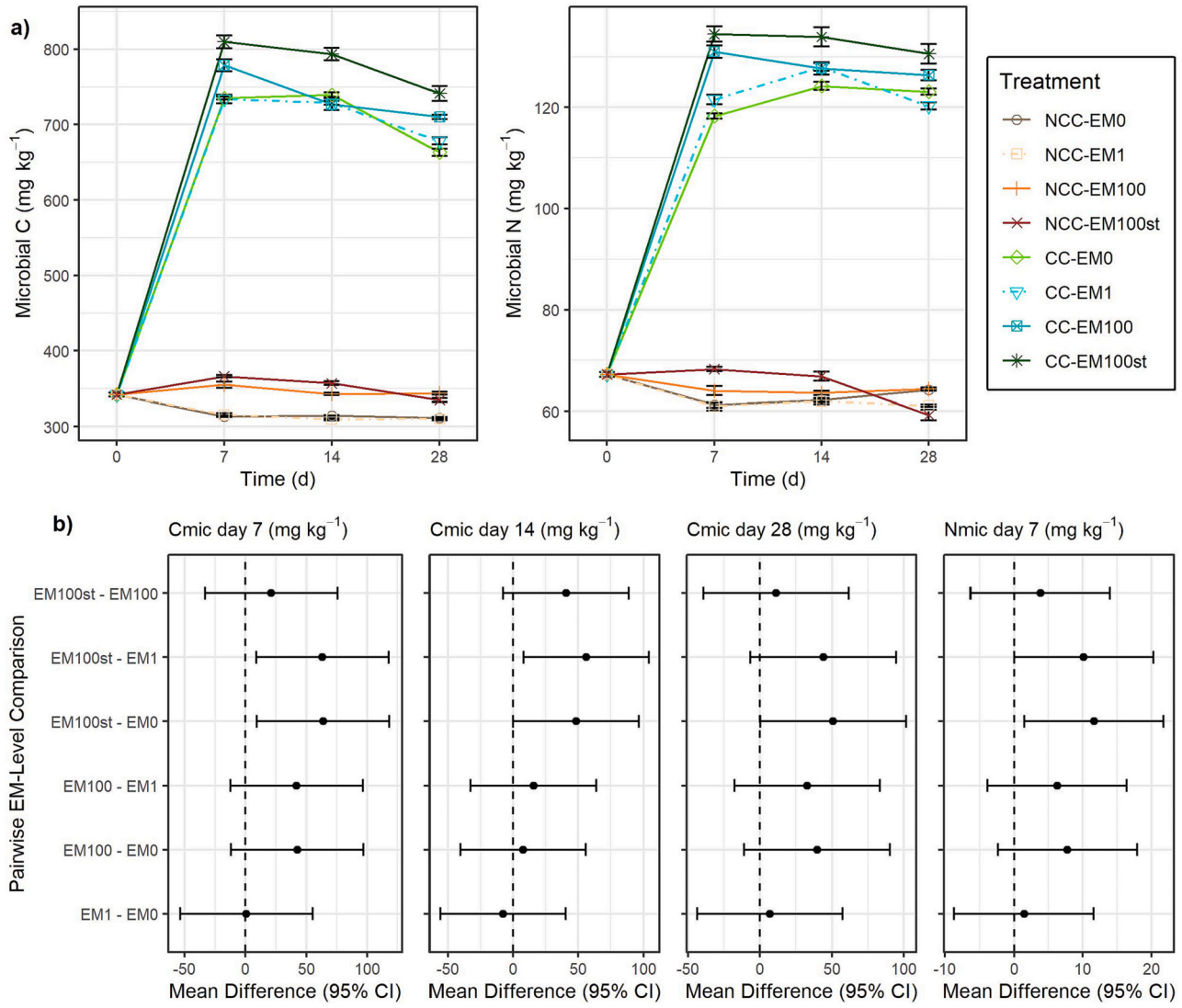

Fig. 3. a) Microbial C and N during the 28-day incubation experiment with 4 measurement time points. Means of four replicates and standard errors are shown. b) Tukey's mean differences between EM-levels for the ANOVA microbial C (or N) ~ Cover crop * EM-level. Only days with significant EM-level effect are presented. Mean differences are indicated with 95 % confidence interval (CI) and significances are marked based on alpha = 0.05.

OTUs of this genus were observed, jointly accounting for >99 % of applied bacterial OTUs. *Acetobacteraceae and Clostridiaceae* were identified in negligible amounts. Bacterial OTUs 4440 and 4994 were most abundant with 72.6 and 16.4 % of applied bacterial OTUs (Table 3). After 7 days of incubation the structure of soil bacterial and microbial communities was compared via permanova, revealing a weak effect on bacterial community structure ($p = 0.046$) and no effect on fungal community structure ($p = 0.816$), based on Bray-Curties dissimilarities matrices. For the bacterial community, pairwise permanova further revealed significant difference between CC-EM0 and CC-EM100 ($p = 0.032$), while no other treatment pair significantly differed from each other. Principally, bacterial community structure was dominated by *Actinobacteriota* and *Proteobacteria, w*hile fungal communities mainly comprised *Mortierellamycota* (Fig. 4). Neither fungal and bacterial richness nor Shannon diversity showed a significant effect of experimental treatments seven days after incubation (Table S3 in the supplementary material).

OTUs identified within the EM solution were traced within the identified bacterial and fungal communities (Fig. 5). While the recommended dose of EM application did not yield an observable increases of inoculated EM taxa, a slight increase could be detected in the 100 times the recommended application dose for bOTU4440 and bOTU4994. Still relative abundances of these OTUS were below 1 %. For fungal communities, there was no effect in the treatments except for the CC-EM1 treatment where the recommended application dose increased fOTU5 (Fig. 5).

3.4. Soil pH

The initial soil pH of 7.12 ± 0.02 was influenced by the different treatments. Addition of acidic solutions of EM1 (pH = 3.98), EM100 (pH = 3.55) and EM100st (pH = 3.58) decreased soil pH only when added in combination with cover crop input (Fig. 6). By day 28, soil pH increased to about 7.2 in all treatments, except for the NCC-EM0 (7.04 ± 0.06) treatment, which was significantly lower than the NCC-EM100 and NCC-EM100st treatment.

3.5. Permanganate oxidizable C

Concentrations of POXC decreased from 544 ± 3 mg kg^{-1} on day zero to values between 445 and 510 mg kg^{-1} within the first days of the incubation experiment and remained stable from day seven onwards (Fig. S2). The CC-EM100st treatment showed a larger decrease within the first four days but also stabilized after day seven in the same range as

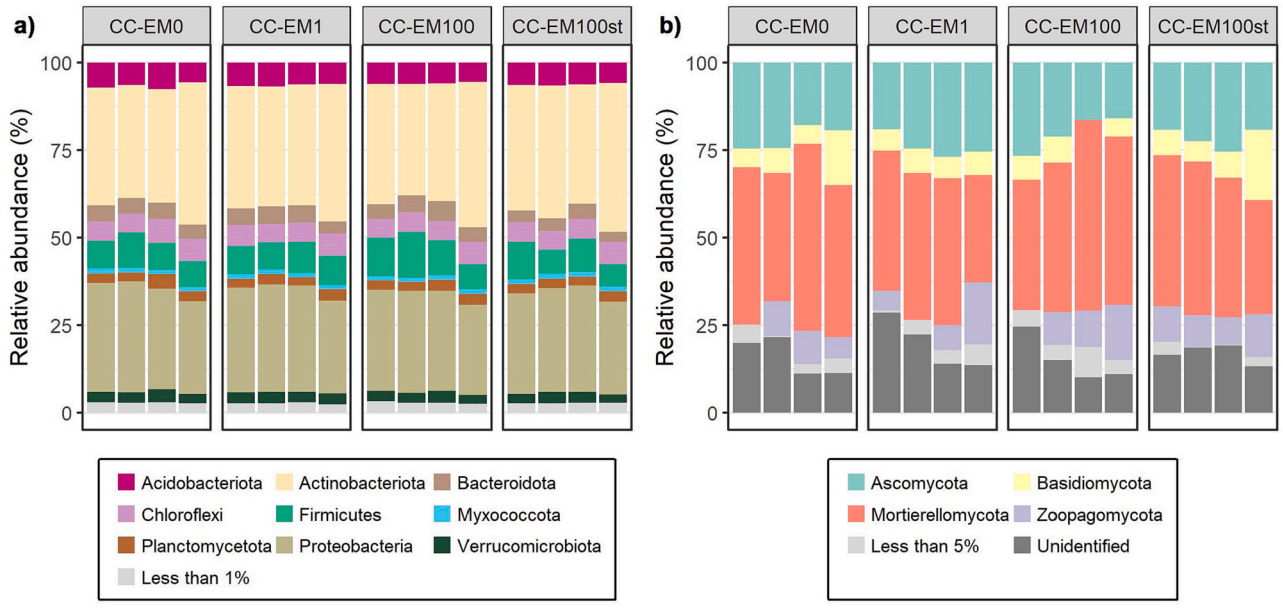

Fig. 4. Relative abundance of bacterial (a) and fungal (b) phyla after 7 days of incubation in all four replicates per treatment.

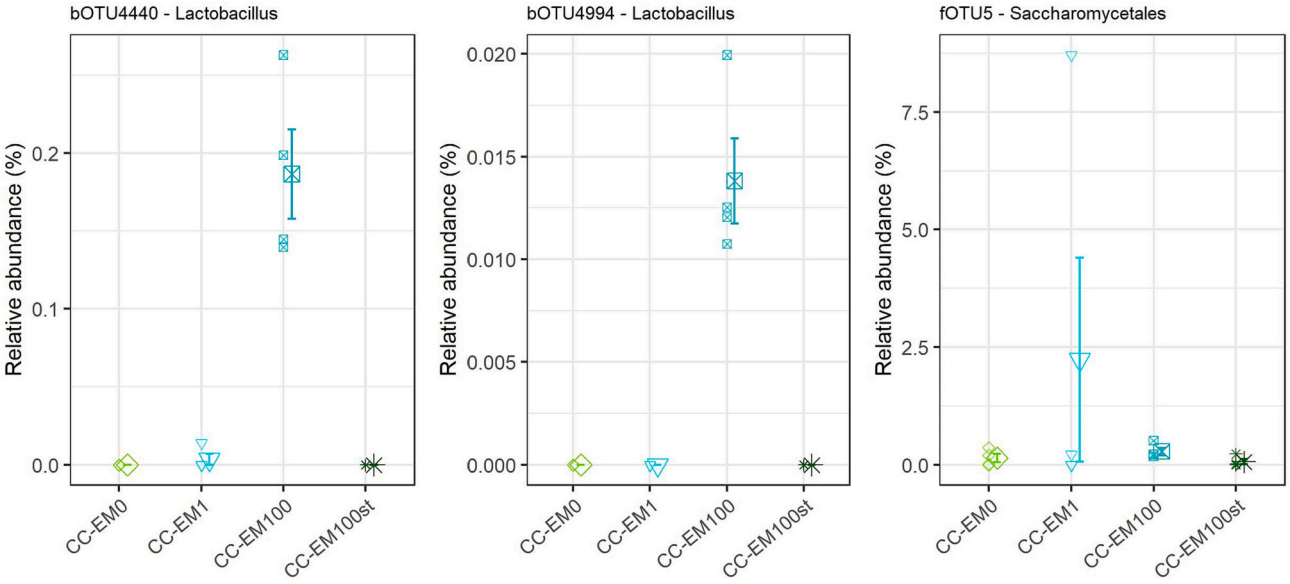

Fig. 5. Relative abundance of bOTU4440 Lactobacillus, bOTU4994, fOTU5 Saccharomycetales of the four treatments with cover crop addition seven days after the start of the incubation. Average and standard errors as well as the values of the four replicates per treatment are indicated.

the other treatments. For POXC, the factors cover crop and EM-level showed significant interactions on all measurement days except day 14 and day 28. Nevertheless, the significantly different treatments as indicated by the Tukey test were not consistent over time.

3.6. Water-soluble ions

The concentrations of the analyzed water-soluble ions were influenced by EM and cover crop addition (Fig. 7 and corresponding statistics in Table S4 in the supplementary material). For F^-, Cl^-, Na^+, K^+, Mg^{2+}, and Ca^{2+} we observed significantly higher concentrations in the treatments with cover crop biomass input. This effect was in general clearer at the beginning (day 7) of the incubation and decreased towards the end (day 28). Cl^-, SO_4^{2-}, Na^+, Mg^{2+}, and Ca^{2+} were often significantly higher in the EM100 and EM100st than in the EM0 and EM1 levels. We only observed a few differences between the EM1 and EM0 level suggesting that the application of EM at the recommended dose did not influence the concentration of water-soluble ions. However, CC-EM1 showed higher Mg^{2+} concentration on day 7 but lower K^+ concentration on day 28 than CC-EM0. More consistent was the difference between the EM100 and the EM100st level. The CC-EM100st treatment showed at least at one time point higher ion concentrations than the CC-EM100 treatment for F^-, Cl^- and SO_4^{2-}. For Cl^- this effect was also observed for the NCC treatments and NCC-EM100st showed significantly higher concentrations than NCC-EM100. For NO_3^-, we found higher concentrations in NCC-EM0 and NCC-EM1 treatments than in all other treatments.

3.7. Water-soluble elements

The inputs of water-soluble elements through cover crop biomass or EM addition can be seen in Table 4 in absolute numbers and relative to

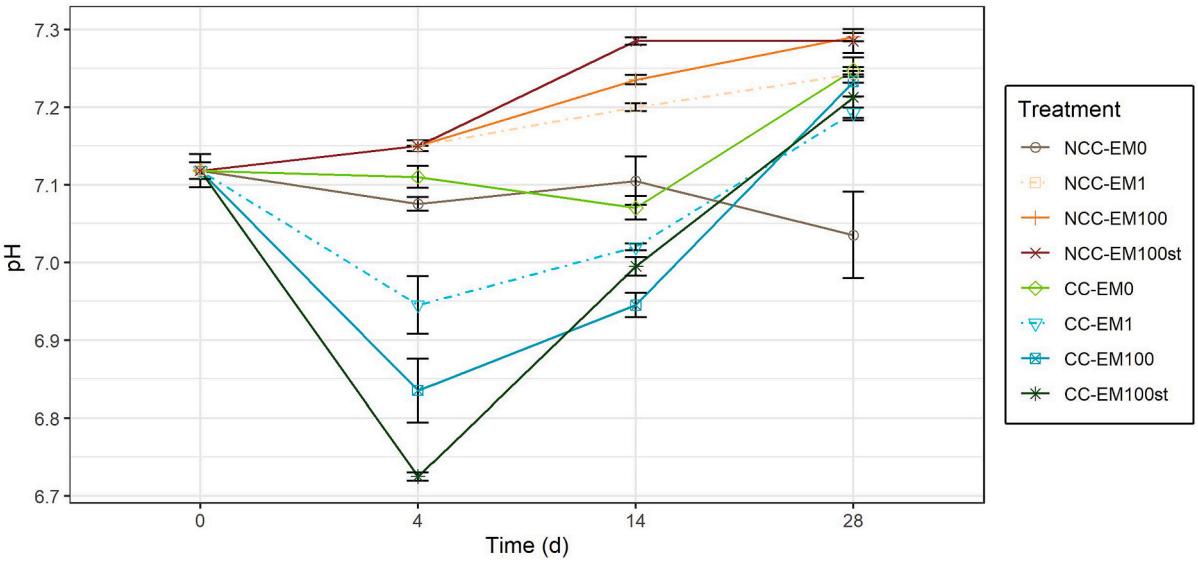

Fig. 6. Soil pH during the 28-day incubation experiment with four measurement time points. Values show means of four replicates (except for day 4 with only 2 replications per treatment). Error bars indicate the standard error.

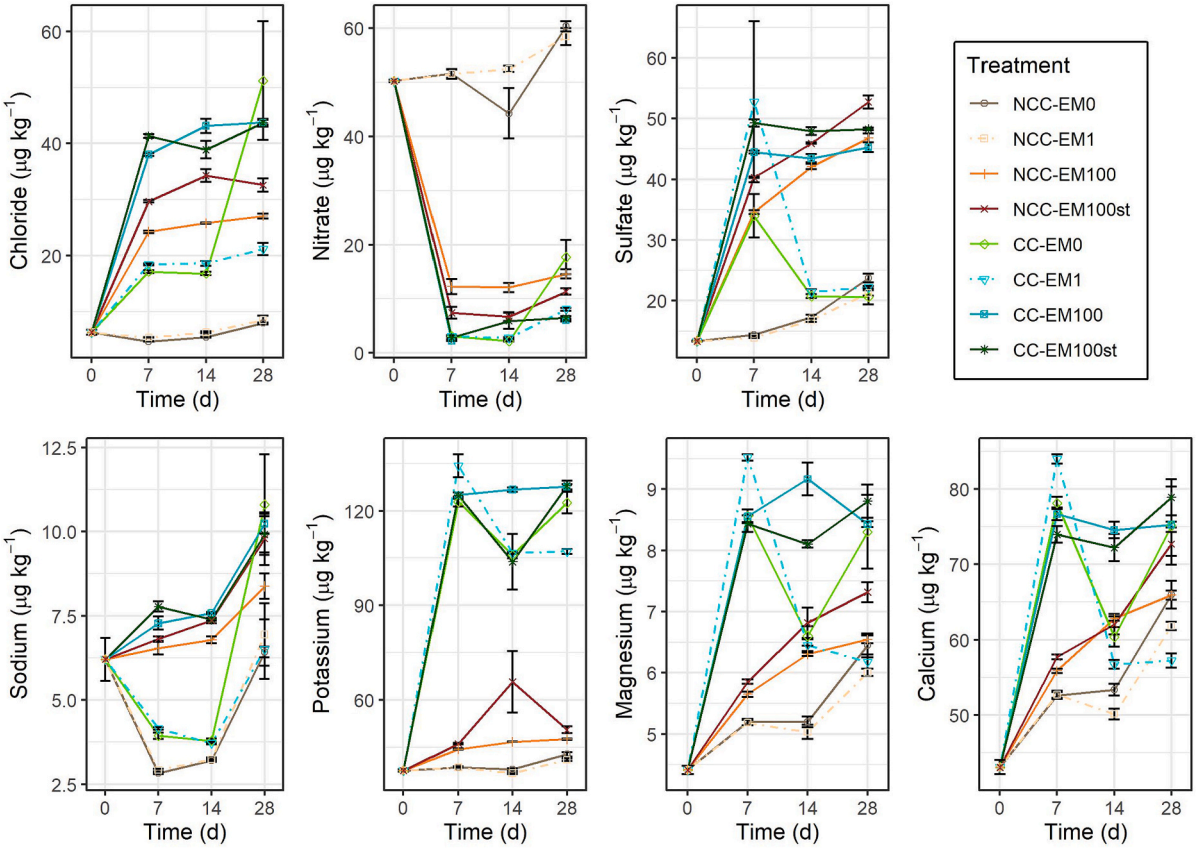

Fig. 7. Concentration (µg per kg soil) of water-soluble ions that showed a significant main effect of EM-level (chloride, nitrate, sulfate, sodium, potassium, magnesium, and calcium) during the 28-day incubation experiment with 4 measurement time points. Values show mean of four replicates and error bars the standard error.

the initial water-soluble concentration in the soil. For all analyzed elements the input through the cover crop biomass was higher than through the EM addition. The concentrations of the analyzed water-soluble elements were influenced by EM and cover crop addition at least at one of the four measurement time points (Fig. 8 and corresponding statistic in Table S5 in the supplementary material). Cover crop input significantly increased concentrations of water soluble Pb, Cd, Cr, Ni, Al, Ag, Mn, Fe, Cu, Zn and U compared to the NCC treatments at least at one timepoint. There were only minor and non-systematic differences in measured concentrations of water-soluble elements between EM-levels and they only occurred in the treatments with cover crop addition. The application of EM in high dose (EM100, EM100st) did

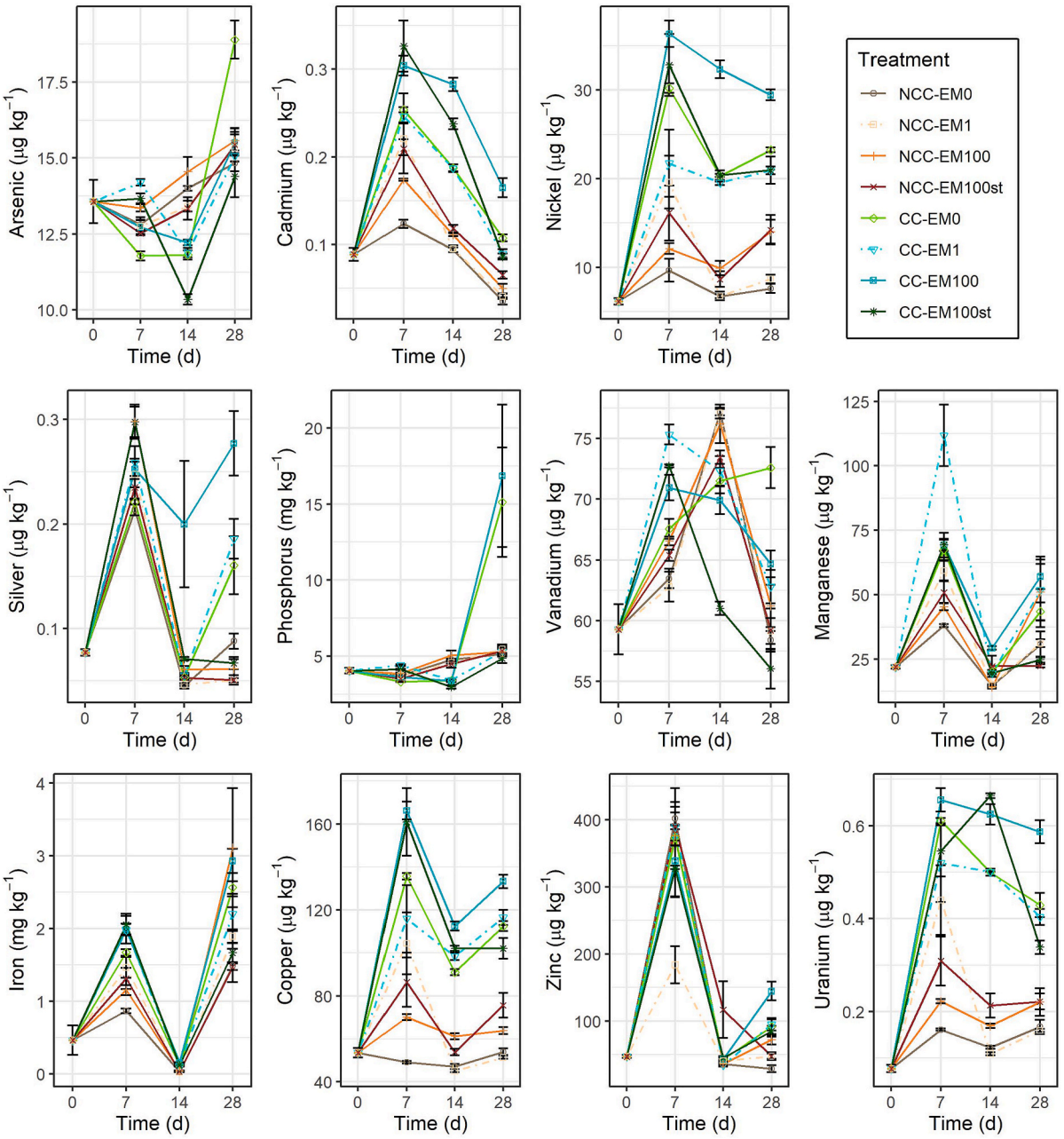

Fig. 8. Concentration (µg or mg kg^{-1} soil) of water-soluble elements that showed a significant main effect of EM-level (arsenic, cadmium, nickel, silver, phosphorus, vanadium, manganese, iron, copper, zinc, and uranium) during the 28-day incubation experiment with 4 measurement time points. Error bars indicate the standard error.

not consistently influence the concentration of water-soluble elements during the soil incubation experiment. The only effects of EM1 compared to EM0 that were statistically significant were on day 7, where the CC-EM1 treatment showed higher concentrations in As and P than the CC-EM0 treatment. The comparison between sterilized and living EM revealed at least at one timepoint significantly higher concentrations of Cd, Ni, Ag, P, Cu and U in the CC-EM100 compared to the CC-EM100st treatment. In the NCC treatments, no significant difference was identified between NCC-EM1 and NCC-EM0 or NCC-EM100 and NCC-EM100st.

4. Discussion

4.1. EM application at recommended dose (EM1)

Application of EM at the recommended dose (EM1) showed no effect on critical soil properties such as soil respiration (Fig. 2) or the development of microbial biomass (Fig. 3) compared to the control treatments (EM0), in both the CC as well as the NCC treatments. This is in line with findings of Schenck zu Schweinsberg-Mickan and Müller (2009) who did not observe any influence of living EM addition on soil respiration compared to a sterilized EM control treatment. The lack of observed results was confirmed by the absence of traced EM-taxa in the soil, as only 1 out of 4 replicates in the CC-EM1 treatment showed slightly higher relative abundance in bOTU4440 (*Lactobacillus*) and fOTU5 (*Saccheromycetales*) than the CC-EM0 treatment. The few statistically

significant differences in water-soluble ions and elements that occurred at specific time points during the incubation experiment were inconsistent over time. In other studies, (Hu et al., 2018) observed higher available phosphorus and potassium contents in EM-compost while other studies reported a slightly higher N content in EM-compost compared to traditional compost (Daur, 2016; Jusoh et al., 2013; Van Fan et al., 2018; Zhong et al., 2018), yet, these investigations were up to now missing for soil. Furthermore, we did not observe any consistent change in POXC throughout the incubation experiment suggesting that EM addition had no effect on already existing labile soil organic matter in the soil that could cause additional release of nutrients of PTTEs. Similarly, a recent review (Safwat and Matta, 2021) also found little evidence to confirm the beneficial effects of EM on composting of organic matter.

The soil incubation mimicked spring-like field conditions in temperate climates (12 °C and 0.2 g H_2O g^{-1} soil) that would be relevant for enhanced cover crop decomposition through EM application. Nevertheless, soil temperature, moisture and water filled pore space during spring are typically highly variable. This would consequently also affect the establishment of EM, that might require very specific conditions for their establishment. On the field scale some experiments have reported higher yield and nutrient efficiency when green manure, farmyard manure or chemical fertilizer were applied in combination with EM under mainly under subtropical climates (Hu and Qi, 2013; Hussain et al., 1999; Javaid and Bajwa, 2011; Khaliq et al., 2006; Youssef et al., 2021). However, in regions with temperate climates, the few existing field studies could not determine any effects on crop yields or soil quality that could be traced to the application of EM to soil (Mayer et al., 2010; Pranagal et al., 2020). This was supported by the results of our study, demonstrating no effect of EM addition at typical application rates on soil properties.

4.2. EM application in high dose (EM100, EM100st)

The addition of EM at 100 times higher than recommended dose showed some effects on soil properties, e.g., on soil respiration (Fig. 2) or microbial C (Fig. 3). However, these changes took place regardless of whether the solution was sterilized (EM100st) or not (EM100), and can thus clearly be assigned to a substrate effect, and not an actual EM effect. The amount of carbon added with the EM100 and EM100st application level was about 0.2 g C per kg of soil (Table 2). This closely matched the difference in cumulated respired C compared to the EM0 level in both the CC and the NCC treatments (Fig. 2b). Since the EM solution was acidic (pH 3.6; Table 1), some of the released CO2 may have originated from the dissolution of carbonates in the alkaline soil. Yet, assuming that all the added acid of the EM solution was buffered by CaCO3 and released as CO2, this would equal to a C release of only 1.6 mg C kg^{-1} soil, i.e., to negligible amounts compared to basal soil respiration (Fig. 2a). The amount of C added at the EM100 treatment (0.2 g C per kg of soil) was much lower than C added with cover crop biomass (5.4 g C per kg soil). Cover crop addition also caused a slight increase in microbial biomass and likely resulted in an immobilization of NO_3^-, which explains the significantly higher NO_3^- concentration in the treatments without high-dose EM or cover crop biomass (NCC-EM0 and NCC-EM1; see Fig. 7 and Table S4). Additionally, at certain time points, higher concentrations of Cl^-, SO_4^{2-}, Na^+, Mg^{2+}, and Ca^{2+} were observed in EM100 and EM100st levels compared to the EM1 and EM0 levels, regardless of cover crop input (Fig. 7, Table S4), suggesting that these ions were part of the EM solution. This suggests that the higher concentrations in those water-soluble ions were also a result of the substrate effect, even though this cannot be fully confirmed since the original EM solution could not be analyzed for these water-soluble ions due to high organic impurities. In contrast, the analysis of the original EM solution for a wide range of water-soluble elements (Table 4) showed that these inputs at the 100 times application level were still minor compared to the inputs by the cover crop biomass. Inputs of potentially harmful elements from the EM solution into the soil system can therefore be ruled out.

Table 1
Characteristics of the arable soil used in this study. Means and standard deviation are presented.

Property	Unit	Value (SD)
Sand†	mass %	50
Silt†	mass %	29
Clay†	mass %	21
Maximum water holding capacity‡	g water per g soil	0.36
pH ($CaCl_2$)		7.12 (0.09)
Total C§	g C kg^{-1} soil	28.6 (0.1)
Inorganic C§	g C kg^{-1} soil	9.04 (0.19)
Organic C§	g C kg^{-1} soil	19.5 (0.3)
Permanganate oxidizable C#	mg C kg^{-1} soil	543 (12)
microbial C††	mg C kg^{-1} soil	342 (18)
Total N§	g N kg^{-1} soil	2.12 (0.01)
microbial N††	mg N kg^{-1} soil	67.2 (3.8)
Magnesium‡‡	g kg^{-1} soil	6.02 (0.16)
Aluminum‡‡	g kg^{-1} soil	9.44 (0.63)
Phosphorus‡‡	g kg^{-1} soil	1.32 (0.66)
Manganese‡‡	g kg^{-1} soil	0.88 (0.02)
Iron‡‡	g kg^{-1} soil	17.8 (0.9)
Copper‡‡	mg kg^{-1} soil	43.2 (0.8)
Zinc‡‡	mg kg^{-1} soil	69.9 (2.7)
Lead‡‡	mg kg^{-1} soil	38.6 (1.5)

† Improved integral suspension pressure method (ISP+) (Durner and Iden, 2021).
‡ The maximum water holding capacity was determined gravimetrically after a water saturated sample lost all gravitational water.
§ Dry combustion with CNS analyzer. For the determination of inorganic C, the samples were first ignited at 550 °C.
# According to Protocol of (Weil et al., 2003).
†† Chloroform fumigation according to the protocol of (Vance et al., 1987).
‡‡ Extracted from soils using nitric acid microwave digestion and measured using an inductively coupled plasma - mass spectrometer.

4.3. Microbial composition and establishment in soil upon addition to soil

The application of EM in high dose, accompanied by a sterilized

Table 2
Description of the levels for the factor cover crop (CC, NCC) and factor EM-level (EM0, EM1, EM100, EM100st) that were combined to a fully orthogonal experimental design.

Level	Input g (kg soil)$^{-1}$	C input g (kg soil)$^{-1}$	N input mg (kg soil)$^{-1}$	Dilution factor	pH	Remarks
CC	12.8 (dry matter)	5.4	300			Dried and cut to 2 mm pieces
NCC	0	0	0			
EM0	0	0	0	Only water	7.00	
EM1	0.256	0.002	0.075	1: 121	3.98	Living EM applied at recommended dose
EM100	25.6	0.2	7.5	1: 1.21	3.55	Living EM applied in 100 times higher quantity than recommended dose
EM100st	25.6	0.2	7.5	1: 1.21	3.58	Sterilized EM applied in 100 times higher quantity than recommended dose

Table 3
Taxonomy and relative share of fungal and bacterial OTUs within the applied EM solution.

	Kingdom	Phylum	Class	Order	Family	Genus	Mean relative abundance (%)	SE
fZOTU5	*Fungi*	*Ascomycota*	*Saccharomycetes*	*Saccharomycetales*			*93.3*	0.8
fZOTU375	*Fungi*	*Mortierellomycota*	*Mortierellomycetes*	*Mortierellales*	*Mortierellaceae*	*NA*	*1.9*	0.3
ffZOTU1425	*unidentified*						*1.3*	0.1
fZOTU9	*unidentified*						*0.7*	0.0
fZOTU1533	*Fungi*	*Mortierellomycota*	*Mortierellomycetes*	*Mortierellales*			*0.6*	0.1
fZOTU66	*Fungi*	*Basidiomycota*	*Tremellomycetes*	*Filobasidiales*	*Piskurozymaceae*	*Solicoccozyma*	*0.5*	0.2
fZOTU23	*Fungi*	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Gibberella*	*0.5*	0.1
fZOTU941	*Fungi*	*Mortierellomycota*	*Mortierellomycetes*	*Mortierellales*	*Mortierellaceae*		*0.4*	0.1
fZOTU1975	*Fungi*	*Ascomycota*	*Sordariomycetes*				*0.4*	0.0
fZOTU2087	*Fungi*						*0.3*	0.0
bZOTU4440	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*72.6*	0.0
bZOTU4994	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*16.4*	1.9
bZOTU3653	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*5.8*	1.2
bZOTU3325	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*3.9*	1.0
bZOTU2664	*Bacteria*	*Proteobacteria*	*Alphaproteobacteria*	*Acetobacterales*	*Acetobacteraceae*	*Acetobacter*	*0.6*	0.2
bZOTU7663	*Bacteria*	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*0.5*	0.0
bZOTU2304	*Bacteria*	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium_sensu_stricto_1*	*0.2*	0.1

Table 4
Concentration of water-soluble elements in cover crop biomass and effective microorganisms and the applied concentrations in the incubation experiment as absolute numbers and as percentage of the initial water-soluble concentration in the soil (Standard deviation of three measurements in brackets).

	Cover crop			Effective microorganisms			
	Concentration in dry matter	Added concentration to soil	Added concentration compared to initial water-soluble concentration in soil (day 0)	Concentration in purchased liquid	Added concentration to soil in EM1-level	Added concentration to soil in EM100-level	Added concentration of EM100 com-pared to initial water-soluble concentration in soil (day 0)
Unit	[mg kg^{-1}]	[µg kg^{-1}]	[%]	[µg L^{-1}]	[ng kg^{-1}]	[ng kg^{-1}]	[%]
As	0.044 (0.005)	0.558 (0.06)	4.1 (0.4)	2.69 (0.04)	0.687 (0.011)	68.7 (1.1)	0.5 (7.8)
Pb	0.204 (0.007)	2.62 (0.08)	241.4 (7.7)	Below detection limit			
Cd	0.011 (0.000)	0.146 (0.003)	165.1 (3.82)	Below detection limit			
Cr	4.31 (0.16)	55.2 (2)	2429.1 (89.5)	7.5 (0.25)	1.91 (0.07)	191.9 (6.5)	8.5 (0.3)
Ni	0.635 (0.03)	8.15 (0.38)	132.4 (6.2)	12.7 (1.4)	3.26 (0.36)	326 (36)	5.3 (0.6)
Ag	0.013 (0.000)	0.163 (0.000)	211.6 (0.1)	2.94 (0.09)	0.751 (0.024)	75.1 (2.4)	97.3 (3.1)
Al	35.4 (2.1)	454 (27)	18.4 (1.1)	173 (33)	44.3 (8.3)	4430 (833)	0.18 (0.03)
P	Not measured						
V	0.079 (0.002)	1.01 (0.02)	1.7 (0.0)	7.05 (0.23)	1.80 (0.05)	181 (6)	0.3 (0.01)
Mn	35.2 (0.7)	451 (9)	2056.3 (39.8)	96.3 (1.5)	24.7 (0.4)	2466 (38)	11.2 (0.2)
Fe	Not measured						
Cu	7.7 (0.24)	99 (3)	184.8 (5.7)	11.9 (0.5)	3.05 (0.13)	305.0 (13)	0.57 (0.02)
Zn	Not measured						
U	0.003 (0.001)	0.036 (0.009)	47.0 (12.3)	0.704 (0.017)	0.180 (0.004)	18.0 (0.4)	23.5 (0.6)

control, enabled the identification of potential effects caused by living microorganisms and distinguish them from substrate effects. In our study, three main organisms were traced and identified from the EM solution. Among them, two Lactobacillus-taxa (bOTU4440 and bOTU4994) showed a much higher relative abundance in the CC-EM100 treatment and were not found in the treatments without living EM. However, their presence constituted <1 % of the total bacterial community.

Effective microorganisms are distributed worldwide, multiplicated and processed into various end products with different additives. This variability poses challenges in comparing different EM studies because the inoculant itself might vary (Dos Santos et al., 2020) and, in most studies, the microbial community was not analyzed. In our study, we analyzed the EM solution via amplicon sequencing of taxonomic marker genes, which revealed bacterial and fungal OTUs assigned to the bacterial genus *Lactobacillus* and fungal order of *Saccheromycetales*. However, we did not identify photosynthetic bacteria or highly abundant *Ascomycota* within the applied EM solution, although these taxa were described as part of the EM consortia (Ahn et al., 2014). Nevertheless, since *Lactobacillus* and *Saccharomycetales* have the potential to conduct anaerobic fermentation, which is the main suggested mechanism through which EM influences the decomposition of organic matter (Higa and Parr, 1994), we conclude that we tested a representative product within this study.

5. Conclusion

The addition of EM at the recommended application dose (EM1) to soil, with or without cover crop biomass, did not lead to any consistently effect on any of the monitored biological or chemical soil properties. When applied at a dose 100 times higher than recommended (EM100), an increased soil respiration and microbial biomass was observed, however, similar effects were observed in the sterilized control treatments (EM100st) and can thus be fully explained by a substrate induced effect. The soil microbial community remained largely unaffected upon EM addition. The analysis of ten water-soluble ions did not reveal any significant effect from the addition of EM solution on the mineralization of organic matter or the release of nutrients. Furthermore, the analysis of 14 water-soluble nutrients and elements showed that none of the analyzed compounds contained in the EM solution are present at harmful concentrations when applied at the recommended doses. However, there was also no significant effect in mobilizing or immobilizing selected compounds in the soil. We therefore conclude that added EM solution themselves did not alter the cover crop decomposition nor any other soil process beyond the carbon, nutrients or other substances added with the EM solution.

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CRediT authorship contribution statement

Simon Oberholzer: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Christa Herrmann:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Natacha Bodenhausen:** Writing – review & editing, Methodology, Investigation, Data curation. **Hans-Martin Krause:** Writing – review & editing, Methodology, Investigation, Data curation. **Adrien Mestrot:** Writing – review & editing, Supervision, Project administration. **Chinwe Ifejika Speranza:** Writing – review & editing, Supervision, Funding acquisition. **Klaus A. Jarosch:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105358.

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