SOIL ORGANISMS 92 (2) · August 2020 pp. 99–108

Effects of steam sterilization on soil abiotic and biotic properties

Peter Dietrich[1,2,*], Simone Cesarz[2,3], Nico Eisenhauer[2,3] and Christiane Roscher[1,2]

[1] UFZ, Helmholtz Centre for Environmental Research, Physiological Diversity, Permoserstrasse 15, 04318 Leipzig, Germany
[2] German Centre of Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany
[3] Institute of Biology, Leipzig University, Deutscher Platz 5e, 04103 Leipzig, Germany
* Corresponding author, e-mail: peter.dietrich@idiv.de

Received 5 March 2020 | Accepted 16 June 2020
Published online at www.soil-organisms.de 1 August 2020 | Printed version 15 August 2020
DOI 10.25674/so92iss2pp99

Abstract

Experiments under natural conditions are becoming increasingly important to investigate the impacts of global change on biodiversity and ecosystem functioning, but field experiments are not always feasible. Climate or biodiversity chamber experiments can be an alternative, which, however, require large amounts of soil substrate. If only low amounts of target soil are available, high quantities of background soil must be sterilized and inoculated with target soil. One of the commonly used methods to sterilize large amounts of background soil is steam sterilization, because it is simple, fast and cheap. However, there is a lack of knowledge, whether steam sterilization is an effective method to completely eliminate all organisms in the soil (in particular heat-resistant organisms) as well as if and how it alters soil abiotic conditions like nutrient concentrations. Therefore, we tested which organisms survived the sterilization treatment and if the effectiveness can be improved by repeated steam sterilization. Additionally, we checked whether steam sterilization changes soil pH, carbon and nutrient concentrations, and whether this is strengthened by a double sterilization treatment. To study this, we steam-sterilized 2 m^3 sand-soil mix (1:4) for 150 min, stored it for 12 days at ambient temperature (for the germination of heat-resistant organisms) and repeated the sterilization procedure. We found a 27% reduction in microbial biomass carbon after the first sterilization treatment and a 51% reduction after the second sterilization treatment compared to untreated soil. Nematodes were almost completely eliminated (97% after second treatment), while rotifers largely remained unchanged. Soil pH and plant-available phosphorus concentration increased after the first sterilization treatment (pH: from 7.44 to 7.79; phosphorus: +28%). Phosphorus concentration increased further after the second sterilization treatment (+53% compared to untreated soil), while pH remained unchanged (7.77). Plant-available potassium and total carbon concentrations decreased after the first treatment (potassium: -19%; carbon: -5%), while total carbon further decreased (-8% compared to untreated soil) and potassium remained unchanged after the second treatment. Taken together, our study highlights that (single and double) steam-sterilization treatments were only partially effective, i.e. non-complete elimination of soil organisms, and additionally influenced soil properties. Nevertheless, steam sterilization is a fast and cost-effective alternative to other sterilization methods, especially when large amounts of soil substrate are needed. Therefore, if so, we recommend to use steam sterilization, but to sterilize the soil twice to significantly reduce the number of soil organisms, and further consider potential side effects, such as an increase in plant-available phosphorous concentration.

Keywords microbial biomass | nematodes | plant-available phosphorus | plant-available potassium | soil biodiversity

ISSN 1864-6417 (print) · **ISSN** 2509-9523 (online)

1. Introduction

There is a rising need for experiments under controlled environmental conditions studying the effect of global change on above- and belowground organisms and on their interactions (Van der Heijden et al. 2008, Blankinship et al. 2011, Van der Putten et al. 2013). Such experiments should be performed under natural field conditions as far as possible (Van der Putten et al. 2016), but this is not always feasible (e.g. when investigating the impact of increased atmospheric CO_2 concentration) or preliminary or partial experiments are necessary. In such cases, the field studies can be prepared or supplemented with greenhouse, climate chamber or biodiversity chamber experiments (Naeem et al. 1994, Eisenhauer & Türke 2018). These experiments require large amounts of soil substrate, which is prepared by inoculating sterilized soil (= background soil) with target soil, if only low amounts of target soil are available, e.g. due to small size of research plots, long-distance transport or nature conservation requirements. Common sterilization methods to produce background soil are autoclaving, gamma- or steam sterilization (Trevors 1996, Berns et al. 2008). The latter one is often used when high quantities of soil substrate are needed, because it is fast and cheap, compared to the other two methods. Thereby, hot water steam flows across the soil with the aim of eliminating all viable organisms (soil biota, plant seeds, etc.), but there is a risk that heat-resistant organisms might survive (Trevors 1996). Furthermore, it is well known that sterilization can lead to nutrient flushes (Skipper & Westermann 1973, Trevors 1996, Berns et al. 2008), which could influence the outcome of the experiment. Despite this knowledge, there is a lack of experiments exploring the efficiency of steam sterilization in eliminating soil biota (in particular heat-resistant organisms) and evaluating its influence on soil properties.

We tested in this study whether the effectiveness of steam sterilization can be improved by repeated sterilization. We steam-sterilized soil for 150 min at ~80°C and then stored the substrate for 12 days at ambient temperature. This incubation was intended to lead to the germination of heat-resistant spores of protozoa, bacteria and fungi and hatching of nematodes from resistant eggs (Trevors 1996). The second sterilization treatment (same sterilization procedure) was then performed to eliminate the germinated/remaining soil biota (Trevors 1996). To evaluate the effectiveness of single and double steam-sterilization treatments, we investigated soil microbial properties (microbial biomass, microbial basal respiration, microbial community composition) as well as abundance of nematodes and rotifers before and after the treatments. Furthermore, we tested whether single steam sterilization causes nutrient flushes (soil total nitrogen, plant-available phosphorus and potassium) or changes in soil properties (soil carbon, soil pH, soil water content), and if so, whether this is strengthened by a double steam-sterilization treatment.

We hypothesized, that (1) the abundance and activity of viable organisms is partly reduced after the first steam-sterilization treatment and completely reduced after second sterilization treatment. Furthermore, we expected (2) a gradual increase in soil carbon, nutrient concentrations and soil water content with each sterilization treatment, while soil pH is not significantly affected.

2. Materials and methods

Sterilization of background soil

In May 2017, soil substrate (1.6 m^3) for the steam sterilization was collected from a biodiversity experiment (Jena Experiment) in Jena (Thuringia, Germany, 50° 55′N, 11° 35′E, 130 m a.s.l.) nearby the river Saale. The soil of the study site is a Eutric Fluvisol which ranges from sandy loam to silty clay with increasing distance to the river (sand content: 40–7%; silt: 44–69%; clay: 16–24%; Roscher et al. 2004). Before the establishment of the biodiversity experiment in 2002, the site was used as arable land for about 40 years and heavily fertilized (Roscher et al. 2004). We used a mix of excavated soil material from different experimental plots for this study, which was stored for several years outside at the experimental area. The soil substrate was transported to the Helmholtz research station Bad Lauchstädt (Saxony-Anhalt), sieved to 10 mm and mixed with 0.4 m^3 quartz sand (0.25–0.5 mm grain size, WF 33, Quarzwerke GmbH, Walbeck, Germany). After mixing, we took a soil sample (around 200 g), which was stored in a fridge (4°C) until further processing (untreated soil with 20% sand = sample A).

On 29 June 2017, the soil-sand mix was steam-sterilized for 150 minutes at ~80°C (= single steam-sterilization treatment) using a modified tipping trailer (EDK 36, Münz Fahrzeugbau GmbH & Co KG, Pliezhausen, Germany) with a steam boiler (S 250, Möschle-Seifert-Dämpftechnik AG, Durbach, Germany) equipped with an oil burner (WL 30 Z, Max Weishaupt GmbH, Schwendi, Germany) and an insulating plastic cover to retain the heat (Fig. 1). After cooling down, the substrate was stored in four closeable plastic boxes (each of 0.6 m^3 size). Four soil samples (around 50 g from each box) were taken, pooled and stored in a fridge (4°C; immediately

after the first sterilization = sample B). The soil substrate was let rest for 12 days at ambient temperature to allow heat-resistant permanent states of soil biota to germinate. On 11 July 2017, i.e. 12 days later, the soil was steam-sterilized a second time using the same procedure as before (= double steam-sterilization treatment). The soil was again stored in the plastic boxes, which were sterilized with a potassium hypochlorite solution (Eau de Javel: 2.6 g KClO to 100 ml water; 1:1) before filling. After the second steam-sterilization treatment as well as after two additional weeks of storage in the boxes at ambient temperature, four soil samples per box were taken (50 g each), pooled per treatment (immediately after second sterilization (= sample C) and two weeks after second sterilization (= sample D)) and stored in the fridge (4°C). Microbial community composition was analyzed via phospholipid fatty acid analysis (PLFA); therefore, 25 g soil per sample (samples A–D) were frozen at -80°C until further processing.

Soil biota

To determine community composition and activity of soil biota before as well as after first and second steam sterilization, we used soil samples of A, B, C and D. All samples (A–D) were replicated five times with the exception of PLFA analysis (three replicates per sample). For analyses of soil microbial properties, the soil was sieved at 2 mm. Basal respiration (BAS) characterizing soil microbial activity and soil microbial biomass carbon (C_{mic}) as important soil ecosystem functions (Eisenhauer et al. 2018) were measured after Scheu (1992) using an O_2-microcompensation apparatus. Furthermore, determination of the soil microbial community was done via phospholipid fatty acid analysis (PLFA) following the protocol of Frostegård et al. (1991). For analyses of free-living nematodes and rotifers, the soil was sieved to 4 mm. Animals were extracted with a modified Baermann method (Ruess 1995) using 25 g soil per funnel (Cesarz et al. 2019). After extraction, soil samples were dried at 50°C for 48 h and weighed. Animals were counted per sample under the microscope using 400x magnification (DMI4000 B, Leica, Wetzlar, Germany), and 50 nematode individuals extracted from untreated soil and all nematodes extracted from soil samples after sterilization were identified to genus level and assigned to trophic groups (Bongers 1988, Yeates et al. 1993).

Soil characteristics

Soil characteristics before and after treatments were determined from soil samples of A, B and C. All samples (A–C) were replicated five times. Samples were air dried and sieved to 2 mm. Plant and animal residuals were removed using tweezers. Soil pH of the samples was determined in a 0.01 M calcium chloride

Figure 1 Photograph of the tipping trailer with steaming system, steam boiler and the insulating plastic cover (Photo: P. Dietrich).

suspension (pH Meter 766, Knick, Berlin, Germany). To determine total soil carbon concentrations and nitrogen concentrations, samples were ground to a fine powder with a mixer mill (MM2000, Retsch, Haan, Germany) and analyzed with an elemental analyzer (Vario EL Element Analyzer, Elementar, Hanau, Germany). Concentrations of soil carbonate were determined volumetrically with a calcimeter according to Scheibler. Soil organic carbon concentrations were calculated as the difference between total soil carbon concentrations and carbonate concentrations. For measurement of plant-available phosphorus concentrations of the samples, soil was extracted with 0.5 M sodium hydrogen carbonate solution (pH 8.5) according to Olsen P method (Olsen 1954) and afterwards analyzed with a plate reader (Varioskan LUX, Thermo Electron LED GmbH, Osterode am Harz, Germany) using the phosphomolybdate blue method (Murphy & Riley 1962). To determine plant-available potassium concentrations, soil samples extracted with 1 M calcium-acetate-lactate were analyzed with an inductively-coupled plasma optical emission spectrometer (ICP-OES, SPECTRO ARCOS, SPECTRO Analytical Instruments GmbH, Kleve, Germany). Soil water content was calculated as the difference of fresh and dried (50°C for 48 h) weight of soil samples used for nematode extraction.

Statistical analysis

Differences in soil biota (BAS, C_{mic}, nematodes, rotifers) and soil characteristics (pH, total nitrogen, total carbon, organic carbon, phosphorus, potassium concentrations, soil water content) among soil treatments (factor with four levels for soil biota: A–D; factor with three levels for soil characteristics: A–C) were tested using analysis of variance (ANOVA) and Tukey's HSD test. Additionally, we used a standardized principal component analysis (PCA) to visualize PLFA results. For all calculations, we used the statistical software R (version 3.6.1, R Development Core Team, http://www.R-project.org) including *ggplot2* (Wickham et al. 2016) and *ggfortify* (Tang et al. 2016) for PCA.

3. Results

Soil biota

Soil biota significantly changed after steam sterilization (Tab. 1). Microbial biomass carbon (C_{mic}) was reduced to 73 % (as measured by the mean) after the first steam-sterilization treatment and to 49 % after the second steam-

Table 1. Summary of properties of untreated soil and ANOVA results for the effect of steam-sterilization treatments (four levels for soil biota: untreated soil, after first and after second sterilization, two weeks after second sterilization; three levels for soil characteristics: untreated soil, after first and after second sterilization) on soil biota (microbial biomass carbon (C_{mic}), basal respiration (BAS), nematodes and rotifers) and soil characteristics (pH, water content, total carbon concentration, organic carbon concentration, total nitrogen concentration, plant-available phosphorus concentration, plant-available potassium concentrations). Listed are mean values, standard deviations (±SD) and units of variables for untreated soil as well as degrees of freedom (Df), mean sums of squares (MS), F ratios (F) and p-values (P) for ANOVA results. Significant effects ($P < 0.05$) are given in bold.

	Properties of untreated soil			ANOVA results			
	Mean	±SD	Unit	Df	MS	F	P
Soil biota							
Microbial biomass carbon	333.84	± 18.71	µg C_{mic} g_{dw}^{-1} soil	3	39096	71.03	**< 0.001**
Basal respiration	0.62	± 0.05	µl O_2 h^{-1} g_{dw}^{-1} soil	3	0.72	32.03	**< 0.001**
Nematodes	1.19	± 0.27	Ind. g_{dw}^{-1} soil	3	1.65	71.39	**< 0.001**
Rotifers	0.76	± 0.71	Ind. g_{dw}^{-1} soil	3	0.18	0.20	0.894
Soil characteristics							
Soil pH	7.44	± 0.03	-	2	0.19	19.70	**< 0.001**
Soil water content	0.13	± < 0.01	g g_{dw}^{-1} soil	2	< 0.01	959.20	**< 0.001**
Total carbon	25.82	± 1.16	mg g_{dw}^{-1} soil	2	5.52	5.64	**0.019**
Organic carbon	8.27	± 1.16	mg g_{dw}^{-1} soil	2	1.90	1.94	0.187
Total nitrogen	1.08	± 0.08	mg g_{dw}^{-1} soil	2	0.02	2.46	0.127
Plant-available phosphorus	0.02	± < 0.01	mg g_{dw}^{-1} soil	2	< 0.01	6.87	**0.010**
Plant-available potassium	0.10	± < 0.01	mg g_{dw}^{-1} soil	2	< 0.01	26.01	**< 0.001**

sterilization treatment compared to untreated soil (Tab. 1; Fig. 2a). After two-weeks storage, microbial biomass carbon was totally reduced to 41 % of the original value before sterilization (Fig. 2a). Basal respiration (BAS) was significantly increased after the first sterilization, but decreased continuously after the following treatments to a level equal to natural soil (Fig. 2b). Nematodes were completely reduced after the first sterilization (N = 0), but recolonized the soil afterwards (Fig. 2c). In the sterilized soil samples (B–D; N=15), we found in total 10 nematodes. All were juveniles and belonged to five different genera: *Cephalobus* (bacterial feeders), *Aporcelaimellus* (omnivores), *Aglenchus*, *Hoplotylus* and *Pratylenchus* (all three genera are plant feeders; Tab. 2). Abundance of rotifers was not significantly changed in response to sterilization (Fig. 2d). PCA of PLFA showed that the concentrations of most fatty acids decreased with each steam-sterilization event, with the exception of a15:0 (gram-positive bacteria), which showed a strong increase after steam-sterilization treatments (Tab. 3; Fig. 3).

Soil characteristics

We found significant differences between untreated soil and soil after the first and second sterilization treatment, respectively (Tab. 1). Soil pH, soil water content and plant-available phosphorus concentration increased after first steam-sterilization treatment (pH: from 7.44 to 7.78; water: +3 %; phosphorus: +28 %; Tab. 1; Fig. 4a, b, c). After second steam-sterilization treatment, soil pH remained unchanged (7.76), while soil water content and phosphorus concentration further increased by 19 %, respectively, compared to single sterilized soil (Fig. 4a, b, c). Plant-available potassium and total carbon concentrations decreased after the first steam-sterilization treatment (potassium: -19 %; total carbon: -5 %; Tab. 1; Fig. 4d, e). After the second sterilization treatment, total carbon concentration further decreased (-3 % compared to single sterilized soil), while potassium concentration remained unchanged (Fig. 4d, e). Soil organic carbon and total nitrogen concentrations did not differ among the treatments (Tab. 1; Fig. 4f).

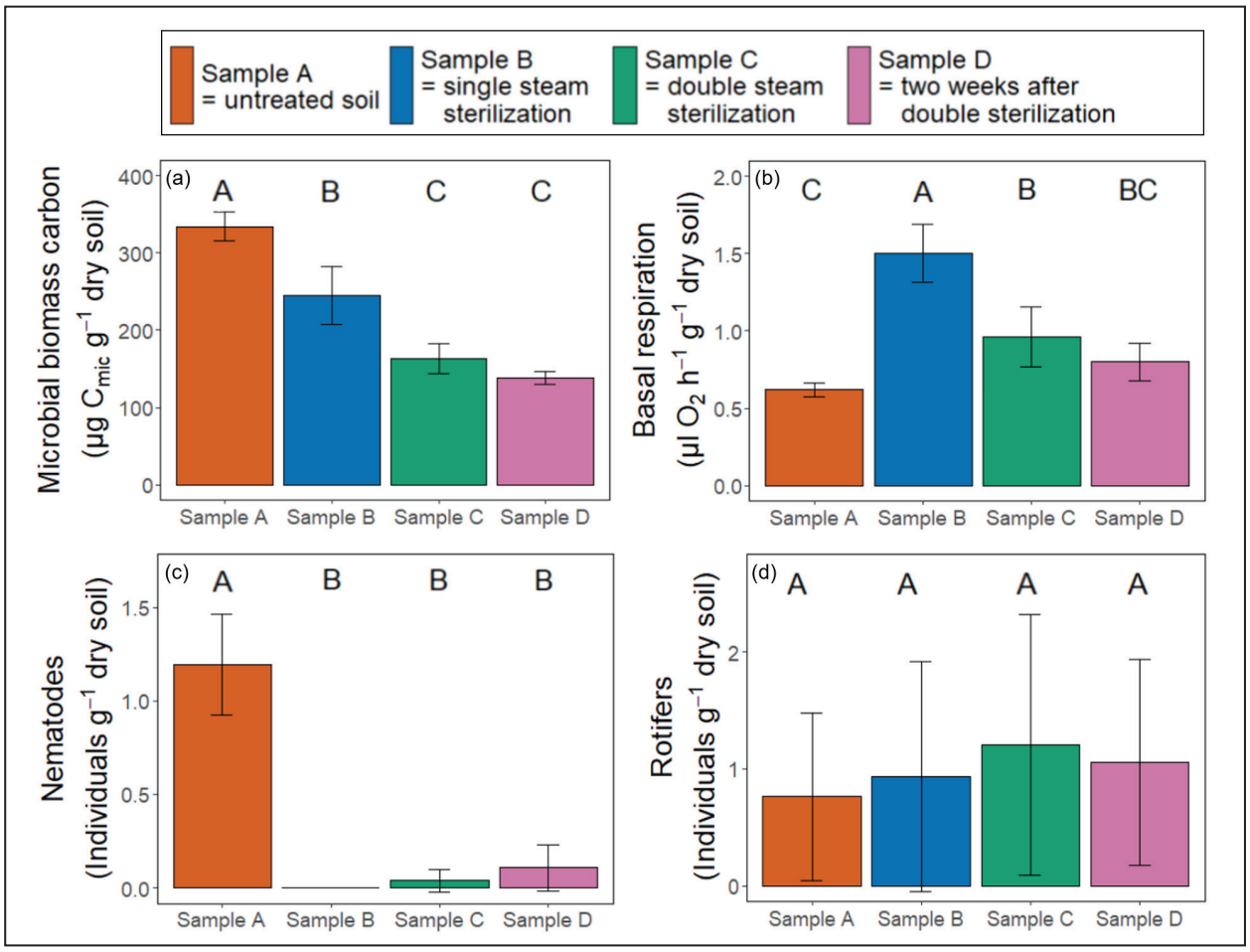

Figure 2. Microbial biomass carbon (C_{mic}; a), basal respiration (BAS; b), number of nematodes (c) and number of rotifers (d) of soil before steam sterilization (Sample A) and after the first (Sample B) and second steam-sterilization treatment (Sample C), as well as two weeks after second steam sterilization (Sample D; five replicates per sample). Bars show mean values (± 1 SD); letters above bars indicate significant ($P < 0.05$) differences among treatments (Tukey's HSD test).

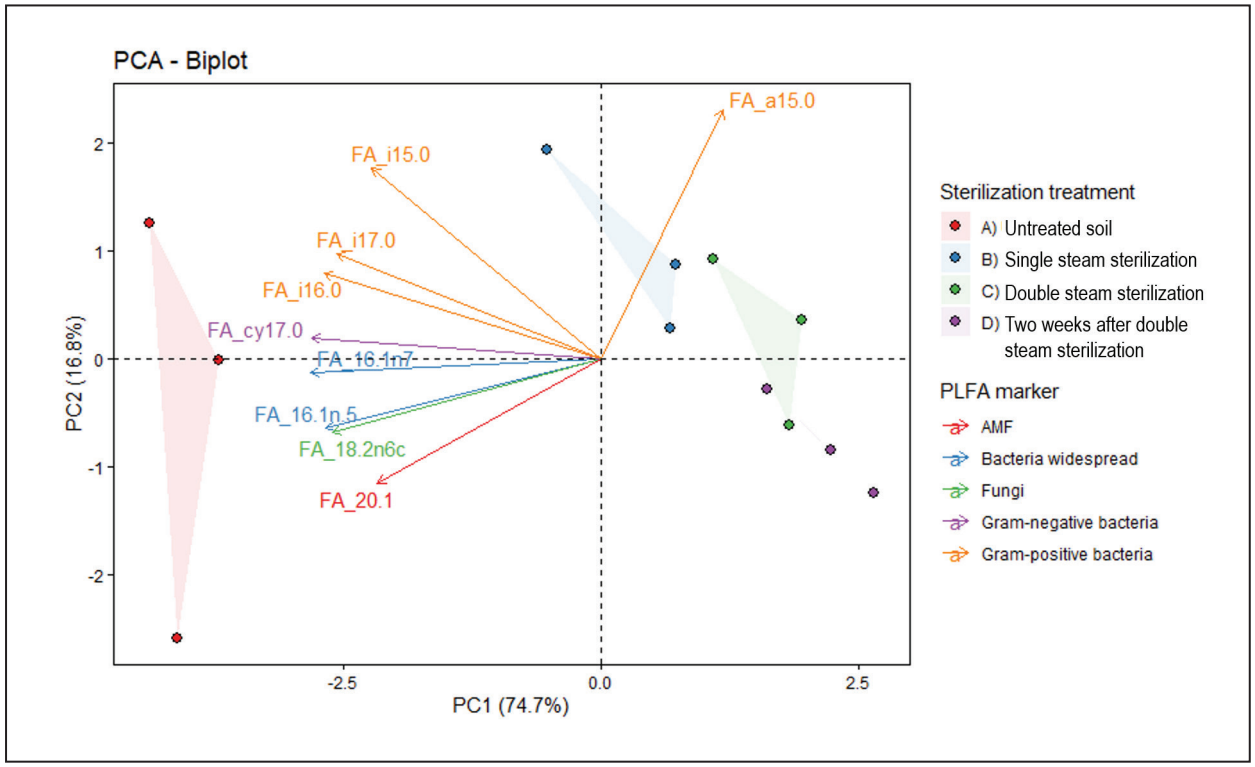

Figure 3. Standardized principal components analysis (PCA; first vs. second axes) of soil before steam sterilization (Sample A) and after the first (Sample B) and second steam-sterilization treatment (Sample C), as well as two weeks after second steam sterilization (Sample D; three replicates per sample) characterized by concentrations of PLFA markers (fatty acids = FA). Color of dots represent sterilization treatments. Arrows indicate the individual PLFA markers.

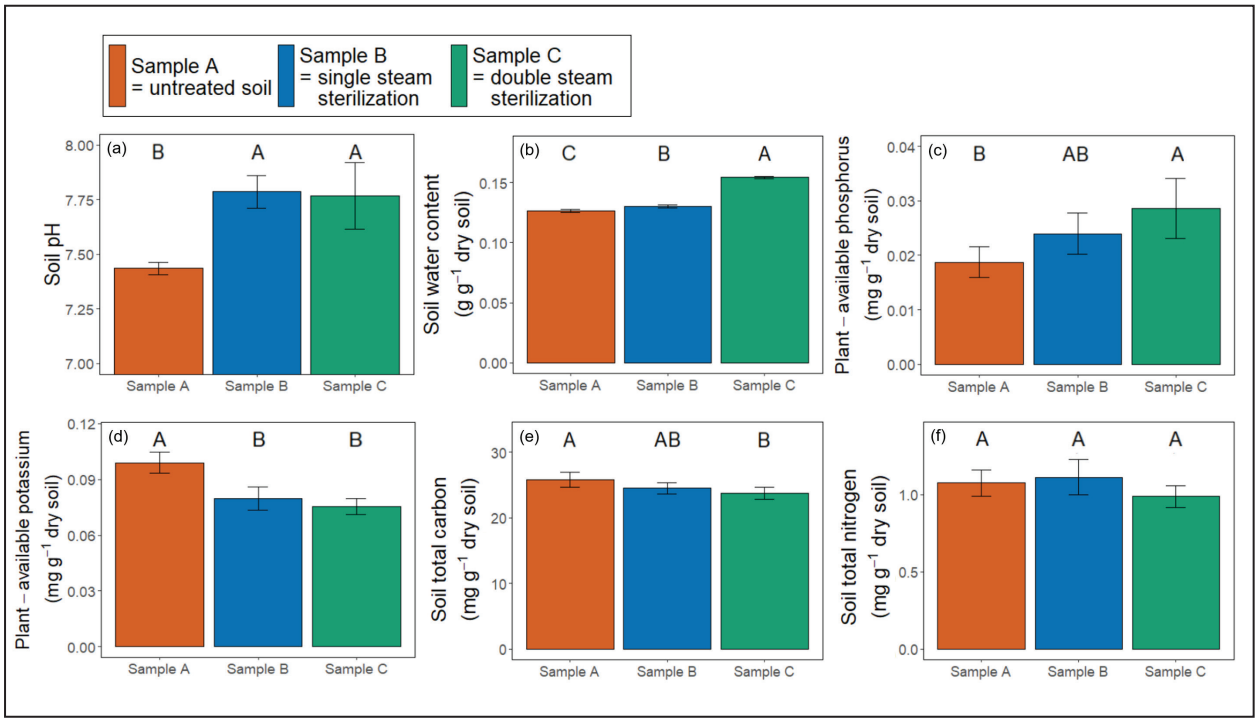

Figure 4 Soil pH (a), soil water content (b), plant-available phosphorus concentration (c), plant-available potassium concentration (d), total carbon concentration (e), and total nitrogen concentration (f) of soil before steam sterilization (Sample A) and after the first (Sample B) and second steam-sterilization treatment (Sample C; five replicates per sample). Bars show mean values (± 1 SD); letters above bars indicate significant ($P < 0.05$) differences among treatments (Tukey's HSD test).

Table 2. Summary table of nematode analysis before and after steam-sterilization treatments. Listed are nematode genera, trophic groups and number of individuals before and after sterilization.

Nematode community		Before sterilization	After sterilization
Genera	Trophic groups	Number of individuals	
Acrobeloides	Bacterial feeders	19	0
Cephalobus	Bacterial feeders	1	2
Eucephalobus	Bacterial feeders	1	0
Aphelenchus	Fungal feeders	1	0
Tylencholaimus	Fungal feeders	1	0
Eudorylaimus	Omnivores	1	0
Thornia	Omnivores	3	0
Aporcelaimellus	Omnivores	0	2
Aglenchus	Plant feeders	4	1
Boleodorus	Plant feeders	1	0
Paratylenchus	Plant feeders	4	0
Tylenchus	Plant feeders	8	0
Bitylenchus	Plant feeders	2	0
Helicotylenchus	Plant feeders	1	0
Hoplotylus	Plant feeders	0	1
Pratylenchus	Plant feeders	3	4

4. Discussion

Single steam-sterilization treatment reduced the abundance of soil biota; however, the effectiveness was lower than expected. Although nematodes were completely eliminated, soil microbial biomass carbon was only reduced to 73 % compared to untreated soil, and the number of rotifers was unchanged. Moreover, basal respiration of microorganisms strongly increased after the first sterilization treatment, which may have been caused by increased soil moisture due to steam condensing and phosphorus flush stimulating microbial activity of the surviving individuals. In general, the results show that a single steam-sterilization event is not sufficient to significantly reduce the number of soil microorganisms in background soil. The second steam-sterilization treatment showed a stronger reduction of soil biota: soil microbial biomass was reduced to 49 % compared to untreated soil, and basal respiration was significantly lower than after the first sterilization treatment (although soil water content and phosphorus concentration further increased). Nevertheless, the abundance of rotifers was still high and nematodes recolonized the soil after the second steam-sterilization treatment, which was possibly due to individuals which survived as eggs. Although we also expected nematodes to survive as dauer larvae, nematodes forming these resting stages (cp1) were not found in the present study. Higher numbers of nematodes after steam sterilization were observed in a study by McSorley et al. (2006) indicating that especially bacterial feeding nematodes strongly increased after an initial strong reduction due to steam sterilization. At day 73 after

Table 3. Summary table of PLFA analysis of untreated soil, soil after first and second steam-sterilization treatments and soil, which was stored for two weeks after the second sterilization treatment. Listed are PLFA markers, soil organism groups and amount of PLFA markers (ng/g_{soil}) detected.

PLFA markers	Soil organism group	Untreated soil		After first sterilization		After second sterilization		Two weeks after second sterilization	
		Mean ± SD (ng/g_{soil})		Mean ± SD (ng/g_{soil})		Mean ± SD (ng/g_{soil})		Mean ± SD (ng/g_{soil})	
i15:0	Gram positive	947.52	± 186.25	846.45	± 130.20	696.88	± 89.57	567.60	± 65.50
a15:0	Gram positive	557.07	± 139.06	1054.60	± 144.94	858.56	± 98.13	715.46	± 71.56
i16:0	Gram positive	454.71	± 49.45	321.15	± 47.44	296.15	± 39.45	251.38	± 40.86
i17:0	Gram positive	254.41	± 59.40	153.82	± 21.21	137.34	± 28.23	101.20	± 26.08
16:1n7	Bacteria	1937.60	± 117.21	1087.20	± 45.85	824.97	± 18.29	775.26	± 20.80
16:1n-5	Bacteria	575.29	± 65.29	330.53	± 23.84	208.81	± 23.27	232.57	± 50.64
cy17:0	Gram negative	289.99	± 32.97	135.57	± 17.63	75.36	± 15.87	75.84	± 14.16
18:2n6c	Fungi	746.56	± 147.45	502.56	± 29.64	364.98	± 64.66	345.78	± 21.00
20:1	AMF	233.32	± 77.76	149.90	± 32.02	141.35	± 28.35	133.79	± 24.83

sterilization, the number of bacterial-feeding nematodes was significantly higher than in the non-treated soil in their study. We also detected bacterial-feeding nematodes in our study (two individuals of the genus *Cephalobus,* a common genus in soil), which survived the double steam-sterilization treatment. Moreover, six out of the 10 nematodes were plant feeders (four of the genus *Pratylenchus*), while number of antagonists, such as omnivorous nematodes, were small in sterilized soil. Using this background soil for a plant experiment without further inoculation of soil biota, including predators, could lead to a reduced growth of plants due to an accumulation of plant feeders as shown in McSorley et al. (2006).

The PCA showed similar results for microorganisms: the abundance of most microorganisms gradually decreased with every sterilization event, but some gram-positive bacteria benefitted from steam sterilization. This abundance increase of gram-positive bacteria after steam sterilization was also shown in the study by McSorley et al. (2006). Our results indicate that the steam-sterilization treatment reduces a large range of soil biota, but also that specific species are still alive in background soil (and even benefitted from sterilization). Thus, steam sterilization can reduce soil biota but the subsequent community will be likely dominated by stress resistant organisms and fast-growing opportunistic species. After two-week storage of soil in plastic boxes, we found a small increase in the number of nematodes, but also a further decline in microbial biomass carbon and basal respiration. Therefore, longer storage of soil after steam sterilization may be recommendable, if nematodes are not problematic for the subsequent experiment.

Moreover, our results indicate that steam sterilization has a significant impact on abiotic soil properties. Double sterilization treatment increased plant-available phosphorus concentration by 53 % compared to untreated soil. This is in line with other studies showing that heating of soil can cause a flush in plant-available phosphorus (Skipper & Westermann 1973, Serrasolses et al. 2008). However, it should be noted that the soil we used was rich in calcium carbonate and had high pH, which can cause a reduced availability of plant-available phosphorus for plants (Blume et al. 2016). Due to sterilization, phosphorus retention could be relaxed leading to a higher concentration of plant-available phosphorus, which is in line with a study by Serrasolses et al. (2008). Furthermore, our study shows that soil pH changed from 7.44 to 7.77 after double steam sterilization, possibly due to the release of bases from the organic matter. This increase in pH was also shown in a previous study (Tanaka et al. 2003). Soil pH is an important soil property influencing growth rates and diversity of soil biota (Bååth & Arnebrant 1994, Zhalnina et al. 2015) and nutrient availability for plants (McCauley et al. 2009); however, the increase in pH was small, which probably has no influence on the subsequent experiment.

Contrary to our expectations, plant-available potassium and soil total carbon concentrations decreased after steam-sterilization treatment. Plant-available potassium in soils is to the large extent held by negative charges on clay particles (Sharpley 1989), while the binding capacity can differ depending on type, structure and particle size of phyllosilicates, and amount of complexing organic acids and inorganic cations (Singh & Goulding 1997). Heating of soil possibly changed the structure of silicates or the quantity of specific soil chemicals, which may have affected the binding capacity and thus the availability of potassium. Furthermore, the decrease of soil total carbon could be explained by the fact that carbon turned into gas due to high heat and evaporated, because the steaming system was not completely closed.

Our study shows that steam sterilization changed abiotic and biotic soil properties of background soil, which is in line with studies on the effects of autoclaving (Williams-Linera & Ewel 1984, Trevors 1996, Berns et al. 2008). Regarding the efficiency of steam sterilization, we recommend to perform double steam-sterilization treatments to reduce soil biota in background soil under 50 %. Regarding soil properties, our results indicate that it does not matter whether the soil is sterilized once or twice, because soil properties are influenced already after the first sterilization event. Only plant-available phosphorus concentration and soil water content increased even further after the second sterilization event in our study. Nevertheless, repeating the steam-sterilization treatment depends on the soil properties, and aim and design of the subsequent experiment (Trevors 1996). For instance, for experiments studying plant-mycorrhizal interactions, it may be better to sterilize the soil only once, because mycorrhizae are not very heat-resistant (Endlweber & Scheu 2006) and phosphorus flush would be lower.

In conclusion, our study highlights that steam sterilization eliminated many soil organisms, but also that a few groups of organisms survived and propagated. Furthermore, steam sterilization caused an increase in phosphorus and soil pH as well as a decrease in potassium, already after the first sterilization treatment. Nevertheless, steam sterilization is a fast and cost-effective alternative to other sterilization methods, especially when large amounts of soil substrate are needed. Therefore, we recommend to run steam sterilization, but to sterilize soil twice, and to consider potential side effects, such as the accumulation of

specific soil organisms or the change in plant-available phosphorus and potassium concentrations.

5. Acknowledgments

We thank the technicians of the experimental research station Bad Lauchstädt for the transport of soil from Jena to Bad Lauchstädt and preparation of background soil. Furthermore, we are grateful to Petra Hoffmann, Konstantin Albrecht, Laura Bergmann, Alfred Lochner and Lika Kudrjasova for measurements in the laboratory. We also thank Volker Grimm (UFZ) and three anonymous reviewers for helpful comments on earlier versions of this paper. The Jena Experiment is funded by the German Research Foundation (FOR 1451). Further support was provided by the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, funded by the German Research Foundation (FZT 118), and by the Heinrich-Böll-Foundation (PhD scholarship).

6. References

Bååth, E. & K. Arnebrant (1994): Growth rate and response of bacterial communities to pH in limed and ash treated forest soils. – Soil Biology and Biochemistry **26**: 995–1001.

Berns, A., H. Philipp, H. D. Narres, P. Burauel, H. Vereecken & W. Tappe (2008): Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. – European Journal of Soil Science **59**: 540–550.

Blankinship, J. C., P. A. Niklaus & B. A. Hungate (2011): A meta-analysis of responses of soil biota to global change. – Oecologia **165**: 553–565.

Blume, H.-P., H. W. Brümmer, R. Horn, E. Kandeler, I. Kögel-Knabner, R. Kretzschmar, K. Stahr & B.-M. Wilke (2016): Scheffer/Schachtschabel: Lehrbuch der Bodenkunde, Vol. 16. – Springer-Verlag: 569 pp.

Bongers, T. (1988): De nematoden van Nederland: een identificatietabel voor de in Nederland aangetroffen zoetwater-en bodembewonende nematoden. – Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging: 408 pp.

Cesarz, S., A. E. Schulz, R. Beugnon & N. Eisenhauer (2019): Testing soil nematode extraction efficiency using different variations of the Baermann-funnel method. – Soil Organisms **91**: 61–72.

Eisenhauer, N. & M. Türke (2018): From climate chambers to biodiversity chambers. – Frontiers in Ecology and the Environment **16**: 136–137.

Eisenhauer, N., J. Hines, F. Isbell, F. Van der Plas, S. E. Hobbie, C. E. Kazanski, A. Lehmann, M. Liu, A. Lochner & M. C. Rillig (2018): Plant diversity maintains multiple soil functions in future environments. – ELife 7: e41228.

Endlweber, K. & S. Scheu (2006): Establishing arbuscular mycorrhiza-free soil: a comparison of six methods and their effects on nutrient mobilization. – Applied Soil Ecology **34**: 276–279.

Frostegård, Å., A. Tunlid & E. Bååth (1991): Microbial biomass measured as total lipid phosphate in soils of different organic content. – Journal of Microbiological Methods **14**: 151–163.

McCauley, A., C. Jones & J. Jacobsen (2009): Soil pH and organic matter. – Nutrient management module **8**: 1–12.

McSorley, R., K. Wang, N. Kokalis-Burelle & G. Church (2006): Effects of soil type and steam on nematode biological control potential of the rhizosphere community. – Nematropica **36**: 197–214.

Murphy, J. & J. P. Riley (1962): A modified single solution method for the determination of phosphate in natural waters. – Analytica chimica acta **27**: 31–36.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton & R. M. Woodfin (1994): Declining biodiversity can alter the performance of ecosystems. – Nature **368**: 734–737.

Olsen, S. R. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. – US Department of Agriculture.

Roscher, C., J. Schumacher, J. Baade,W. Wilcke, G. Gleixner, W. W. Weisser, B. Schmid & E.-D. Schulze (2004): The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. – Basic and Applied Ecology **5**: 107–121.

Ruess, L. (1995): Studies on the nematode fauna of an acid forest soil: spatial distribution and extraction. – Nematologica **41**: 229–239.

Scheu, S. (1992): Automated measurement of the respiratory response of soil microcompartments: active microbial biomass in earthworm faeces. – Soil Biology and Biochemistry **24**: 1113–1118.

Serrasolses, I., J. Romanyà & P. Khanna (2008): Effects of heating and autoclaving on sorption and desorption of phosphorus in some forest soils. – Biology and Fertility of Soils **44**: 1063–1072.

Sharpley, A. N. (1989): Relationship between soil potassium forms and mineralogy. – Soil Science Society of America Journal **53**: 1023–1028.

Singh, B. & K. W. T. Goulding (1997): Changes with time in the potassium content and phyllosilicates in the soil of the Broadbalk continuous wheat experiment at Rothamsted. – European Journal of Soil Science **48**: 651–659.

Skipper, H. D. & D. T. Westermann (1973): Comparative effects of propylene oxide, sodium azide, and autoclaving on selected soil properties. – Soil Biology and Biochemistry **5**: 409–414.

Tanaka, S., T. Kobayashi, K. Iwasaki, S. Yamane, K. Maeda & K. Sakurai (2003): Properties and metabolic diversity of microbial communities in soils treated with steam sterilization compared with methyl bromide and chloropicrin fumigations. – Soil science and plant nutrition **49**: 603–610.

Tang, Y., Horikoshi, M. & Li, W. (2016): ggfortify: unified interface to visualize statistical results of popular R packages. – The R Journal **8**: 478–489.

Trevors, J. (1996): Sterilization and inhibition of microbial activity in soil. – Journal of Microbiological Methods **26**: 53–59.

Van der Heijden, M. G., R. D. Bardgett & N.M. Van Straalen (2008): The unseen majority: soil microorganisms as drivers of plant diversity and productivity in terrestrial ecosystems. – Ecology Letters **11**: 296–310.

Van der Putten, W. H., R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski & J. A. Schweitzer (2013): Plant–soil feedbacks: the past, the present and future challenges. – Journal of Ecology **101**: 265–276.

Van der Putten, W. H., M. A. Bradford, E. Pernilla Brinkman, T. F. van de Voorde & G. F. Veen (2016): Where, when and how plant–soil feedback matters in a changing world. – Functional Ecology **30**: 1109–1121.

Wickham, H., W. Chang & M. H. Wickham (2016): Package 'ggplot2'. – Create Elegant Data Visualisations using the Grammar of Graphics Version **2**: 1–189.

Williams-Linera, G. & J. J. Ewel (1984): Effect of autoclave sterilization of a tropical andept on seed germination and seedling growth. – Plant and Soil **82**: 263–268.

Yeates, G. W., T. Bongers, R. De Goede, D. W. Freckman & S. Georgieva (1993): Feeding habits in soil nematode families and genera—an outline for soil ecologists. – Journal of nematology **25**: 315.

Zhalnina, K., R. Dias, P. D. de Quadros, A. Davis-Richardson, F. A. Camargo, I. M. Clark, S. P. McGrath, P. R. Hirsch & E. W. Triplett (2015): Soil pH determines microbial diversity and composition in the park grass experiment. – Microbial ecology **69**: 395–406.