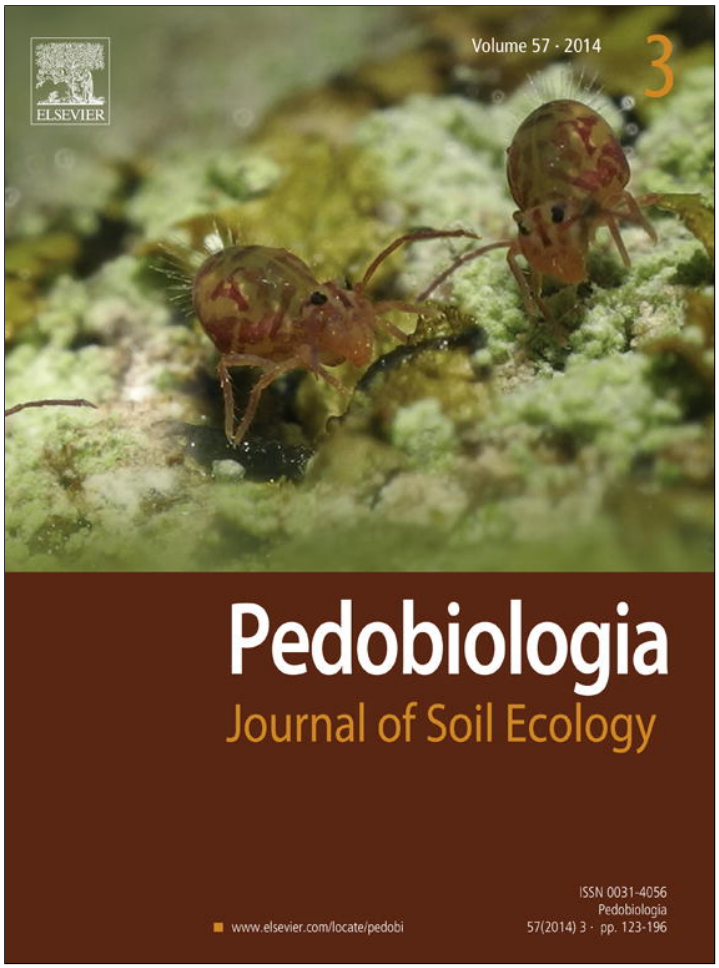

Pedobiologia 57 (2014) 123–130

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.de/pedobi

Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings

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ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form
18 December 2013
Accepted 20 December 2013

Keywords:
EPS-producing bacteria
Gamma-irradiation
Rhizosheaths
Root morphology
Soil autoclaving
Wheat

ABSTRACT

Sterilized soils are frequently used in experiments related to soil biology. Soil sterilization is known to alter physicochemical characteristics of soil, plant growth and community structure of the newly developed bacterial population. However, little information exists regarding soil sterilization effects on belowground processes mediated through root–microbe–soil interactions, e.g., development of rhizosheaths which significantly promote the plant growth under stress environments. The present study was conducted to elucidate effects of soil sterilization on wheat root growth and formation of rhizosheaths in relation to chemical changes caused by soil sterilization and the proportion of expolysaccharide (EPS)-producers in bacterial population recolonizing the sterilized soils. Wheat plants were grown for two weeks under greenhouse conditions either in the unsterilized soil or in soils sterilized by autoclaving (121 °C, 1 h) or by gamma (γ)-irradiation (50 kGy). While soil sterilization had no effect on the release of macronutrients, both sterilization procedures significantly increased the electrical conductivity, water-soluble carbon and DTPA-extractable Mn. Seedlings grown in sterilized soils produced higher root biomass and the rhizosheath soil (RS) mass as compared to those grown in the unsterilized soil. Soil sterilization also increased the root length, surface area, volume and number of tips. In bulk soil, RS and on roots, the proportion of EPS-producers in the total bacterial population was higher in sterilized treatments than in the unsterilized. Amending the unsterilized soil with glucose-C increased the root biomass, whereas adding Mn II increased the RS mass. The results showed that soil sterilization by autoclaving or γ-irradiation increases the root growth and RS mass of wheat seedlings. The water-soluble C and DTPA-extractable Mn released upon sterilization, and the increased proportion of EPS-producers in the bacterial population recolonizing the sterilized soils were involved in the observed effects. The results may have implications in studies using autoclaved or γ-irradiated soils to investigate soil–plant–microbe interactions and signify the need to account for intrinsic stimulatory effects of soil sterilization.

Introduction

Soil sterilization is frequently used to eliminate or reduce microbial activity in studies pertaining to microbial inoculations, soil enzymes, and degradation/sorption/mobility of pesticides and other xenobiotics (Degrange et al. 1997; Luo et al. 2001; Liebich et al. 2006). Methods of soil sterilization include heat treatments (e.g., dry heat and autoclaving), fumigation (e.g., with formaldehyde, propylene oxide, chloroform and methyl bromide) and high-level γ-irradiation (Trevors 1996; McNamara et al. 2003). Due to toxic residual effects the use of fumigants will be withdrawn by 2015 (Alphei and Scheu 1993), whereas autoclaving and γ-irradiation are relatively safe and effective methods for soil sterilization (Alphei and Scheu 1993; McNamara et al. 2003).

An ideal sterilization method should not adversely affect soil properties. However, autoclaving as well as γ-irradiation are known to alter physicochemical characteristics of soil (Trevors 1996; McNamara et al. 2003; Berns et al. 2008). Autoclaving is known to decrease (Darbar and Lakzian 2007) or increase (Alphei and Scheu 1993) the soil pH, to decrease the cation-exchange capacity (CEC) (Sandler et al. 1988), and to increase the dissolved organic carbon (DOC) (Lynch 1982; Serrasolsas and Khanna 1995a; Darbar and Lakzian 2007). Besides increasing the electrical conductivity (EC) of soil (Darbar and Lakzian 2007), autoclaving is known to increase available/exchangeable/extractable nutrients e.g., N (NH_4^+ and NO_3^-), P (Skipper and Westermann 1973; Lopez and Wollum

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http://dx.doi.org/10.1016/j.pedobi.2013.12.005

1976; Alphei and Scheu 1993; Serrasolsas and Khanna 1995a,b; Darbar and Lakzian 2007), Mg, Mn and Fe (Lopez and Wollum 1976; Wolf et al. 1989). While high level γ-irradiation is an effective biocide, it also affects soil physicochemical properties. However, γ-irradiation is relatively less disruptive and thus preferable to other methods of soil sterilization (McNamara et al. 2003; Berns et al. 2008). Although γ-irradiation up to 20 kGy generally eliminates actinomycetes, fungi, invertebrates and bacteria in most soils, radio-resistant bacteria may require a dose higher than 70 kGy (McNamara et al. 2003). Effects of γ-irradiation on soil CEC and pH are variable with no consistent trends (Wolf et al. 1989; Thompson 1990; Alphei and Scheu 1993). The generally observed effects of γ-irradiation on soil chemistry include a decrease in the CEC (Bank et al. 2008) and NO_3^--N (Alphei and Scheu 1993; Tuominen et al. 1994), and an increase in the DOC (Lynch 1982; Marschner and Bredow 2002; Berns et al. 2008). Gamma-irradiation of soil is also known to increase available/exchangeable/extractable nutrients e.g., NH_4^+-N (Alphei and Scheu 1993; Tuominen et al. 1994), K (Bowen and Cawse 1964; Dalton et al. 1989), P (Thompson 1990; Alphei and Scheu 1993), Fe (Bank et al. 2008) and Mn (Bowen and Cawse 1964; Wolf et al. 1989). Effects of γ-irradiation on soil chemical properties are generally dose-dependant and are more drastic on moist compared to dry soils (McNamara et al. 2003).

Studies on plant growth in autoclaved and γ-irradiated soils generally pertain to effects of microbial inoculations, whereas only few deal with intrinsic effects of soil sterilization on plant growth. The most frequently observed effect of steam sterilization of soil is the reduced plant growth due to toxicity of plant-available Mn released from the organic fraction, and due to elimination of microbes that transform the available Mn into higher oxides (Boyd 1971; Williams-Linera and Ewel 1984). Plant growth in autoclaved soils may also be reduced due to P deficiency induced by killing of symbiotic myccorrhizae involved in P absorption (Wallace et al. 1973). Plant growth in γ-irradiated soils may either be slightly reduced (Bowen and Rovira 1961), or considerably increased mainly due to increased exchangeable N (Jenkinson et al. 1972). Since soil sterilization may alter the root growth and community structure of the newly developed bacterial population (Marschner and Rumberger 2004; Wertz et al. 2007), we hypothesize that it may also influence belowground processes mediated through root–microbe–soil interactions, e.g., development of rhizosheaths. Rhizosheaths are common in plants and are particularly observed in grasses and cacti growing under drought stress. Although their function is not fully understood, rhizosheaths are known to play important role in conserving water under drought stress (Watt et al. 1994), in alleviating salt stress by restricting Na uptake (Ashraf et al. 2004), and in associative nitrogen fixation (Bergmann et al. 2009). Consequently, an increase in the mass of rhizosheaths may significantly promote plant growth under stress environments (Amellal et al. 1998; Ashraf et al. 2004). Inoculating efficient strains of EPS-producing bacteria is known to increase rhizosheaths in wheat plants grown under non-axenic conditions (Amellal et al. 1998; Ashraf et al. 2004). Keeping in view the altered root growth and structure of the bacterial community recolonizing sterilized soils, soil sterilization as such may also affect the formation of rhizosheaths in plants grown under non-axenic conditions. However, studies pertaining to intrinsic effects of soil sterilization on the development of rhizosheaths have been lacking. The present study was conducted to elucidate effects of soil sterilization on the growth, root morphology and the development of rhizosheaths in wheat seedlings grown non-axenically in autoclaved and γ-irradiated soils. The population densities of EPS-producing and total bacteria recolonizing sterilized soils were also measured and compared with those of unsterilized soil. Besides, since water-soluble C and extractable Mn have been consistently reported to increase in the autoclaved and γ-irradiated soils, experiments were conducted to examine their possible role in modifying the root growth/morphology and the mass of rhizosheaths.

Materials and methods

Soil

The soil (Typic Ustocrepts, Hafizabad series; hereafter referred as Hafizabad sandy loam) used in most experiments was collected from an experimental field in the Nuclear Institute for Agriculture & Biology, Faisalabad. The field has been under a mungbean–wheat rotation for the past 20 years. Some physicochemical characteristics of the arable (0–20 cm) soil layer were: total N, 0.07%; organic matter, 0.85%; maximum water-holding capacity (WHC), 30.5%; $CaCO_3$, 1.8%; pH (1:1, soil water), 7.2; EC (1:1, soil water), 672 $\mu S\,m^{-1}$; CEC, 9.6 meq g^{-100}; sand 63.0%; silt, 20.7% and clay, 16.3%. The soil was air dried, sieved (<2 mm) and stored at room temperature. Before autoclaving, the soil was moistened to 45% WHC and conditioned at room temperature for 48 h. The soil (2-cm thick layer in glass dishes) was autoclaved at 121 °C for 1 h. For γ-irradiation, 5-kg portions of the air-dried soil were packed in cardboard boxes lined with polyethylene sheet and irradiated at 50 kGy using a ^{60}Co commercial gamma irradiator.

Plant growth experiments

All experiments were conducted in PVC plastic cylinders (8 cm inner diameter × 18 cm deep) accommodating 1 kg of soil. To facilitate the recovery of intact root system, cylinders were vertically split into two halves, which were combined together with adhesive tape and the base sealed with polyethylene sheet. After desired treatments, the final soil moisture content was adjusted to 50% of WHC and maintained throughout the experiment period. Eight seeds of wheat (*Triticum aestivum* cv. Iqbal-2000) were sown, and three days after germination the plant population reduced to four pot^{-1}. With this experimental set up, roots remained within the soil and did not approach the pot bottom during the two-week growth period. For harvesting, cylinders were cut open, the soil spread in a tray and seedlings recovered along with intact root system including the soil adhering to roots. Plant growth experiments were carried out in a completely randomized design with three replicate pots for each treatment. Plants (four) from each replicate pot were pooled before analyses. All experiments were conducted in a greenhouse under natural conditions during December to March. Climatic conditions during different experiments were as follows: day length, 10.9–12.2 h; temperature, 8–13 °C (minimum) and 22–28 °C (maximum); relative humidity, 73–100% (morning) and 41–68% (mid-day); and photon flux density, 114–517 $\mu mol\,m^2\,s^{-1}$ (morning) and 535–1293 $\mu mol\,m^2\,s^{-1}$ (mid-day). Although in all experiments plants were grown for two weeks, the shoot and root biomass and the mass of rhizosheaths varied in different experiments due to variation in climatic conditions.

Effects of soil sterilization on plant growth and rhizosheaths

Two experiments were conducted to study effects of soil autoclaving and γ-irradiation on plant growth, root morphology and the mass of rhizosheath soil (RS) of wheat seedlings grown without nutrient amendment. In the first experiment, besides determining the plant biomass and the mass of RS, root morphology was studied and the RS analyzed for water-soluble and insoluble saccharides. In the second experiment, effects of soil autoclaving and γ-irradiation were studied on the shoot biomass/nutrient concentration, mass of covered and bare root components, and the mass of RS. Additional pots were maintained under similar conditions for measuring the

Table 1
Physicochemical properties of the Hafizabad sandy-loam as affected by autoclaving and γ-Irradiation.

Soil property	Treatment[a]				LSD	
	Unsterilized (air-dried)	Unsterilized (conditioned)	Autoclaved	γ-Irradiated	$P<0.05$	$P<0.01$
pH (1:1, soil:water)	7.23 ± 0.01 d[b]	7.41 ± 0.01 b	7.60 ± 0.02 a	7.36 ± 0.01 c	0.04	0.06
EC (μS m^{-1})	683 ± 6 c	672 ± 3 c	778 ± 5 a	713 ± 12 b	23.1	33.6
CEC (meq g^{-100})	9.6 ± 0.0 a	9.6 ± 0.0 a	8.9 ± 0.5 a	8.7 ± 0.5 a	NS	
Water soluble carbon (mg kg^{-1})	81 ± 1 c	48 ± 5 d	157 ± 3 a	119 ± 8 a	16.3	23.8
NH_4^+-N (mg kg^{-1})	8.0 ± 0.9 a	5.9 ± 1.6 ab	5.6 ± 1.0 ab	3.4 ± 0.2 b	3.41	4.96
NO_3^--N (mg kg^{-1})	51.4 ± 0.5 a	55.5 ± 2.6 a	49.9 ± 1.1 a	42.7 ± 2.0 b	5.70	8.29
Olson's P (mg kg^{-1})	8.5 ± 0.5 a	8.6 ± 0.3 a	8.7 ± 0.8 a	8.6 ± 0.2 a	NS	
NH_4OAc-extractable K (mg kg^{-1})	200 ± 6 ab	206 ± 3 a	188 ± 3 b	191 ± 3 b	13.3	19.3
KCl-extractable Al (mg kg^{-1})	Nd[c]	Nd	Nd	Nd		
DTPA-extractable Mn (mg kg^{-1})	1.3 ± 0.1 c	0.8 ± 0.0 c	13.2 ± 0.3 a	4.1 ± 0.1 b	0.53	0.77
DTPA-extractable Cu (mg kg^{-1})	1.1 ± 0.1 b	1.0 ± 0.0 c	0.9 ± 0.1 d	1.7 ± 0.0 a	0.11	0.16
DTPA-extractable Zn (mg kg^{-1})	1.1 ± 0.2 a	1.0 ± 0.3 a	0.9 ± 0.2 a	1.1 ± 0.1 a	0.69	1.01
DTPA-extractable Fe (mg kg^{-1})	3.3 ± 0.1 b	2.5 ± 0.1 d	2.8 ± 0.1 c	3.8 ± 0.1 a	0.29	0.41

[a] For autoclaving, the soil was conditioned for 72 h whereas air-dried soil was used for γ-irradiation.
[b] Mean of 3 replicates ± SE; figures in a row followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$); LSD = least significant difference.
[c] Not detected.

population density of total and EPS-producing bacteria in the bulk soil, RS, and on roots.

Effects of C and Mn amendments on plant growth and RS mass

Since soil sterilization significantly increased the water-soluble C and the DTPA-extractable Mn (Table 1), experiments were conducted to elucidate the possible role of soluble C and Mn in the observed variation in wheat seedling growth and the RS mass. In the first experiment, the unsterilized soil besides receiving N (at 100 mg kg^{-1} as NH_4NO_3) and P (at 50 mg kg^{-1} as KH_2PO_4) was amended with glucose at 0, 125, 250 or 500 mg C kg^{-1}. In the second experiment, the unsterilized soil besides receiving N and P was amended with $MnSO_4{\cdot}H_2O$ at 0, 5, 15 or 50 mg Mn kg^{-1}. In the Hafizabad sandy-loam that was used in all previous experiments, autoclaving released relatively smaller amount of DTPA-extractable Mn (net release, 12 mg kg^{-1}) than generally reported in the literature. Therefore, in the third experiment, we explored another soil (Hafizabad clay loam) to study if higher amounts of DTPA-extractable Mn released upon autoclaving might differently affect the plant growth or the RS mass. In this experiment, plant growth and RS mass in the autoclaved soil were compared with unsterilized soils in which the water-soluble C and/or Mn contents were either not changed, or were brought equal to those of the autoclaved soil using glucose and $MnSO_4{\cdot}H_2O$.

Isolation of the rhizosheath soil

Plants were shaken on a vibrating-arm shaker (1 min) to remove the loosely adhering soil from roots. The soil tightly adhering to roots (i.e., the RS) was recovered by washing roots with distilled water. As done for shoots and roots, the RS of four plants from each replicate pot was pooled. Plant material and RS were dried at 70 °C until constant weight.

Root morphology

Root morphology of wheat seedlings grown in unsterilized and sterilized soils was compared using an image-analysis technique. Roots were stained (1 min) in methyl violet (0.1 g l^{-1}), washed with distilled water and spread in water contained in a transparent tray. The tray was placed on a desktop scanner (AGFA SNAP SCAN 1236) and the image recorded for further analysis using WinRHIZO Pro v. 2003B software (Regent Instrument Inc. Montréal, QC, Canada).

Bacterial population density

Population densities of total and EPS-producing bacteria in soil (bulk and RS) and on roots were determined by the plate count method. One g soil (fresh weight) was shaken with nine ml of sterile saline (30 min, 200 rpm) and the suspension serially diluted. Root samples were washed with sterile water and the excess water absorbed on sterile filter paper. One g (fresh weight) roots were homogenized with pestle and mortar in nine ml of sterile saline and serially diluted. A 100-μl of the selected serial dilution was spread on LB (for total bacteria) or on RCV (for EPS-producing bacteria) agar media plates. Plates were incubated at 30 °C and the number of colonies counted after 48 h on LB agar and after seven days on RCV agar. For determining the dry weight, a separate set of soil and root samples was dried at 70 °C.

Analyses

Soil pH and EC were measured after shaking soil (30 min) with distilled water (soil:water, 1:1), whereas CEC was determined by the NH_4OAc method. For extraction of water-soluble C, 20 g soil was shaken (2 h) with 100 ml of distilled water, centrifuged (10 min, 1500 rpm) and the supernatant filtered (Sartorius Minisart, 0.2 μm). Organic C in the filtrate was determined by an acid-dichromate method (Riehm and Ulrich 1954). For determination of saccharides in the RS, RS-water suspension was centrifuged, the supernatant filtered (Sartorius Minisart, 0.2 μm) and the residual RS air dried. Water-soluble saccharides in the filtrate and insoluble saccharides in the residual RS were measured as glucose-equivalents using phenol–sulphuric acid method (Šafařik and Šantrůčková 1992). Soil mineral N was determined by a micro-Kjeldahl method after extraction with 2 N KCl (Keeney and Nelson 1982), whereas total N of plant material was determined by a micro-Kjeldahl method as described by Bremner and Mulvaney (1982). Soils were extracted for determination of available P (0.5 M $NaHCO_3$; soil:extractant 1:5), K (1 N NH_4OAc; soil:extractant 1:5), Al (1 N KCl; soil:extractant 1:5) and micronutrients (AB-DTPA; soil:extractant 1:2). For determination of total P and cations (K, Zn, Cu, Fe and Mn), powdered plant material was digested in a mixture of HNO_3:$HClO_4$:H_2SO_4 (10:4:1). Available P in the soil extract was determined according to Olsen et al. (1954), whereas total P in the acid digest was analyzed by vanadomolybdophosphoric method (Jackson 1962). Cations in the acid digest and in soil extracts were measured by ICP-OES.

Table 2
Dry matter yield of shoot, root and rhizosheaths soil, and root morphology of wheat seedlings grown in unsterilized, autoclaved and γ-irradiated Hafizabad sandy loam.[a]

Parameter	Treatment			LSD	
	Unsterilized	Autoclaved	γ-Irradiated	$P<0.05$	$P<0.01$
Dry matter yield					
Shoot (mg pot^{-1})	121 ± 5 a[b]	138 ± 5 a	151 ± 13 a	NS	
Root (mg pot^{-1})	84 ± 8 b	148 ± 18 a	138 ± 6 a	41.1	62.3
Rhizosheath soil mass (RS)					
g pot^{-1}	2.4 ± 0.5 c	4.1 ± 0.2 b	6.4 ± 0.3 a	1.16	1.76
g (m root)$^{-1}$	0.3 ± 0.0 c	0.4 ± 0.0 b	0.5 ± 0.0 a	0.06	0.09
Water soluble saccharides in RS					
mg g^{-1}	1.6 ± 0.1 a	1.0 ± 0.1 b	0.9 ± 0.0 b	0.21	0.32
mg pot^{-1}	3.5 ± 0.1 c	4.2 ± 0.2 b	5.4 ± 0.2 a	0.53	0.80
Water insoluble saccharides in RS					
mg g^{-1}	7.9 ± 0.4 a	6.6 ± 0.3 b	8.1 ± 0.0 a	0.95	1.44
mg pot^{-1}	18.0 ± 1.7 c	26.8 ± 0.6 b	51.2 ± 1.7 a	4.87	7.37
Root morphology					
Length (m pot^{-1})	7.1 ± 0.3 b	10.4 ± 0.9 a	12.6 ± 1.0 a	2.74	4.16
Surface area (cm^2 pot^{-1})	65 ± 10 b	102 ± 8 a	130 ± 13 a	36.0	54.6
Volume (cm^3 pot^{-1})	0.49 ± 0.1 b	0.80 ± 0.1 a	1.07 ± 0.1 a	0.29	0.44
Average diameter (mm)	0.30 ± 0.04 a	0.31 ± 0.01 a	0.33 ± 0.01 a	NS	
Tips (pot^{-1})	3256 ± 413 c	4884 ± 169 b	6536 ± 621 a	1527	2314
Specific root length [m (g root DW)$^{-1}$]	86 ± 7 ab	71 ± 4 b	91 ± 3 a	17.9	27.3
Specific root tip density (g root DW^{-1})	43,140 ± 2319 a	32,824 ± 593 b	47,255 ± 3483 a	84,423	12,792

[a] Experiment conducted without additional nutrient application.
[b] Mean of 3 replicates ± SE; figures in a row followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$).

Data were subjected to analysis of variance followed by Duncan's multiple range test (DMRT) using COSTAT software. All results are reported on the basis of oven-dry weight and are means of three replicates.

Results

Chemical changes in soil due to sterilization

Since soil was moistened and conditioned before autoclaving and an air-dried soil was used for γ-irradiation, changes due to sterilization were compared with the respective unsterilized soil. Both sterilization procedures caused significant changes in some chemical characteristics of the soil; changes were more pronounced due to autoclaving than by γ-irradiation (Table 1). Although CEC was not affected, both sterilization methods significantly increased the EC ($P<0.05$). The soil pH either slightly decreased due to autoclaving or slightly increased by γ-irradiation ($P<0.05$). Water-soluble C increased due to autoclaving (3.3-fold increase; $P<0.01$) as well as by γ-irradiation (1.5-fold increase; $P<0.01$). Plant-available macronutrients (NH_4^+, NO_3^- and P) were not affected by autoclaving though it caused a slight (9%) decrease in the extractable K. While γ-irradiation had no effect on available P and K, it caused a 58% reduction in NH_4^+ ($P<0.05$) and a 17% reduction in NO_3^- ($P<0.01$). Among micronutrients, the most pronounced effect of soil sterilization was on DTPA-extractable Mn, which increased due to autoclaving (17-fold increase; $P<0.01$) and γ-irradiation (3-fold increase; $P<0.01$).

Effects of soil sterilization on plant growth and RS mass

In the first experiment carried out without additional N and P, shoot growth was not affected by soil sterilization (Table 2), whereas the root biomass significantly increased due to autoclaving (1.8-fold increase; $P<0.01$) and γ-irradiation (1.7-fold increase; $P<0.05$). Both soil sterilization procedures significantly increased the mass of rhizosheaths (Fig. 1, supplementary material; Table 2). The increase in the pot^{-1} mass of RS was more pronounced with γ-irradiation (2.6-fold increase; $P<0.01$) than with autoclaving (1.7-fold increase, $P<0.05$). The specific mass of RS i.e., (m root)$^{-1}$ also increased due to soil sterilization (Table 2); again the increase being higher due to γ-irradiation (50%; $P<0.01$) than due to autoclaving (18%; $P<0.01$). Coinciding with the increased (pot^{-1}) mass of RS, both sterilization methods also increased the amount of water-soluble and insoluble saccharides in the RS.

Analysis of root morphology confirmed the stimulatory effect of soil sterilization on root growth as revealed by a significant increase in the root length, number of tips, surface area and volume. However, while soil sterilization had no effect on the average root diameter, autoclaving significantly reduced the specific root tip density (24% reduction, $P<0.05$; Table 2). Stratifying the root morphology data into different root-diameter classes indicated that the majority of root tips (representing laterals) belonged to the finest roots (diameter up to 0.2 mm) without significant effects of soil sterilization (Fig. 2, supplementary material). More than 75% of the root length comprised fine roots having diameter up to 0.4 mm, whereas almost 50% of the root surface area and 23–33% of the root volume belonged to this root diameter class. Thicker roots (diameter, 0.4–1.0 mm) contributed more to the root surface (40–49%) and volume (56–63%) than to the root length (18–23%). Roots belonging to the diameter class 0–6.0 mm contributed to 91–94% of the total root length, 73–83% of the total surface area and 51–66% of the total root volume; the length, surface area and volume of this root population were not significantly different in plants grown in unsterilized or sterilized soils.

In the second experiment conducted without nutrient amendments, effects of soil sterilization on plant growth were reproduced; i.e., no effect on the shoot biomass and a significant increase in the root biomass (1.4-fold increase, $P<0.05$; Table 3). The observed increase in the root biomass was in the component that was covered with rhizosheaths (2.2–2.4-fold increase, $P<0.05$), whereas the mass of bare roots was not affected by soil sterilization. Consequently, the RS mass [whether expressed as pot^{-1}, as (g root)$^{-1}$ or as (g covered root)$^{-1}$] was significantly higher in plants grown in sterilized soils than in the unsterilized ($P<0.05$, Table 3). Besides, plants grown in sterilized soils showed significantly higher concentrations of N and K in shoots as compared to those grown in the unsterilized soil ($P<0.01$, Table 3).

Table 3
Plant growth, shoot nutrient concentration and mass of rhizosheath soil of wheat grown in unsterilized, autoclaved and γ-irradiated Hafizabad sandy loam.[a]

Parameter	Treatment			LSD	
	Unsterilized	Autoclaved	γ-Irradiated	$P<0.05$	$P<0.01$
Dry matter yield					
Shoot (mg pot^{-1})	135 ± 1 a[b]	132 ± 8 a	141 ± 2 a	NS	
Root (mg pot^{-1})	53 ± 6 b	74 ± 6 a	77 ± 3 a	17.8	26.9
Covered roots (mg pot^{-1})	15 ± 1 b	38 ± 6 a	33 ± 2 a	13.7	20.7
Bare roots (mg pot^{-1})	38 ± 5 a	37 ± 1 a	44 ± 3 a	NS	
Rhizosheath soil mass					
g pot^{-1}	0.4 ± 0.0 b	2.9 ± 0.5 a	3.1 ± 0.2 a	1.03	1.56
g (g root)$^{-1}$	7 ± 0 b	42 ± 10 a	40 ± 5 a	21.1	32.0
g (g covered root)$^{-1}$	23 ± 1 b	92 ± 28 a	93 ± 9 a	58.8	89.1
Shoot nutrient concentration					
N (%)	4.5 ± 0.0 c	7.2 ± 0.2 a	5.3 ± 0.1 b	0.40	0.60
P (%)	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	NS	
K (%)	3.9 ± 0.1 c	4.6 ± 0.2 b	5.5 ± 0.1 a	0.44	0.67
Zn (mg kg^{-1})	62.6 ± 0.4 a	62.7 ± 8.1 a	48.5 ± 2.6 a	NS	
Cu (mg kg^{-1})	20.4 ± 1.8 ab	23.0 ± 0.3 a	17.3 ± 0.1 b	3.69	5.59
Fe (mg kg^{-1})	429 ± 5 a	385 ± 5 b	330 ± 8 c	21.2	32.1
Mn (mg kg^{-1})	24.1 ± 1.3 b	29.4 ± 0.7 a	21.7 ± 1.0 b	3.54	5.36

[a] Experiment conducted without additional nutrient application.
[b] Mean of 3 replicates ± SE; figures in a row followed by different letter are significantly different by Duncan's multiple range test ($P<0.05$).

Table 4
Effects of autoclaving and γ-irradiation of Hafizabad sandy loam on population density of total and exopolysaccharide (EPS)-producing bacteria in soil and on wheat roots.[a]

Component	Bacterial type	Treatment		
		Unsterilized	Autoclaved	γ-Irradiated
		Bacterial counts [$\times 10^5$ (g DW)$^{-1}$][b]		
Bulk soil	Total	10.3 ± 0.1 g[c]	43.3 ± 4.5 de	29.7 ± 2.4 ef
	EPS-producers	4.2 ± 0.3 h	49.8 ± 11.6 de	22.1 ± 1.3 f
		(0.41)[d]	(1.15)	(0.75)
Rhizosheath soil	Total	9.4 ± 0.5 g	65.8 ± 14.7 d	50.5 ± 5.6 d
	EPS-producers	2.1 ± 0.1 i	57.2 ± 16.8 d	41.7 ± 5.6 de
		(0.23)	(0.83)	(0.83)
Roots	Total	3935 ± 494 a	1205 ± 270 c	1588 ± 202 bc
	EPS-producers	2394 ± 223 b	1383 ± 379 c	1464 ± 92 c
		(0.61)	(1.15)	(0.92)

[a] Experiment conducted without additional nutrient application.
[b] At the time of sowing wheat, no bacteria were detected in the autoclaved and γ-irradiated soils, whereas in the unsterilized soil the total and EPS-producing bacterial population [$\times 10^5$ (g DW)$^{-1}$] was 9.8 ± 0.2 and 2.7 ± 0.1, respectively.
[c] Mean of 3 replicates ± SE; figures in a row or a column followed by different letter(s) are significantly different by Duncan's multiple range test ($P<0.05$).
[d] Figures in parentheses indicate the ratio of EPS-producing bacteria/total bacteria.

The increase in shoot N was higher in the autoclaved (59%) than in the γ-irradiated soil (17%), whereas the increase in shoot K was higher in the γ-irradiated (40%) than in the autoclaved soil (17%). Among micronutrients, the shoot Fe concentration was significantly reduced by soil sterilization (20–23% reduction, $P<0.01$; Table 3). However, due to increased availability of Mn in the autoclaved soil, shoots grown in the autoclaved soil showed significantly higher Mn concentration as compared to those grown in the unsterilized soil (22% increase, $P<0.05$).

After a two-week experiment period, the population density of total culturable bacteria in the bulk soil of the unsterilized treatment was similar to that recorded at the beginning, whereas it significantly increased in sterilized treatments (3–4.4-fold increase, $P<0.05$; Table 4). However, the population density of EPS-producers in the bulk soil increased after two weeks; the increase was highest (18.8-fold) in the autoclaved soil, followed by γ-irradiated (8.3-fold) and was least (1.6-fold) in the unsterilized soil. In the unsterilized and autoclaved soils, the bulk and RS soils did not differ with respect to total bacterial population density. In the RS of γ-irradiated treatment, however, the population density was almost twice as much as in the bulk soil ($P<0.05$; Table 4). Comparing the bulk and rhizosheath soils for the population density of EPS-producers, the density was similar in the autoclaved treatment; it was almost half in the RS of non-sterile treatment but was almost twice high in the RS of γ-irradiated treatment. Although population densities of total and EPS-producing bacteria in the bulk soil and in RS were lower in unsterilized compared to sterilized treatments, population densities on roots were almost twice high in the unsterilized treatment. In soil as well as on roots, the proportion of EPS-producers in the total bacterial population was significantly higher in sterilized treatments than in the unsterilized ($P<0.05$; Table 4).

Effects of C and Mn on plant growth and RS mass

Applying glucose-C to unsterilized soil had no effect on the RS mass, whereas at high concentration (500 mg kg^{-1}) it increased shoot and root biomass ($P<0.05$; Table 5). Although shoot and root biomass were not affected by Mn, amending the unsterilized soil with Mn at ≥15 mg kg^{-1} significantly increased the RS mass whether expressed as pot^{-1} or as (g root)$^{-1}$ ($P<0.05$; Table 5). We explored another soils viz. Hafizabad clay loam to study if variation in the amount of Mn released by autoclaving may differently affect the plant growth and the RS mass. The net release of soluble C due to autoclaving was lesser in the Hafizabad clay loam (80 mg kg^{-1}; Table 6) than that observed in the Hafizabad sandy loam (109 mg kg^{-1}; Table 1). However, autoclaving the clay loam released much higher amount of Mn (net release, 81 mg kg^{-1};

Table 5
Plant growth and rhizosheath soil mass of wheat seedlings grown in unsterilized Hafizabad sandy loam amended with different levels of glucose or $MnSO_4 \cdot H_2O$.

Amendment[a]	Dry matter yield (mg pot^{-1})		Rhizosheath soil mass	
	Shoot	Root	g pot^{-1}	g (g root)$^{-1}$
Glucose (mg C kg^{-1})				
0	177 ± 2 b[b]	55 ± 1 b	3.6 ± 0.1 a	67 ± 3 ab
125	178 ± 10 b	56 ± 7 b	4.1 ± 0.2 a	72 ± 4 a
250	168 ± 13 b	50 ± 1 b	3.7 ± 0.3 a	73 ± 4 a
500	236 ± 11 a	73 ± 2 a	4.2 ± 0.3 a	58 ± 4 b
LSD				
$P < 0.05$	33.1	12.8	0.73	9.3
$P < 0.01$	48.2	18.6	1.06	13.5
$MnSO_4 \cdot H_2O$ (mg Mn kg^{-1})				
0	161 ± 2 a[b]	66 ± 3 a	2.8 ± 0.1 c	41 ± 2 c
5	188 ± 7 a	62 ± 4 a	3.3 ± 0.1 bc	53 ± 5 b
15	189 ± 9 a	58 ± 4 a	4.2 ± 0.6 ab	76 ± 6 a
50	161 ± 12 a	59 ± 5 a	4.5 ± 0.2 a	77 ± 5 a
LSD				
$P < 0.05$	26.3	13.9	1.01	16.8
$P < 0.01$	38.3	20.3	1.47	24.5

[a] In both experiments, additional N (as NH_4NO_3) and P (as KH_2PO_4) were applied at 100 and 50 mg kg^{-1}, respectively.
[b] Mean of 3 replicates ± SE; for each amendment figures in a column followed by different letter are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 6
Wheat seedling growth and mass of rhizosheath soil (RS) as affected by autoclaving and addition of Mn and C in the Hafizabad clay loam.[a]

Parameter	Treatment[b]				LSD	
	Autoclaved	Unsterilized	Unsterilized Mn + C balanced[c]	Unsterilized Mn balanced	$P < 0.05$	$P < 0.01$
pH (1:1, soil:water)	8.71 ± 0.02 a[d]	8.55 ± 0.01 b	–	–	0.11	0.25
EC (μS m^{-1})	529 ± 8 a	351 ± 3 b	–	–	43.3	99.9
Water-soluble C (mg kg^{-1})	128 ± 9 a	48 ± 3 b	–	–	27.6	45.8
NH_4^+-N (mg kg^{-1})	9.9 ± 1.7 a	9.5 ± 1.4 a	–	–	NS	
NO_3^--N (mg kg^{-1})	4.8 ± 0.5 a	3.6 ± 0.4 a	–	–	NS	
Olson's P (mg kg^{-1})	11.6 ± 0.8 b	14.9 ± 0.1 a	–	–	1.47	2.44
Extractable K (mg kg^{-1})	429 ± 6 a	439 ± 10 a	–	–	NS	
Extractable Mn (mg kg^{-1})	86 ± 0 a	5 ± 1 b	–	–	1.4	2.4
Shoot dry weight (mg pot^{-1})	215 ± 22 a	232 ± 6 a	229 ± 17 a	131 ± 20 b	56.7	82.5
Root dry weight (mg pot^{-1})	103 ± 1 b	105 ± 5 b	137 ± 9 a	87 ± 6 b	19.6	28.6
RS dry weight (g pot^{-1})	5.1 ± 0.5 a	2.6 ± 0.2 b	4.8 ± 0.4 a	4.2 ± 0.1 a	1.16	1.69
RS dry weight [g (g root)$^{-1}$]	49 ± 5 a	25 ± 1 c	35 ± 1 b	48 ± 3 a	9.6	13.9

[a] The soil has been under cotton-wheat rotation.
[b] All treatments received additional N (at 100 mg kg^{-1} as NH_4NO_3) and P (at 50 mg kg^{-1} as KH_2PO_4).
[c] Manganese and water-soluble C contents of the unsterilized soil were brought equal to that of the autoclaved soil by adding $MnSO_4 \cdot H_2O$ and glucose, respectively.
[d] Mean of 3 replicates ± SE; figures in a row followed by different letter are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 6) as compared to the sandy loam (12 mg kg^{-1}; Table 1). The shoot and root biomass were not affected by autoclaving the Hafizabad clay loam (Table 6). However, as observed in experiments with the Hafizabad sandy loam, autoclaving significantly increased the RS mass also in the Hafizabad clay loam ($P < 0.01$; Table 6). Moreover, adding Mn (alone or with glucose-C) to the unsterilized Hafizabad clay loam significantly increased the RS mass ($P < 0.01$).

Discussion

Although soil sterilization effects on physicochemical properties vary greatly with soil types, the release of soluble C and that of Mn are the most commonly observed changes caused by autoclaving and γ-irradiation. Such changes are more pronounced in autoclaved compared to γ-irradiated soils and this was observed in the present study too. However, probably due to very low (<1%) organic C content of the soils used, the amount of water-soluble C released upon sterilization was much lower than that reported in some earlier studies (Lynch 1982; Shaw et al. 1999; McNamara et al. 2003; Darbar and Lakzian 2007). Besides, autoclaving compared to γ-irradiation of the Hafizabad sandy loam though released 4-times higher DTPA-extractable Mn, and autoclaving the Hafizabad clay loam released 7-times more Mn than the Hafizabad sandy loam, magnitudes of Mn released were much lower than those reported in earlier studies (Skipper and Westermann 1973; McNamara et al. 2003). Neither autoclaving nor γ-irradiation increased the level of available macronutrients in the soil as generally reported in the literature (Jenkinson et al. 1972; Alphei and Scheu 1993; Serrasolsas and Khanna 1995a,b; McNamara et al. 2003). A slight decrease in the NO_3^- content of the γ-irradiated soil is attributable to its reduction into NO_2^- (Cawse and Cornfield 1969). Although the shoot biomass was generally not affected by soil sterilization, shoot N and K concentrations were higher in wheat seedlings grown in sterilized soils (without added N and P), most probably due to increased root biomass.

The increased RS mass in sterilized soils may be attributed to enhanced root exudation caused by Mn toxicity (Mora et al. 2009). Although the observed maximum shoot Mn level (29 mg kg^{-1}) was negligible as compared to the toxic level reported for wheat tissues (e.g., 720 mg kg^{-1}; Fageria 2001), the soil Mn level was probably high enough, at least in autoclaved soils, to trigger exudation of carboxylates to detoxify Mn. Soil sterilization might have eliminated microbes which transform available Mn into higher oxides and thus the accumulated Mn was probably implicated in the observed effects. As increased root growth and the RS mass were consistently observed in sterilized soils, we tried to explore whether similar effects can be produced in unsterilized soil by adding glucose-C and/or Mn (II). Stimulatory effect of the added glucose-C on the

root growth was indeed observed in the Hafizabad sandy loam, whereas adding Mn to unsterilized soil increased the RS mass in the Hafizabad sandy loam as well as in the Hafizabad clay loam (Tables 5 and 6). However, we speculate that in contrast to effects produced in sterilized soils, the observed effects of Mn (II) in unsterilized soils should be of short duration. This is because in unsterilized neutral to alkaline soils Mn (II) concentration may decline to zero within few days (Silber et al. 2008). Regarding the role of soluble C in increasing the root growth, relatively low amounts of soluble C released in the autoclaved and γ-irradiated soils were as much effective as almost 4-times higher amount of glucose-C applied to the unsterilized soil (compare Tables 1 and 5). Therefore, it was not only the content of soluble C but probably also its composition/structure, which might have been altered due to autoclaving and γ-irradiation (Berns et al. 2008). Besides, higher root biomass and the RS mass were recorded in the γ-irradiated than autoclaved Hafizabad sandy loam (Tables 2 and 3) despite much lower amounts of soluble C and Mn released in the γ-irradiated compared to autoclaved soil (Table 1). This indicates that factors other than soluble C and Mn were probably also involved in the observed effects.

The development of rhizosheaths is mediated through an interaction of soil particles with root hair and polysaccharides exuded either by roots or those synthesized by root-colonizing bacteria (Watt et al. 1994; Amellal et al. 1998). While soil sterilization as such is known to cause changes in the structure of bacterial communities developing on roots (Marschner and Rumberger 2004; Wertz et al. 2007), the increased root exudation due to Mn, and the soluble C released upon soil sterilization probably also favoured the growth of efficient EPS-producing bacteria thus causing increased soil aggregation around roots. Although we did not analyze the bacterial community structure, sterile compared to non-sterile treatments indeed showed much higher proportion of EPS-producers in the total bacterial population. Although population densities of total and EPS-producing bacteria on roots were higher in unsterilized compared to sterilized treatments, the bacterial community structure on roots probably differed in unsterilized and sterilized soils. Root exudates as well as bacterial EPSs may vary greatly in their ability to form stable soil aggregates (Traoré et al. 2000; Kaci et al. 2005). Therefore, it is possible that sterile compared to non-sterile treatments favoured the EPS-producing bacterial community which synthesized efficient exopolysaccharides thus increasing the mass of rhizosheaths. Besides, the new bacterial population developed in sterilized soils probably also included strains capable of producing growth promoting substances thus increasing the root growth (Kaci et al. 2005; Zemrany et al. 2007). We may not rule out the possibility that the bacterial community that was not culturable by the methods employed, probably also differently developed in unsterilized and sterilized soils.

Results of the present study suggested that soil sterilization by autoclaving and γ-irradiation promotes root growth and the development of rhizosheaths in wheat seedlings through interaction of various factors including soluble C and Mn released upon soil sterilization, and probably altered community structure of the bacterial population recolonizing sterilized soils. More studies are desirable to ascertain whether the observed effects of autoclaving and γ-irradiation can be generalized for different soil types/plant species.

Acknowledgements

We thank Mohammad Asad Mehmood and Mohammad Sajid for valuable technical assistance, and W.M. Kaiser and M.S. Mirza for critically reading the manuscript. Equipment grant by the Alexander-von-Humboldt Foundation, Germany is also gratefully acknowledged.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pedobi.2013.12.005.

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