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

T. Mahmood[a,*], S. Mehnaz[b], F. Fleischmann[c], R. Ali[a], Z.H. Hashmi[a], Z. Iqbal[a]

[a] Soil Science Division, Nuclear Institute for Agriculture & Biology, Jhang Road, Faisalabad 38000, Pakistan
[b] Department of Biological Sciences, Forman Christian College, Ferozepur Road, Lahore 54600, Pakistan
[c] Fachgebiet für Pathologie der Waldbäume, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 1, 85354 Freising, Germany

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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

* Corresponding author. Tel.: +92 0419201786.
E-mail address: tmahmood@niab.org.pk (T. Mahmood).

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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