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Effect of autoclave sterilization of a tropical andept on seed germination and seedling growth

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Summary Steam sterilization of a Typic Dystrandept in Costa Rica resulted in a six-fold increase in extractable Mn, to levels often considered toxic. Seeds of eight species, comprised of six successional taxa and two cultivars (soybean, *Glycine max* and raddish, *Raphanus sativus*) were planted in the sterilized soil and in unsterilized soil after delays of 1, 8, 15, and 28 days. Germination and mortality were not different in the two soils, indicating that steam-sterilized soil can safely be used in seed traps. Six species (including both cultivars) grew better in unsterilized soil, but two of the native taxa (*Phytolacca rivinoides* and *Bocconia frutescens*) grew significantly faster in sterilized soil.

Introduction

Soil sterilized in an autoclave is frequently used in plant-growth experiments. Sterilization decreases populations of soil microorganisms and seeds, and affects growth and species richness of subsequent microbial colonists[19]. Steam sterilization also changes some of the chemical properties of the soil, and thereby affects the higher plants growing in it. In a comparative study of three soils, autoclaving produced variable changes in pH (increased in one soil, decreased in another, and did not change in the third), K (increased in two, decreased in one), N and P (each increased in two and did not change in one), and no change in Ca[18]. Other researchers reported that organic matter and Mg decreased, while the effects on P K and Ca were small and variable[11]. A third experiment produced increases in pH (0.1 units) and Mg but no significant changes in exchangeable Al, Ca, and K, extractable P, total N and organic matter[14]. Preliminary analysis of the same soil used in this study showed no significant differences in pH, organic matter, N, P, K, Ca, Mg, S, Cu, Zn, and Fe between sterilized and unsterilized soil.

Regardless of their variable findings with respect to many soil properties, all these investigators have reported one change in common: a great increase in extractable Mn. When five Hawaiian soils were steam sterilized, exchangeable Mn increased from a few ppm to approximately 3000 ppm[8,9]. Furthermore, the concentration of Mn was 2 to 12 times higher in plants grown in sterilized soils than in those grown in unsterilized soils. The authors concluded that the change in Mn availability caused by steam sterilization might affect plant growth either positively or negatively, depending on the Fe:Mn ratio of the soil.

Our interest in determining the effect of soil steam sterilization on seed germination and seedling establishment arose during research in which seeds were trapped in trays containing autoclaved soil. These traps were placed for 4 weeks in different ecosystems, after which they were moved to a shadehouse where seed germination and seedling growth were monitored. We wanted to determine whether or not the increase of extractable Mn caused by autoclaving was affecting seed germination, seedling survival, and the initial stages of seedling growth.

Table 1. Concentration of extractable manganese in soil subjected to steam sterilization and unsterilized controls. Values are means ($mg*kg^{-1}$) of three samples. Means in the same column accompanied by the same superscript do not differ significantly at alpha = 0.01

Time after sterilization, days	Steam	No steam
1	113^{a}	14^{a}
8	101^{a}	19^{a}
15	117^{a}	30^{a}
28	88^{c}	17^{a}
Mean	105	18

Table 2. Height (mm) of plants grown for 60 days in steam-sterilized and unsterilized soils. Values are averages of all individuals growing in three trays. For each species, means in the same row accompanied by the same superscript are not significantly different at alpha = 0.01. All total means (all delays combined) for each species, except those for *I. sultanii* and *G. max* are significantly different

		Delay, days				
Species	Treatment	1	8	15	28	Mean
P. rivinoides	steam	67^{a}	79^{a}	69^{a}	39^{b}	68
	no steam	29^{a}	19^{c}	24^{b}	23^{bc}	23
S. jamaicense	steam	6^{a}	6^{ab}	5^{ab}	5^{b}	6
	no steam	11^{a}	9^{ab}	5^{b}	6^{ab}	9
I. sultanii	steam	26^{a}	24^{a}	24^{a}	19^{a}	24
	no steam	26^{a}	18^{b}	21^{ab}	26^{a}	23
I. tiliacea	steam	50^{a}	41^{a}	51^{a}	37^{a}	45
	no steam	163^{a}	98^{a}	211^{a}	39^{a}	138
M. micrantha	steam	14^{a}	12^{a}	14^{a}	13^{a}	13
	no steam	37^{a}	27^{b}	29^{b}	42^{a}	34
B. frutescens	steam	39^{b}	50^{a}	40^{b}	44^{ab}	45
	no steam	32^{b}	44^{a}	35^{b}	39^{ab}	38
G. max	steam	89^{c}	207^{b}	226^{b}	318^{a}	223
	no steam	212^{c}	245^{b}	197^{c}	285^{a}	233
R. sativus	steam	22^{c}	43^{b}	64^{a}	59^{a}	48
	no steam	60^{c}	75^{b}	90^{a}	57^{c}	71

Methods

The soil used in this study was a Typic Dystrandept (Colorado Series) developed from aged pyroclastic materials of the Irazu-Turrialba volcano complex[1,15]. It is high in allophane and organic C, has good structure, and is deep and well drained.

The soil was taken from the 0–15 cm depth, mixed, and placed in 24 trays. Twelve of these 35 by 50 cm trays were autoclaved at 1 kg of pressure for 30 min. Then all 24 trays were moved to a shadehouse and randomly separated into four groups, each consisting of three sterilized trays and three unsterilized trays.

Because we suspected that the effects of steam sterilization might diminish with time and exposure of the soil to weather, we assigned each group of trays to a different post-sterilization sampling delay. The first group was sampled 1 day after sterilization, the second after 8 days, the third after 15 days, and the fourth after 28 days. Soil samples were oven dried at 55°C for 24 h, ground, and passed through a 2 mm sieve. Additional soil samples from the third group of trays were air-dried to compare the effects of air-drying and oven-drying on extractable

Mn. Manganese was extracted with a $NaHCO_3$ modified-extractant solution and measured with an atomic absorption spectrophotometer[5].

The trays in each group were planted at the same time their soil was sampled. Six successional species and two cultivars (soybeans and radish) were selected for this study. The successional species were selected because they were known to have been caught in the seed traps and were readily available at the time this experiment was conducted. The cultivars were selected because they were reported to be sensitive to elevated concentrations of Mn[16,17]. The species (and the number of seeds planted in each tray) were *Glycine max* (10), *Raphanus sativus* (10), *Solanum jamaicense* (15), *Bocconia frutescens* (10), *Phytolacca rivinoides* (10), *Impatiens sultanii* (15), *Mikania micrantha* (8), and *Ipomoea tiliacea* (10).

All trays were watered daily. The following were recorded for each species. number of germinated seeds, seedling mortality, and seedling height. This was done daily for the first 3 weeks and periodically thereafter until 61 to 63 days following planting.

Because of their rapid growth, *G. max* and *R. sativus* were harvested 20 days after planting. The aboveground dry mass of these two species was measured and their plant tissues were analyzed for total Mn by atomic absorption spectrophotometry after digestion in perchloric acid/nitric acid. The other species were harvested 60 days after planting and the leaf area of each seedling was measured to 1.0 mm^2 with an area meter.

Results and discussion

Steam sterilization increased the extractable Mn in the soil approximately four- to eight-fold (Table 1); this is consistent with the findings of other investigators[3,4,8,9,11,14,17,18]. The highest Mn concentrations were found 1 and 15 days after sterilization, while soil sampled 28 days after sterilization had the lowest concentration. Because the different forms of active Mn in soil (water soluble, exchangeable, and higher oxides) are in dynamic equilibrium with one another and the release of Mn by autoclaving is a reversible reaction[2,6,7,12], we anticipated a decrease in extractable Mn with time after soil sterilization. However, even after 28 days the Mn concentration was still five-fold higher in sterilized than in unsterilized soil. The reverse transformation of extractable Mn into insoluble oxides occurs slowly in these soils, even though the samples were warm and well watered.

Others have reported a slight increase in exchangeable Mn after air-drying and a large increase following oven-drying[8]. In our study, air-dried soil samples had a slightly lower mean concentration of extractable Mn than those that were oven-dried, but the difference was not significant.

Seeds and growing plants vary greatly in their sensitivity to the action of steam-treated soils[10] but, in general, seed germination and seedling mortality did not differ between sterilized and unsterilized soil in the eight species we studied. No species had a positive germinaton response to steam sterilization, and only *P. rivinoides* (planted 28 days after sterilization) and *M. micrantha* (planted after 1 day) had significantly higher germination in non-sterilized soil. Seedling mortality did not differ significantly ($p > 0.05$) between heated and unheated soils for any species or time delays.

In general, there were two well-defined growth responses to autoclave sterilization (see representative examples in Fig. 1), and most species showed the same trend in both height growth (Table 2) and leaf-area growth (Table 3). Two species (*P. rivinoides* and *B. frutescens*) grew better in sterilized soil than in unsterilized soil; the other six species grew better in unsterilized soil. *Solanum jamaicense, I. tiliacea* and *M. micrantha* grew taller and had more leaf area in unsterilized soil, while *I. sultanii* had more leaf area, but did not grow taller. Seedlings of *G. max* and *R. sativus* grown in unsterilized soil grew taller, had higher aboveground biomass, and had lower Mn concentration in their tissues than did seedlings grown in sterilized soil (Table 4). Furthermore, leaves of both species showed symptoms of Mn toxicity (marked chlorosis and brown necrotic spots)[13] when grown in autoclaved soil.

The two species that responded positively to steam-sterilized soil – *P. rivinoides* and *B.*

Table 3. Foliar area of plants grown in sterilized and unsterilized soils. Values are averages (mm^2) of all individual growing in three trays. Means in the same row accompanied by the same superscript are not significantly different at alpha = 0.01. Overall treatment means (steam or no steam, all delays combined) for each species are significantly different

		Delay, days				
Species	Treatment	1	8	15	28	Mean
P. rivinoides	steam	8219^{b}	10729^{a}	11262^{a}	8825^{a}	10007
	no steam	1717^{a}	854^{b}	1893^{a}	1904^{a}	1474
S. jamaicense	steam	56^{ab}	37^{bc}	28^{c}	73^{a}	47
	no steam	736^{a}	343^{a}	31^{a}	222^{a}	570
I. sultanii	steam	587^{a}	527^{a}	545^{a}	381^{a}	534
	no steam	1071^{a}	475^{b}	944^{ab}	1415^{a}	986
I. tiliacea	steam	1452^{a}	1162^{a}	1843^{a}	1629^{a}	1528
	no steam	3938^{a}	4477^{a}	5495^{a}	1529^{a}	4103
M. micrantha	steam	47^{a}	39^{a}	68^{a}	55^{a}	54
	no steam	932^{b}	625^{b}	701^{b}	1711^{a}	1000
B. frutescens	steam	1089^{b}	2243^{a}	1234^{b}	2095^{a}	1783
	no steam	896^{b}	1937^{a}	1136^{b}	1287^{ab}	1362

a > b > c, alpha = 0.01

Table 4. Mass (g) and total manganese ($mg*kg^{-1}$) in aboveground tissues of *G. max* and *R. sativus* grown in steam-sterilized and unsterilized soil. Values are means of seedlings planted in 12 trays. The members of all four pairs of means differ significantly at alpha = 0.05

	Mass g		Manganese concentration $mg*kg^{-1}$	
Species	steam	no steam	steam	no steam
G. max	0.213^{b}	0.254^{a}	833^{a}	353^{b}
R. sativus	0.018^{b}	0.035^{a}	2253^{a}	504^{b}

a > b, alpha = 0.05

frutescens – share ecological characteristics: both have seeds with long life in the soil, and both colonize disturbed or burned sites very rapidly. Furthermore, *P. rivinoides* (a member of the Phytolaccaceae) is known to be non-mycorrhizal. The mycorrhizal status of *B. frutescens* (a woody member of the Papaveraceae) is uncertain, but we have not observed mycorrhizal fungi in any of about a dozen root preparations of this species examined to date. It is likely that the response of these two species is related to changes in the soil microbial community caused by sterilization, in addition to changes in soil chemistry.

Because germination and seedling mortality were relatively unaffected by soil autoclaving, steam sterilization can be used to prepare soil for seed traps and similar uses. Alternative trapping media have even greater disadvantages, either because they are completely artificial substrates (vermiculite, empty containers, sticky substances, *etc.*) or because they make it impossible to distinguish newly arrived seeds from contaminants already present in the soil (unsterilized soil, methyl-bromide sterilized soil, *etc.*). We conclude that although steam sterilization can retard growth of many species – presumably due to Mn toxicity – the procedure can be used safely for preparing soil for uses that do not require evaluation of growth.

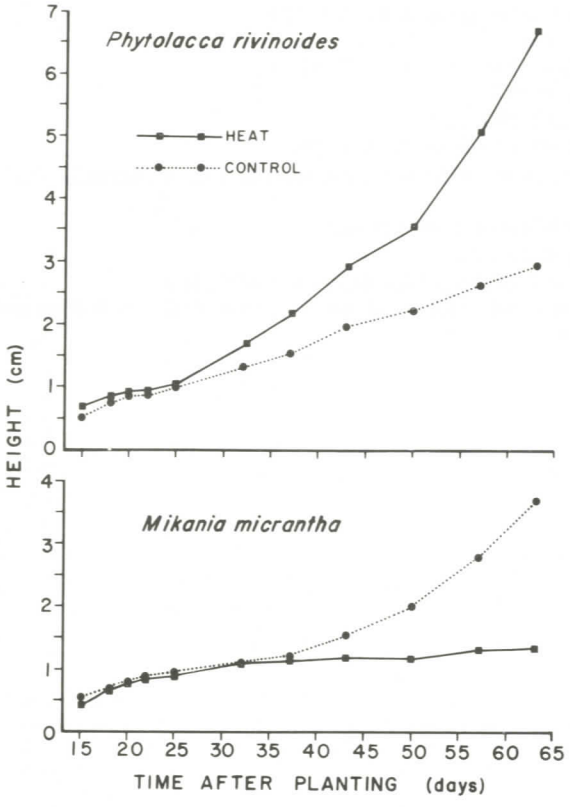

Fig. 1. Representative height-growth curves, showing the two types of responses observed. *Upper* Growth of *P. rivinoides,* one of two species that grew better on steam-sterilized soils. *Lower* Growth of *M. micrantha,* one of six species that grew better on unheated soils. Seeds were planted one day after steam sterilization.

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