

Mineral amendments to improve plant growth after soil sterilization in allelopathy experiments

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(Received in revised form: December 07, 2019)

ABSTRACT

This study aimed to ameliorate the soil physicochemical properties and plant growth after soil autoclaving by adding mineral amendments. Agricultural soil was mixed with sand and perlite in 2 ratios (1:1 and 1:2, w/w), creating 4-treatments that were sterilized by autoclaving. Thereafter, 4 plant species [*Zea mays*, *Lactuca sativa* and *Sinapis alba* (crops) and *Amaranthus retroflexus* (weed)] were individually grown in pots containing the autoclaved soil mixtures. At the end of the assay, physicochemical parameters of soil mixtures (pH, soil texture and structure) and plant morphological traits (leaf area, fully developed leaves, emergence, length, biomass, and rhizosheath) were measured. There was a significant increase in soil pH and a decrease in soil particle density after the addition of perlite. Plant growth (shoot length, root length or biomass) seedling emergence, leaf area and rhizosheath were significantly increased with the use of mineral amendments, especially perlite. The use of inexpensive amendments effectively ameliorated soil physicochemical properties and plant growth after soil autoclaving. Improving the soil properties after sterilization by using mineral amendments would be useful to standardize soil physicochemical conditions and thus, reducing the undesired interference in the evaluation of allelopathic effects.

Keywords: Allelopathy, *Amaranthus retroflexus*, crop, *Lactuca sativa*, mineral amendments, plant development, pot bioassay, *Sinapis alba*, soil autoclaving, soil physicochemical properties, soil sterilization, weed.

INTRODUCTION

The use of soil in experimental assays typically entails disturbing processes as soil extraction, transportation, manipulation and storage (11) that produce negative consequences on soil characteristics. At the laboratory scale, soil alterations include structural changes, the reduction of water potential or the physical impediment of root development, which consequently reduces plant growth (3,4,8). In allelopathic assays, the use of soil is gaining attention, since it is a key factor in disentangling the ecological effects of chemical compounds under natural conditions (19). Using soil as the matrix of allelochemical experiments often requires the inactivation of the microbial community. Without the influence of soil microbiota, it is possible to design manipulative experiments that explore plant-plant and plant-microbe interactions (9,14) or the potential effects of allelopathic compounds (16). Among other roles in ecosystem function, soil microbiota plays a fundamental role as mediators in allelopathic interactions (17). Soil sterilization is widely used in allelopathy studies to distinguish the effects caused by biotic and abiotic components. In sterilization methods, autoclaving is highly used but it changes the soil

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physicochemical properties, limiting plant establishment and difficult the elucidation of allelochemical effects.

Within the many physical and chemical processes used to sterilize the soil, autoclaving is a common and cost-effective method to distinguish the effect of biotic and abiotic components (6,24), and to study the soil microbial community and its effects on plant performance, including allelopathic interactions (6,9,13,14,21,22,33). However, soil autoclaving involve changes in the soil chemistry, soil nutrient availability or soil structure (1,16,18,31,37). These modifications influence the plant-soil feedback driven by resources or chemical compounds as allelochemicals (5).

Although the addition of amendments in different proportions has been used to ameliorate the soil structure (7,9,12,34) but the different mineral amendments were not compared to improve the plant growth after soil autoclaving. Therefore, we hypothesized that autoclaved (sterilized) soils treated with mineral amendments (different proportions of perlite and sand) could ameliorate soil physicochemical characteristics and consequently, improve the plant growth. This study aimed to evaluate (i) the amelioration of physicochemical properties after soil autoclaving, by adding sand and perlite and, (ii) the effects of mineral amendments on plant growth of different species with agronomic interest, widely used in allelopathy experiments.

MATERIALS AND METHODS

Soil sampling and experimental design

In February 2016, the top 20-cm soil was collected from an agricultural field (Sandy loam, top-soil A_p horizon) at Ribadelouro, Spain (29 T 528701 4661544). The soil was kept in plastic bags and immediately transported to the laboratory for further processing. Once in the laboratory, soil samples were air-dried and sieved (0.5 cm) to remove coarse roots, organic debris, and small stones. The physical characteristics of the soil before sterilization were: sand 59.6%, silt 29.2%, clay 11.2% (23), bulk density 1.21 g cm⁻³ (10), particle density 2.53 g cm⁻³ (23). Soil physicochemical characteristics were pH (1:2.5 w:v in distilled water) 4.7; electrical conductivity (EC) < 0.07 mS cm⁻¹; organic matter (OM) 8.3 % (28) ; total N 0.29 % and available P (Olsen method) 20 ppm (28), assimilable K 86 ppm and exchangeable Mg⁺², Ca⁺², Na⁺, K⁺ and Al³⁺ were 0.22, 1.0, 0.14, 0.22 and 1.70 cmol⁽⁺⁾ kg⁻¹, respectively (displacement by NH₄Cl) (28).

The air-dried soil was then combined with mineral amendments to create a total of four treatments together with adequate controls (soil without mineral amendments). Soil was thoroughly mixed with perlite (2-6 mm, Gramo Flor GmbH & Co. Germany, hereafter P) or sand (0.7 mm, commercial sand, hereafter S) in a ratio of 1:1 (P1 and S1) and 1:2 (P2 and S2), respectively. Therefore, the experimental treatments consisted of 2 Factors, (i) Amendments: 4 [P1 (soil + perlite, 1:1), P2 (soil + perlite, 1:2), S1 (soil + sand, 1:1), S2 (soil + sand, 1:2)] and (ii) Test plants: 4 [*Zea mays* L., *Lactuca sativa* L., *Sinapis alba* L. and *Amaranthus retroflexus* L. (weed)]. The four soil treatments together with control were individually sterilized by autoclaving (121 °C, 20 minutes) for three consecutive days (26).

Plastic pots (375 cm³) were then filled with 300 g of different amendments. Test plant species were 3-crops (*Z. mays*, *L. sativa* and *S. alba*) and one common weed (*Amaranthus retroflexus*). As per treatment, 5-seeds of each crop were sown per pot, while for *A. retroflexus*, 3 mg of seeds were sown (due to small seed size). After seedling

emergence, thinning was done to keep 5-plants per pot. In the last week of February 2016, pots were arranged in a completely randomized design with five replications. Pots were kept in glasshouse (photoperiod of 12/12 light/dark conditions and within a range of temperatures that fluctuate between 30-12 °C (max-min). During the experiment, the mean of the relative humidity was 77 % and the light intensity was 1228 $\text{kJ m}^{-2} \text{day}^{-1}$) in our University for 7 weeks. The pots were watered twice a week to provide adequate moisture levels to seedlings.

Soil physicochemical measurements and plant harvest

After 7 weeks, soil pH, conductivity, together with particle density, texture and bulk density were measured. Soil pH was assessed in a soil solution rate of 1:2.5 soil weight/deionized water volume ratio in a Hanna HI-2020 digital pH meter (10). Soil electrical conductivity was measured in a Crison CDTM-523 conductivimeter, dissolving 20 g of soil mixtures in deionized water until a saturated paste was obtained (10). Soil bulk density was calculated using 100 cm^3 cylinders (10). The pycnometer method was used to measure soil particle density (23).

Prior to harvest, in first week of April 2016, the number of living plants were counted to calculate plant emergence, together with the number of fully developed leaves. After that, plants were carefully uprooted and shoot and root length, together with leaf area were measured. Rhizosheath, defined as the soil that remains firmly adhered to roots after uprooting plants (35), was measured following Mahmood *et al.* (21) method. Briefly, roots were introduced in Falcon tubes (15 mL), 9 mL of deionized water were added, and tubes were sonicated (Branson sonifier 250) and dried (70 °C, 24 h). Soil biomass obtained was expressed as dry soil per unit of root length (35). The leaves and roots were dried (70 °C) until constant weight to obtain dry biomass. Specific root length (SRL) was calculated as the ratio of root length and dry root weight [SRL= (root length/root dry weight)/100].

Statistical analysis

Data were subjected to Kolmogorov-Smirnov test to assess normality and Levene's test to check the homogeneity of variances. The effect of mineral amendments on dependent variables was evaluated through One-way ANOVA, using pairwise comparisons as Tukey's HSD, or Dunnett's T3, in case homogeneity of variances was not satisfied, as the *post-hoc* tests. In the case of normality assumptions were not satisfied, mean comparisons were carried out using Kruskal-Wallis as the non-parametric test, and pairwise comparisons through Mann-Whitney U test. Statistical analyses were carried out using the IBM SPSS Statistics v.23.0 software package for Windows (IBM SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Our results indicated that the negative consequences of autoclaving on soil physicochemical parameters could be notably improved by adding mineral amendments. Soil structural changes due to particle disaggregation produced by autoclaving are highly variable depending on the soil type and the intrinsic aggregation stability (6). As we hypothesized, physicochemical correction had important effects on plant responses, mainly in soil treated with P1 and P2. Results observed on seedling growth showed beneficial effects viz., increase in the number of viable seedlings, leaf area, rhizosheath (Table 2), root and shoot growth and plant biomass (Figure 1a, 1b). Although the comprehension of plant-soil microbiota interactions is fundamental to elucidate the ecological role of

allelochemical compounds under natural conditions, the elimination of soil biota is often necessary. The inactivation of the microbial community by sterilization helps to reveal the specific role of chemical compounds in allelopathy bioassays, which could be highly useful in the search for bioactive molecules with industrial purposes.

Soil physicochemical properties

The addition of mineral amendments modified the soil physicochemical properties (Table 1) and increased the soil pH (> 1 unit of pH in P2 treatment) ($P < 0.05$). One of the main consequences of soil autoclaving are changes produced in soil physicochemical properties, including soil pH (20,29). In any case, negative effects produced by soil autoclaving constrain plant growth through soil compaction, changes in soil pH, masking the phytochemical effects and thus making it difficult to interpret the allelochemical potential.

In our study, intrinsic electrochemical properties of perlite (30), especially when higher proportions are applied (in P2), could be responsible for the increase in soil pH. As a consequence, the correction of soil acidity could facilitate nutrients availability, but it also helps to overcome the risk of root exposure to toxic ions as Al^{+3} , Mn^{+2} , or H^{+} (32). An increase in soil pH generally favours the availability of N, P and other key elements (K^{+} , Ca^{+2} , Mg^{+2}), while reducing the solubility of Al^{+3} with potential toxicity (36). This fact could be relevant in allelochemical studies that use acidic soils.

Table 1. Physico-chemical properties (mean \pm SD) of soils treated with different mineral amendments

	Control	S1	S2	P1	P2	$F_{(5,12)}$	p
Sand (%)	49.0 (± 0.26)c	84.1 (± 0.42)a	86.4 (± 4.70)ab	68.1 (± 0.99)b	71.9 (± 1.25)b	114.95	<0.001
Silt (%)	36.7 (± 1.34)a	12.4 (± 5.44)abc	8.8 (± 1.98)c	25.0 (± 5.57)abc	19.8 (± 1.86)b	28.47	<0.001
Clay (%)	14.3 (± 1.64)	3.6 (± 5.09)	4.7 (± 4.12)	6.8 (± 5.93)	8.4 (± 1.00)	3.19	0.068
Bulk density (g cm ⁻³)	1.00 (± 0.04)b	1.56 (± 0.07)a	1.73 (± 0.04)a	1.09 (± 0.13)b	1.01 (± 0.06)b	57.54	<0.001
Particle density (g cm ⁻³)	2.42 (± 0.11)a	2.12 (± 0.35)ab	2.33 (± 0.22)a	2.30 (± 0.08)ab	1.80 (± 0.11)b	4.58	0.023
pH H ₂ O	4.99 (± 0.03)c	5.72 (± 0.05)b	5.56 (± 0.16)b	5.72 (± 0.05)b	6.00 (± 0.12)a	47.65	<0.001
EC (mS cm ⁻²)	0.19 (± 0.02)	0.16 (± 0.05)	0.10 (± 0.02)	0.24 (± 0.10)	0.19 (± 0.01)	2.71	0.091

S1= soil + sand (ratio 1:1), S2= soil + sand (1:2), P1= soil + perlite (1:1); P2= soil + perlite (1:2), EC=Electrical conductivity. Values of P in bold indicate significant differences in One-way ANOVA. Different letters in each row indicate significant differences according to the Tukey's or T3 Dunnett's post hoc tests at the $P \leq 0.05$ level.

Structural changes caused by autoclaving often produce subsidiary effects like the increase in bulk density with the consequent augment in soil compaction (25). As expected, the addition of mineral amendments altered sand and silt in a significant manner. Consequently, soil texture was also modified, changing from loam soils (control soils) to sandy-loam soils (with the addition of perlite) or loamy-fine sand soils (after sand addition). The texture of soils treated with sand showed more noticeable differences than those treated with perlite in comparison with control. However, contrary to our expectations, bulk density was not significantly decreased in P1 and P2 compared with control and particle density declined in P2 compared with S2 and control ($P < 0.05$) (Table 1). These results indicated that P and S amendments more effectively modified the soil texture rather than structure and thereby providing favourable texture for plant growth.

Table 2. Effects of mineral amendments on the seedling growth (true leaves, leaf area, rhizosheath and specific root length (SRL) of different plant species.

	Control	S1	S2	P1	P2	F _(e,19)	X ²	P
Seedlings (n)	<i>Z. mays</i>	3.8 (±1.30)	2.8 (±1.48)	3.6 (±1.14)	4.0 (±0.71)	4.0 (±1.41)	2.818	0.589
	<i>S. alba</i>	1.2 (±0.45) c	3.00 (±0.71) b	1.40 (±1.14) c	3.20 (±1.10) b	4.60 (±0.55) a	18.026	0.001
	<i>L. sativa</i>	1.20 (±0.45) c	4.20 (±0.84) a	4.20 (±0.84) a	3.00 (±0.00) b	4.25 (±0.96) abc	13.158	0.011
Leaves (n)	<i>A. retroflexus</i>	4.80 (±0.45)	4.60 (±0.89)	5.00 (±0.00)	5.00 (±0.00)	5.00 (±0.00)	3.133	0.536
	<i>Z. mays</i>	3.40 (±1.14)	3.75 (±0.50)	3.60 (±1.14)	3.80 (±0.84)	3.80 (±1.10)	0.677	0.954
	<i>S. alba</i>	2.20 (±0.84) b	3.00 (±0.71) ab	2.25 (±1.26) ab	3.40 (±1.14) ab	4.00 (±0.71) a	3.190	0.037
Leaf area (cm ²)	<i>L. sativa</i>	2.40 (±1.14)	4.00 (±0.71)	3.80 (±1.10)	3.00 (±1.00)	3.50 (±0.58)	6.722	0.151
	<i>A. retroflexus</i>	4.20 (±0.84)	4.00 (±1.22)	4.20 (±0.84)	4.20 (±0.84)	4.20 (±0.84)	0.047	0.996
	<i>Z. mays</i>	13.07 (±2.83) ab	11.80 (±2.85) b	18.23 (±6.92) ab	22.94 (±7.78) a	18.86 (±5.80) ab	1.701	0.044
Rhizosheath (mg cm ⁻¹)	<i>S. alba</i>	0.10 (±0.02) c	2.28 (±1.34) b	0.41 (±0.39) c	7.69 (±3.06) a	5.88 (±1.07) ab	31.305	<0.001
	<i>L. sativa</i>	1.12 (±0.70) b	3.31 (±1.47) b	0.98 (0.23) b	8.50 (±4.88) a	10.42 (±2.85) a	11.519	<0.001
	<i>A. retroflexus</i>	0.03 (±0.02) c	0.16 (±0.10) b	0.07 (±0.06) bc	1.26 (±0.30) a	0.65 (±0.24) a	27.212	<0.001
SRL (m g ⁻¹)	<i>Z. mays</i>	2.8 (±0.67) ab	2.5 (±0.69) b	3.0 (±0.44) ab	4.6 (±1.37) a	4.4 (±0.82) a	5.723	0.004
	<i>S. alba</i>	0.1 (±0.2) c	16.1 (±7.5) b	5.0 (±5.9) bc	61.1 (±22.6) a	62.0 (±26.04) a	19.147	0.001
	<i>L. sativa</i>	19.6 (±24.8)	35.5 (±15.3)	39.7 (±20.9)	27.04 (±8.3)	59.9 (±22.5)	7.472	0.113
SRL (m g ⁻¹)	<i>A. retroflexus</i>	<0.01 (±0.00) b	4.5 (±2.4) b	2.3 (±0.1) b	23.7 (±10.2) a	26.1 (±11.7) a	19.648	<0.001
	<i>Z. mays</i>	1.6 (±0.94) ab	2.2 (±0.62) a	0.8 (±0.32) b	0.8 (±0.27) b	1.1 (±0.65) ab	5.530	0.004
	<i>S. alba</i>	220.0 (±173.21) a	12.5 (±13.81) ab	191.3 (±281.15) a	1.6 (±1.33) bc	0.5 (±0.41) c	15.417	0.004
SRL (m g ⁻¹)	<i>L. sativa</i>	109.2 (±120.54) a	7.3 (±6.50) ab	20.9 (±15.55) a	3.0 (±2.57) b	1.4 (±1.44) b	13.718	0.008
	<i>A. retroflexus</i>	41.2 (±32.92) ab	77.3 (±65.22) a	94.9 (±61.54) a	12.1 (±0.59) c	24.2 (±15.70) bc	14.372	0.006

S1: Soil + sand (ratio 1:1), S2: Soil + perlite (1:1), P2: Soil + perlite (1:2), SRL: Specific root length. Values of P in bold indicate significant differences in One-way ANOVA or Kruskal Wallis. Different letters in the same row indicate significant differences between treatments according to Tukey's or Dunnett's T3 post hoc test or Mann-Whitney U test (at P<0.05 level).

Plant growth

The addition of mineral amendments produced beneficial effects on the growth of plants from important families in agriculture as *Poaceae*, *Asteraceae*, *Amaranthaceae* or *Brassicaceae*. Before harvest, the number of viable seedlings of *S. alba* were significantly enhanced with the use of S1, P1 and P2. In *L. sativa*, viable seedlings were enhanced with the use of S1, S2 and P1. Also, the number of well-developed leaves of *S. alba* were significantly increased with the use of P2 (Table 2).

In general, leaf area in *L. sativa*, *S. alba* and *A. retroflexus* was significantly increased when plants grew in soils treated with mineral amendments (Table 2). After harvest, the use of mineral amendments also increased the plant growth and biomass (shoot and root) mainly in P1, P2 and S1 treatments (Fig. 1a, b).

Specific root length (SRL) of *L. sativa* and *S. alba* was higher in control soils and S2 than in perlite treatments (Table 2). Plants with lower root length and biomass showed higher SRL values, while the opposite trend was observed in plants with higher values of root length and biomass (Fig. 1a, b, Table 2). The increase in root length and biomass can be related to the decrease in soil bulk density (15), as roots can easily penetrate in less compacted soil. Nevertheless, root biomass increased more than root length, when plants grew in P1 and P2, as indicated by SRL results (Table 2). Good indicator of environmental changes and used to characterize economic aspects of the root system (27), a decrease in SRL suggest that plants are investing in root biomass instead of root elongation; a decrease in SRL also serves as an indicator of nutrient availability since this value generally decreases under fertilization (27). In our case, better plant performance was facilitated by the decrease in particle density and the change in soil texture, since plants growing in P2 had longer roots and produced more biomass than other treatments. Mineral amendments (sand but mostly perlite) increased the soil particle size (> 0.05 mm fraction), leading to an increase in pore size, reducing the soil compaction, facilitating root access to air and water, and consequently better root growth (12).

Although the general effect of mineral amendments on plant growth was positive (Figure 2), the amelioration of soil physicochemical properties affected the plants in a species-dependent manner. Large and balanced figures in soils treated with mineral amendments indicated better plant development whereas plants growing in control soils presented extremely irregular performance.

The improvement in plant growth was particularly noticeable for *S. alba*, *L. sativa*, and *A. retroflexus*. With the use of P2, species with more fragile and delicate root morphology (*L. sativa* and *A. retroflexus*) were more benefited in terms of root length and biomass (Fig. 2). As a consequence, better root development facilitates plant access to water and nutrients, leading to higher growth (mainly shoot length and biomass) of both species. Increasing the photosynthetic surface through rapid leaf development and higher leaf area facilitate plant access to light energy, becoming independent of the cotyledon reserves and thus, rapidly increasing plant growth. In addition, we observed a general increase in the rhizosphere of plants grown with mineral amendments, especially P1 and P2 (Table 2). Rhizosphere is a specific assembly, where roots, allelochemicals, soil particles and microorganisms are in direct contact. Due to this complex nature, rhizosphere plays a fundamental role in regulating the soil-root-plant water flow, nutrients acquisition and the uptake of allelochemicals compounds (35). Although its function is highly complex and subjected to many biotic and abiotic factors, rhizosphere is a direct marker of

root health, and higher volumes indicate an increase in mucilage production and more development of root hairs (15) that help plant growth under stressful environments (2).

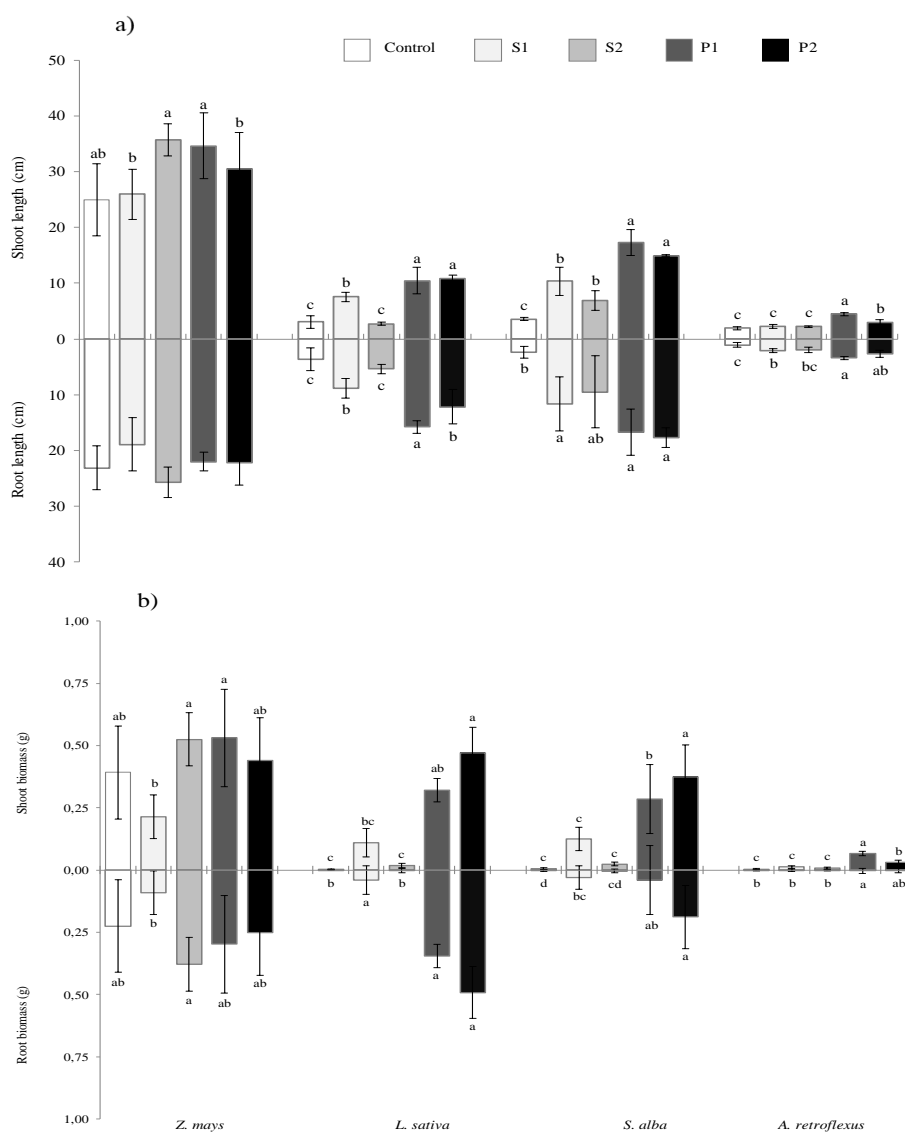


Figure 1. Effects of mineral amendments on (a) shoot and root length and (b) shoot and root biomass in test plant species in sterilised soil in pot culture. Different letters indicate significant differences (at $P \leq 0.05$) in Tukey's or T3 Dunnett's as the post-hoc tests. S1= soil + sand (ratio 1:1), S2= soil + sand (1:2), P1= soil + perlite (1:1); P2= soil + perlite (1:2)

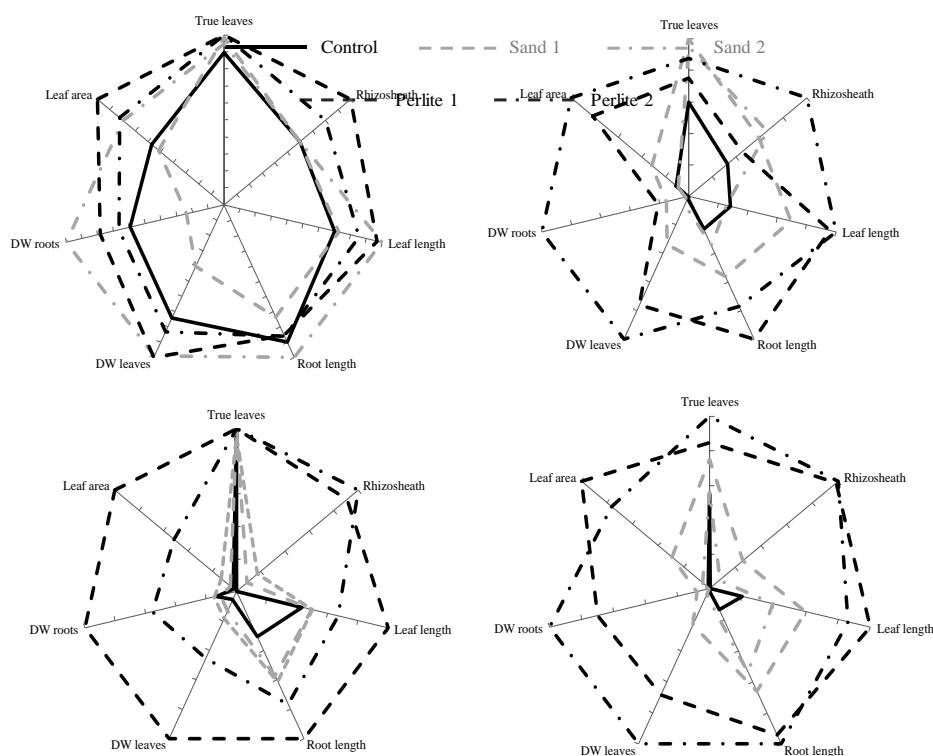


Figure 2. Representation of different morphological traits of *Z. mays*, *L. sativa*, *S. alba* and *A. retroflexus* after the addition of mineral amendments. Radial graphs represent results relative to the highest value (indicated as 100%) of each biometrical parameter (true leaves, leaf area, dry biomass (DW leaves and roots), root and leaf length, and rhizosheath).

CONCLUSIONS

Due to its feasibility, availability, and cost-effectiveness, autoclaving is the most common method used for soil sterilization. Our results evidenced that the use of mineral amendments (particularly perlite) effectively improved soil physicochemical parameters and ameliorated plant responses after soil autoclaving. In terms of plant performance, perlite at different concentrations showed the best results. The sand treatments effectively promoted plant growth in some cases, even at lower doses. The use of mineral amendments alleviated the negative consequences of autoclaving, homogenizing soil conditions for plant growth. Therefore, the use of inexpensive and easily accessible amendments is a valuable tool to improve the soil physicochemical conditions after sterilization, reducing negative effects on plant growth produced by autoclaving. Thus, improvement in plant growth conditions would differentiate between the negative effects produced by sterilization than those produced by chemical compounds in allelopathy bioassays.

ACKNOWLEDGMENTS

We sincerely thank Dr. Maria Luisa Andrade Couce for her helpful comments on the manuscript. We are also grateful to Dr. Flora Alonso, Yaiza Lechuga-Lago, Andrés Rodríguez-Seijo and Manoel Lago-Vila for their collaboration during the soil measurements. We also thank Mariasole Calbi and Jonatan Rodríguez for their cooperation during plant harvest. Alejandra Guisande-Collazo is currently funded by the University of Vigo through Ph.D. Student contract.

FUNDING

This work was funded by Xunta de Galicia, Spain (CITACA Strategic Partnership, Reference: ED431E 2018/07).

REFERENCES

1. Alpei, J. and Scheu, S. (1993). Effects of biocidal treatments on biological and nutritional properties of a mull-structured woodland soil. *Geoderma* **56**: 435-448.
2. Ashraf, M., Hasnain, S., Berge, O. and Mahmood, T. (2004). Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biology and Fertility of Soils* **40**: 157-162.
3. Bengough, A.G., McKenzie, B.M., Hallett, P.D. and Valentine, T.A. (2011). Root elongation, water stress, and mechanical impedance: A review of limiting stresses and beneficial root tip traits. *Journal of Experimental Botany* **62**: 59-68.
4. Bengough, A.G. and Mullins, C.E. (1991). Penetrometer resistance, root penetration resistance and root elongation rate in two sandy loam soils. *Plant and Soil* **131**: 59-66.
5. Bennett, J.A. and Klironomos, J. (2019). Mechanisms of plant-soil feedback: interactions among biotic and abiotic drivers. *New Phytologist* **222**: 91-96.
6. Berns, A.E., Philipp, H., Narres, H.D., Burauel, P., Vereecken, H. and Tappe, W. (2008). Effects 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.
7. Callaway, R.M., Thelen, G.C., Rodriguez, A. and Holben, W.E. (2004). Soil biota and exotic plant invasion. *Nature* **427**: 731-733.
8. Cambi, M., Mariotti, B., Fabiano, F., Maltoni, A., Tani, A., Foderi, C., Laschi, A. and Marchi, E. (2018). Early response of *Quercus robur* seedlings to soil compaction following germination. *Land Degradation and Development*. DOI: 10.1002/ldr.2912.
9. Cardinaux, A., Hart, S.P. and Alexander, J.M. (2018). Do soil biota influence the outcome of novel interactions between plant competitors? *Journal of Ecology*. DOI: 10.1111/1365-2745.13029.
10. Carter, M.R. and Gregorich, E.G. (2007). *Soil Sampling and Methods of Analysis*. 2nd Edn. CRC Press, New York.
11. Cui, H., Wang, C., Gu, Z., Zhu, H., Fu, S. and Yao, Q. (2014). Evaluation of soil storage methods for soil microbial community using genetic and metabolic fingerprints. *European Journal of Soil Biology* **63**: 55-63.
12. Ezawa, T., Yamamoto, K. and Yoshida, S. (2002). Enhancement of the effectiveness of indigenous arbuscular mycorrhizal fungi by inorganic soil amendments. *Soil Science and Plant Nutrition* **48**: 897-900.
13. Getenga, Z.M., Dorfler, U., Reiner, S. and Sabine, K. (2004). Determination of a suitable sterilization method for soil in isotopuron biodegradation studies. *Bulletin of Environmental Contamination and Toxicology* **72**: 415-421.
14. Guisande-Collazo, A., González, L. and Souza-Alonso, P. (2016). Impact of an invasive N₂-fixing tree on arbuscular mycorrhizal fungi and development of native species. *AoB Plants* **8**: 1-11.
15. Haling, R.E., Brown, L.K., Bengough, A.G., Valentine, T.A., White, P.J., Young, I.M. and George, T.S. (2014). Root hair length and rhizosheath mass depend on soil porosity, strength and water content in barley genotypes. *Planta* **239**: 643-651.

16. Heil, J., Liu, S., Vereecken, H. and Brueggemann, N. (2015). Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. *Soil Biology and Biochemistry* **84**: 107-115.
17. Inderjit. (2005). Soil microorganisms: An important determinant of allelopathic activity. *Plant and Soil* **274**: 227-236.
18. Jenkinson, D. and Powelson, D. (1976). The effects of biocidal treatments on metabolism in soil-I. Fumigation with chloroform. *Soil Biology and Biochemistry* **8**: 167-177.
19. Kaur, H., Kaur, R., Kaur, S., Baldwin, I.T and Inderjit (2009). Taking ecological function seriously: Soil microbial communities can obviate allelopathic effects of released metabolites. *PLoS One* **4** : e4700.
20. Lotrario, J.B., Stuart, B.J., Lam, T. and Kosson, D.S. (1995). Effects of sterilization methods on the physical characteristics of soil: Implications for sorption isotherm analyses. *Bulletin of Environmental Contamination and Toxicology* **54**: 668-675.
21. Mahmood, T., Mehnaz, S., Fleischmann, F., Ali, R., Hashmi, Z.H. and Iqbal, Z. (2014). Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. *Pedobiologia* **57**: 123-130.
22. Mauli, M.M., Nóbrega, L.H.P., da Silva Souza Filho, A.P., Meneghetti, A.M., Rosa, D.M. and Tonini, M. (2018). Soil moisture effects on emergence and initial development of *Bidens pilosa*. *Tropical and Subtropical Agroecosystems* **21**: 141-149.
23. McCarty, L.B., Hubbard, L.R. and Quisenberry, V. (2016). *Applied Soil Physical Properties, Drainage and Irrigation Strategies*. Springer International Publishing, Switzerland.
24. Meinhardt, K.A. and Gehring, C.A. (2012). Disrupting mycorrhizal mutualisms: A potential mechanism by which exotic tamarisk outcompetes native cottonwoods. *Ecological Applications* **22**: 532-549.
25. Miransari, M., Bahrami, H.A., Rejali, F., Malakouti, M.J. and Torabi, H. (2007). Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. *Soil Biology and Biochemistry* **39**: 2014-2026.
26. Nazeri, N.K., Lambers, H., Tibbett, M. and Ryan, M.H. (2013). Do arbuscular mycorrhizas or heterotrophic soil microbes contribute to plant acquisition of mineral phosphate? *Plant and Soil* **373**: 699-710.
27. Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Löhmus, K., Majidi, H., Metcalfe, D., Olsthoorn, A.F.M., Pronk, A., Vanguelova, E., Weih, M. and Brunner, I. (2007). Specific root length as an indicator of environmental change. *Plant Biosystems* **141**: 426-442.
28. Page, A.L., Miller, R.H. and Keeney, D.R. (1982). *Chemical and Microbiological Properties*. Second Edition. American Society of Agronomy and Soil Science Society of America, USA.
29. Razavi Darbar, S. and Lakzian, A. (2007). Evaluation of chemical and biological consequences of soil sterilization methods. *Caspian Journal of Environmental Sciences* **5**: 87-91.
30. Samar, M. and Saxena, S. (2016). Study of chemical and physical properties of perlite and its application in India. *International Journal of Science Technology Management and Research* **5**: 1-11.
31. Skipper, H.D. and Westermann, D.T. (1973). Comparative effects of propylene oxide, sodium azide and autoclaving on selected soil properties. *Soil Biology and Biochemistry* **5**: 409-414.
32. Slattery, W., Conyers, M. and Aitken, R. (1999). Soil pH, aluminum, manganese and lime requirement. In: *Soil Analysis: An Interpretation Manual* (Eds., K.I. Peverill, L. Sparrow and D. Reuter), pp. 103-125. CSIRO Publishing, Melbourne.
33. Smith, M.E., Delean, S., Cavagnaro, T.R. and Facelli, J.M. (2018). Evidence for species-specific plant responses to soil microbial communities from remnant and degraded land provides promise for restoration. *Australian Ecology* **43**: 301-308.
34. Vogelsang, K.M. and Bever, J.D. (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology* **90**: 300-407.
35. Watt, M., Mccully, M.E. and Canny, M.J. (1994) Formation and stabilization of rhizosheaths of *Zea mays* L. : Effects of soil water content. *Plant Physiology* **106**: 179-186.
36. Whalen, J.K., Chang, C., Clayton, G.W. and Carefoot, J.P. (2000). Cattle manure amendments can increase the pH of acid soils. *Soil Science Society of America Journal* **64**: 962-966.
37. Wolf, D.C., Dao, T.H., Scott, H.D. and Lavy, T.L. (1989). Influence of sterilization methods on selected soil microbial, physical and chemical properties. *Journal of Environmental Quality* **18**: 39-44.