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SHORT COMMUNICATION

The Effects of Sterilisation on Abiotic Soil Properties and the Challenge this Poses to Untangling the Influence of Abiotic and Biotic Soil Variables on Plant Growth

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Abstract

To separate the effects of the abiotic and biotic soil components on plant growth, researchers can compare plants grown in sterilised and unsterilised soil (unsterilised soil approach). However, using this approach can be problematic if the abiotic component of the soil is affected by the sterilisation treatment, which is often the case. We aimed to determine how often this approach is used by plant-soil interaction studies and demonstrate how it can impact plant growth. We reviewed the relevant literature to determine how often the unsterilised soil approach is used and whether the studies that use it acknowledge the 'soil sterilisation' caveat. We then conducted a glasshouse experiment to demonstrate the effect that soil sterilisation has on selected soil nutrients and consequently on plant growth. Our literature review revealed that of the studies that used the unsterilised soil approach, only 23% measured and reported soil nitrogen or phosphorus before and after sterilisation, with 50% of these reporting a change in one or both nutrients. We then showed that the growth of our study species, *Eucalyptus saligna*, was greater when grown in sterilised soils compared to unsterilised soils. Interestingly, there was a strong positive relationship between the change in ammonium nitrogen and *E. saligna* growth between the sterilised and unsterilised soils. Given our findings, we advocate that studies that use the unsterilised soil approach should measure the abiotic soil properties before and after sterilisation and if differences are found, these studies should use the inoculation approach instead.

Keywords Autoclaving · Dry heat · Ionising radiation · Plant growth · Soil microbes · Soil nutrients

1 Introduction

Both the abiotic and biotic components of soils significantly impact plant growth, from the individual to the community-level (Berendsen et al. 2012; Trivedi et al. 2020). Researchers often use soil sterilisation to reduce the biotic component of the soil to determine the influence of each component on plant growth (Trevors 1996). There are two experimental approaches in which soil sterilisation can be used to achieve this. The first approach compares the growth of plants grown in sterilised soil with and without the addition of inoculum (hereon referred to as the inoculum approach). The second compares the growth of plants grown in sterilised and unsterilised soil (hereon referred to as the unsterilised soil approach).

The strength of the unsterilised soil approach over the inoculation approach is that it allows the assessment of the influence of soil microbial communities at somewhat natural densities and composition (Brinkman et al. 2010; see van de Voorde et al. 2012). However, the weakness of this approach is that the abiotic soil properties may be impacted by the sterilisation treatment, resulting in differences between the sterilised and unsterilised soils. This is because the killed microbes and other soil organisms release their cell contents into the soil after sterilisation, causing a flush of nutrients, amongst other changes (McNamara et al. 2003; Dietrich et al. 2020). If these changes are not accounted for, they may significantly impact the validity of inferences made from

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the data obtained from these studies. For this reason, the inoculation approach is considered to be the preferable of the two approaches (van de Voorde et al. 2012).

We reviewed the recent literature to determine how often plant-soil interaction studies use the unsterilised soil approach and whether these studies consider the known effect soil sterilisation can have on soil nutrients. We then conducted a glasshouse experiment to demonstrate the effect these soil nutrient changes can have on plant growth.

2 Materials and Methods

2.1 Literature Review

We compiled a database of related studies published between January 2013 and April 2023 by searching for the terms 'soil', 'sterili*', 'plant', 'growth' and 'biomass' on Web of Science. Of the 238 studies retrieved, we retained 132 that compared the growth (shoot, root or total biomass) of individual plants grown in sterilised and unsterilised soils (i.e. unsterilised soil approach) or sterilised soil with and without the addition of inoculum (i.e. inoculation approach). The most common reasons for studies not being retained were that they were not plant-focused, did not contain primary data (e.g. review and meta-analysis articles), assessed plant community growth rather than individual plant growth, or did not include a sterilised soil treatment. For each retained study, we first identified the sterilisation experimental approach (i.e. unsterilised soil vs. inoculation vs. both). For the studies that used the unsterilised soil approach, we identified (1) the method of sterilisation, (2) the study species, (3) the growth duration of the study, (4) whether the study measured soil nitrogen and phosphorus before and after sterilisation, (5) the effect of sterilisation on soil nitrogen and phosphorus, (6) the effect of sterilisation on plant growth and (7) the suggested cause (biotic or abiotic) of the plant growth response.

2.2 Glasshouse Experiment

We collected and sieved (5 mm) soil samples from the base of two *Eucalyptus saligna* trees at four locations (Chichester State Forest, Cascade National Park, Richmond Range National Park, Toonumbar National Park) within its native range along the eastern seaboard of New South Wales, Australia (Fig. 1). The soil collected at each location was classified as sandy loam. Each soil sample was split into two subsamples, with one half being sterilised by autoclaving it for 1.5 h on a wet cycle, which peaked at 121 °C for 20 min, and the other half remaining unsterilised. We selected autoclaving as our sterilisation method because it was the most frequently used method in the plant-soil interaction studies.

The ammonium nitrogen, nitrate nitrogen and available phosphorus content of each sterilised and unsterilised soil subsample were measured by the CSBP Soil and Plant Analysis Laboratory (Perth, WA, Australia). Nitrate and ammonium were measured using Raymont and Lyons Method 7C2b (Raymont and Lyons 2010). They were extracted from the soil using a 2 M potassium chloride solution. Ammonium was measured colorimetrically. Nitrate was first reduced to nitrite through a copperised cadmium column and then

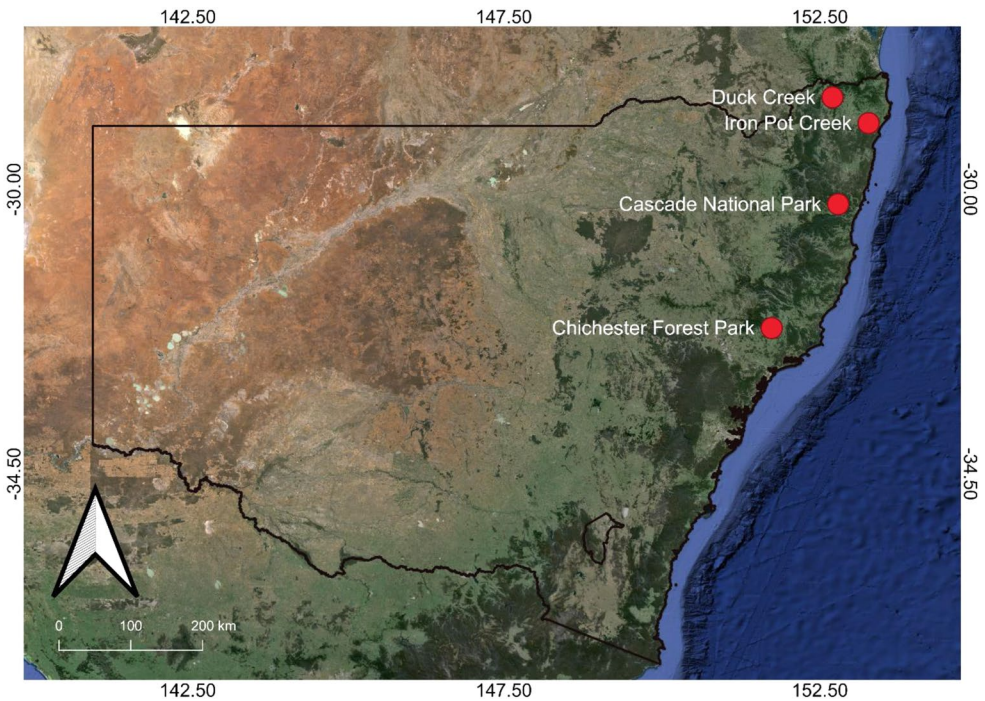


Fig. 1 A map of the locations of the soil collection sites in New South Wales, Australia

measured colorimetrically. Available phosphorus was measured using Raymont and Lyons Method 9B (Raymont and Lyons 2010). A 0.5 M sodium bicarbonate solution adjusted to pH 8.5 was added to the soil samples at a ratio of 100:1 and left for 16 h. The resulting solution was then acidified and measured colorimetrically. Using these data, the percentage change in each soil nutrient between the sterilised and unsterilised paired subsamples was calculated.

For each of the eight soil subsamples, one *E. saligna* seedling was grown from seed in a 3 L container. The seeds were sourced from the same locations where the soil samples were collected. The seedlings were grown for 20 weeks in a climate-controlled glasshouse with the day/night temperature set to 27/19°C. During the growth period, the seedlings received water three times a day through a spike (~ 150 mL/day). No fertiliser was added to the pots to ensure that any sterilisation effect on soil nutrient availability was not masked. The seedlings did not require pest management. After the growth period, the seedlings were harvested, oven-dried and weighed. Using these data, the percentage change in total biomass of the seedlings between sterilised and unsterilised paired subsamples was calculated.

Paired t-tests were conducted to determine if autoclaving changed the total biomass of the *E. saligna* seedlings and soil nutrients in a consistent direction. Linear regressions were then used to determine if there were relationships between the percentage change in total biomass and soil nutrients between sterilised and unsterilised soils. The data analyses were conducted in R (R Core Team 2022) and the regressions were visualised using ggplot2 in R (Wickham 2016).

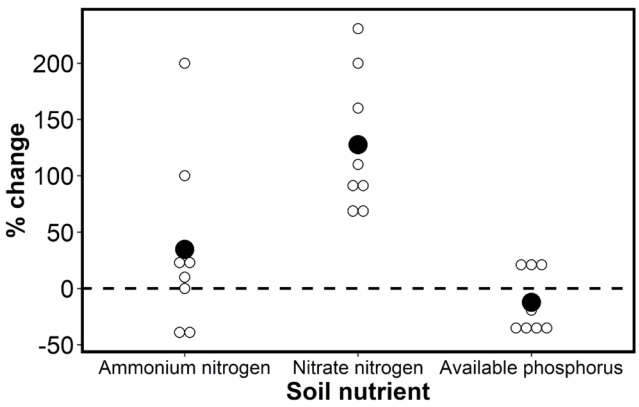


Fig. 2 The mean (●) % change of the soil nutrients between sterilised and unsterilised soils ($n = 8$). When an open dot (○) is above the dashed line, it means the sterilised soil had greater nutrients than the unsterilised soil and vice versa for the dots below the dashed line

3 Results

3.1 Literature Review

The literature review identified 132 studies that met the search criteria, of which 63 used the inoculation approach while 63 used the unsterilised soil approach. A further five studies used both experimental approaches simultaneously and one study did not specify the approach used.

Of the 68 studies (i.e. 63 studies that used the unsterilised soil approach only and 5 that used both approaches) that used the unsterilised soil approach, autoclaving (48/68, 70%) was the most frequently used sterilisation method, followed by ionising radiation (18/68, 26%) and dry heat (1/68, 1%). No studies used chemical fumigation. The sterilisation method of three studies (2/68, 3%) was not reported. One study used both autoclaving and ionising radiation.

Of the 68 studies that used the unsterilised soil approach, 16 (23%) measured soil nitrogen or phosphorus before and after sterilisation. Eight (50%) of these studies reported a significant change (increase or decrease) in one or both nutrients. These eight studies measured the growth responses of 16 plant species, with the response of eight species being related to changes in soil nutrients due to sterilisation.

3.2 Glasshouse Experiment

The *E. saligna* seedlings grown in autoclaved soil produced significantly more total biomass than seedlings grown in unsterilised soil ($T = 4.92$, $p = 0.002$, Fig. 2a). Autoclaving altered all the soil nutrients (Fig. 3) but only nitrate nitrogen had a consistent increase ($T = 3.59$, $p = 0.009$, Fig. 2c). In contrast, ammonium nitrogen ($T = 0.19$, $p = 0.827$, Fig. 2b) and available phosphorus (T=-0.65, 0.535, Fig. 2d) were not altered in a consistent direction.

The percentage change in total biomass of the *E. saligna* seedlings was positively related to the percentage change in the soil ammonium nitrogen ($F_{1,6}$=6.60, $p = 0.042$, $R^2 = 0.52$, Fig. 4) but was not related to the percentage change in soil nitrate nitrogen ($F_{1,6}$=2.05, $p = 0.202$) or available phosphorus ($F_{1,6}$=3.93, $p = 0.095$).

4 Discussion

Researchers often use soil sterilisation to determine the influence of the abiotic and biotic components of soil on plant growth (Trevors 1996). This can be achieved using either the inoculum experimental approach or the unsterilised soil experimental approach. We found that neither experimental approach is favoured by plant-soil interaction studies, which is surprising given the known 'nutrient

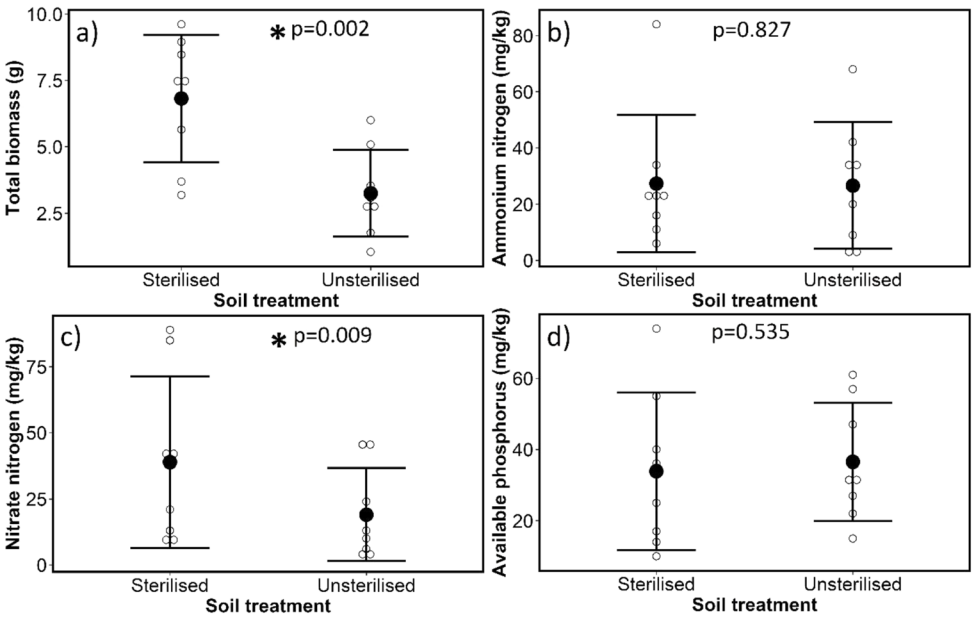


Fig. 3 The mean (●) **(a)** total biomass of the *E. saligna* seedlings and the **(b)** ammonium nitrogen, **(c)** nitrate nitrogen and **(d)** available phosphorus of the sterilised and unsterilised soils they were grown in ($n = 8$). The error bars represent one standard error. Asterisks represent significant stand type differences while letters represent significant study site differences ($p < 0.05$)

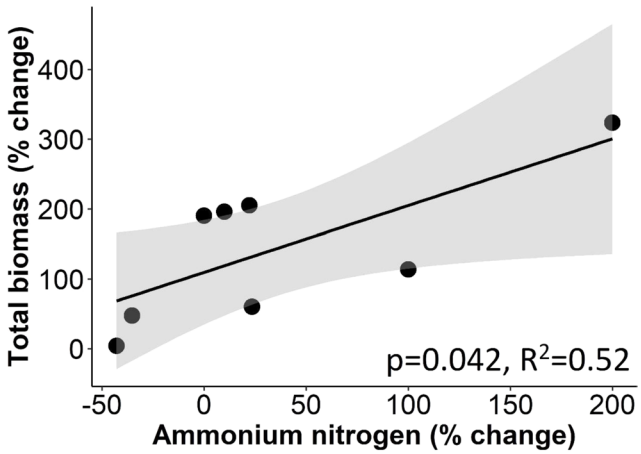


Fig. 4 The relationship between the percentage change in the total biomass between *Eucalyptus saligna* seedlings grown in sterilised and unsterilised soils and ammonium nitrogen percentage change in sterilised and unsterilised soils ($n = 8$)

flush' caveat associated with the unsterilised soil approach (McNamara et al. 2003; Dietrich et al. 2020). There appears to be a clear distinction in the experimental approach used by studies that focus on the effect of specific soil microbes on plant growth compared with those that focus on the effect of whole soil microbial communities. Of the 68 studies that used the inoculation approach (i.e. 63 studies that used the inoculation approach only and 5 that used both approaches), only 24 (36%) tested the effect of whole soil microbial communities on plant growth. The remaining 43 studies tested a specific soil microbe or group of microbes (e.g. arbuscular mycorrhizal fungi). On the other hand, all the studies that used the unsterilised soil approach tested whole soil microbial community effects on plant growth. This was expected because this approach cannot be used to test specific soil microbes. These findings suggest that researchers who are testing whole soil microbial communities are reluctant to use the inoculation approach. This may be because the strength of the unsterilised soil approach is that the soil microbial community does not get diluted, as is the case when using the inoculation approach (Brinkman et al. 2010). However, we suggest that this strength is negated by the fact that soil microbial community structure is strongly driven by the abiotic properties of soils (Chaparro et al. 2012; Serna-Chavez et al. 2013). This means that if the abiotic soil properties differ between the sterilised and unsterilised soil, the soil microbial community is likely to follow suit.

Of the studies that reported using the unsterilised soil approach, autoclaving was the most frequently used sterilisation method. This may be because autoclaving was believed to be the most effective soil sterilisation method in the past (Kale and Raghu 1982). However, with the emergence of bioinformatic technologies (qPCR) that has enabled a more accurate assessment of living microbial biomass in soils after sterilisation, it has been shown that autoclaving is less effective at sterilising soils than other sterilisation methods (Li et al. 2023). Further, it has also been shown that autoclaving is more likely to alter abiotic soil properties such as organic carbon and soil nutrients (Berns et al. 2008; Wentao et al. 2019), particularly at the standard temperature of 121 °C (Serrasolsas and Khanna 1995). However, these findings are not definitive and further comparative studies of the effectiveness of different sterilisation methods are needed. In contrast to autoclaving, chemical fumigation was the least popular sterilisation method, which may be due to the impacts of chemical fumigants on human health

and the environment becoming increasingly recognised (Castellano-Hinojosa et al. 2022).

Although the influence of soil sterilisation on abiotic soil properties has been well documented (McNamara et al. 2003; Dietrich et al. 2020), only a quarter of the studies from our literature review that used the unsterilised soil approach measured soil nitrogen or phosphorus before and after sterilisation. This highlights that plant-soil interaction studies often do not acknowledge that soil sterilisation may alter abiotic soil properties. This is despite half of the studies that did do the measurements reporting a significant change (increase or decrease) in one or both nutrients. These studies measured the growth response of 16 plant species, with the growth response of half of these species being related to the changes in soil nutrients due to sterilisation. That is, if soil nutrients increased after sterilisation, so did plant growth, and vice versa. The results of our glasshouse experiment reinforce these findings. That is, autoclaving altered the nutrient levels of the field-collected soil, with the resulting changes in soil ammonium enhancing the growth of the glasshouse-grown *E. saligna* seedlings. Despite this, the biotic component of the soil was suggested to be responsible in part, if not entirely, for the growth response observed in all eight species. Although beneficial microbes and soil-borne pathogens can impact the growth of plants in unsterilised soil (Katan 2017), it is also likely that the species were responding to changes in soil nutrients caused by sterilisation.

Of the 16 species that had accompanying soil nutrient data before and after sterilisation, only eight had their growth duration reported. Interestingly, the three species that had a growth response related to the changes in soil nutrients due to sterilisation were grown for an average of 97 days while the five species that had a growth response that was not related to sterilisation were grown for an average of 85 days. This demonstrates that the effect of changes in soil nutrients due to sterilisation on plant growth does not diminish with time. The eight species whose growth duration was not reported were from a single study.

Our study aimed to provide an example, rather than a comprehensive exploration, of how soil sterilisation can alter abiotic soil properties, hence why we only focused on soil nitrogen and phosphorus. With that in mind, it needs to be pointed out that there is a range of abiotic soil properties (e.g. other macronutrients, organic carbon, pH), most of which can impact plant growth, that can be altered by soil sterilisation (McNamara et al. 2003; Dietrich et al. 2020). Nevertheless, we have demonstrated that soil sterilisation can significantly alter soil nutrients, which in turn strongly affects plant growth. For this reason, we advocate that studies that use the unsterilised soil approach should first measure the abiotic soil properties before and after sterilisation before proceeding with the study. Ideally, no significant change would occur and the study can proceed, allowing its subsequent findings to be interpreted without a 'soil sterilisation' caveat. Unfortunately, as we have shown, this is often not the case. In some instances, these differences can be minimised. For example, the addition of nutrients to the unsterilised soil can be used to negate the nutrient flush in the sterilised soil, although we did not identify a single study in our literature review that did this. Alternatively, we suggest the inoculation approach may be the more suitable approach. Although the dilution of the soil microbial community associated with inoculation is a weakness of this approach (Brinkman et al. 2010), steps can be taken to reduce its impact. For example, inoculation using sieved soil rather than a soil wash has been shown to result in a more similar microbiome to the source soil after three weeks (Howard et al. 2017). Further, the greater volume of soil that is used to inoculate the more the resulting microbiome will resemble that of the source soil (Howard et al. 2017). The trade-off with this is that too much inoculum may alter the abiotic soil properties.

5 Conclusions

Our findings are concerning, as they show that plant-soil interaction studies that use the unsterilised soil approach generally do not consider the effects of soil sterilisation on abiotic soil properties despite the potentially significant impact these changes can have on plant growth. This is evident from the relatively small proportion of studies that measured abiotic soil properties before and after sterilisation. For those that did measure abiotic soil properties, many attributed the observed growth responses to the influence of biotic variables rather than soil sterilisation. This suggests that these studies may have overstated the influence of soil biotic variables on plant growth. In the future, studies that use the unsterilised soil approach must measure the abiotic soil properties before and after sterilisation. If significant changes occur, the studies should consider using the inoculation approach instead.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42729-024-01882-y.

Author Contributions AM and JJLR came up with the study conception and design. All authors contributed to the data collection. AM performed the data analysis and wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The data is provided as supplementary material.

Declarations

Conflict of Interest The authors have no conflicts of interest to declare.

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