Soil Microbial Feedback on Eupatorium adenophorum Growth and Competition: A Comparison of Different Sterilization Methods (Postprint)

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Date: 2017-10-20T00:00:00+00:00

Abstract

Soil microbial removal is an important approach for verifying the regulatory role of soil microbial feedback in the competitive exclusion of native plant communities by invasive plants. To identify the optimal method for removing soil microorganisms to assess feedback effects and to understand the feedback role of soil microorganisms in competition between Ageratina adenophora and native plants, this study compared the effects of rhizosphere soil from A. adenophora monodominant communities treated with three common sterilization methods (dry heat sterilization, moist heat sterilization, and irradiation sterilization), with and without vermiculite addition, on the growth of A. adenophora and the native plant Rabdosia amethystoides. The results showed that, compared with non-sterilized soil, all three sterilization treatments significantly inhibited the growth of both A. adenophora and R. amethystoides; sterilized soil with added vermiculite significantly promoted the growth of both species compared to sterilized soil without vermiculite; when vermiculite was added to sterilized soil, the biomass of both plants in irradiation-sterilized soil was significantly higher than that in dry heat-sterilized and moist heat-sterilized soils, with A. adenophora biomass increasing by 30.8% and 66.5% under irradiation sterilization compared to dry heat and moist heat sterilization, respectively, and R. amethystoides biomass significantly increasing by 109.5% and 63.4%, respectively. The treatment combining irradiation sterilization with vermiculite addition most accurately reflects the feedback effects of soil microorganisms on plant growth. Further pot experiments were conducted with mixed planting of A. adenophora and R. amethystoides in irradiation-sterilized soil with vermiculite addition versus non-sterilized soil with vermiculite addition. The results demonstrated that soil microorganisms significantly enhanced the competitive advantage of A. adenophora over R. amethystoides, increasing the relative competitive advantage index by 16.0%, indicating that soil microorganisms play a

positive feedback commensal regulatory role in the invasion process whereby A. adenophora competitively excludes native plants.

Full Text

Feedback of Soil Biota on *Ageratina adenophora* Growth and Competitiveness with Native Plants: A Comparison of Different Sterilization Methods

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Abstract

Soil sterilization is commonly employed to investigate feedback effects of soil microbes on plant community succession, particularly in cases of competitive exclusion of native plants by invasive species. To identify the most suitable soil sterilization method for assessing microbial feedback and to elucidate the role of soil microbes in mediating competition between the invasive plant *Ageratina adenophora* and native species, we compared the growth of *A. adenophora* and the native plant *Rabdosia amethystoides* in rhizosphere soils from *A. adenophora*-dominated communities subjected to three sterilization methods: dry-heating, autoclaving, and gamma irradiation, with or without vermiculite amendment. Compared with non-sterilized soil, all three sterilization treatments significantly inhibited growth of both *A. adenophora* and *R. amethystoides*. Vermiculite addition to sterilized soils significantly promoted growth of both species compared with sterilized soils without vermiculite. Under sterilization with vermiculite amendment, biomass of both species was highest in irradiated soils, with *A. adenophora* biomass increasing by 30.8% and 66.5% compared with dry-heated and autoclaved soils, respectively, and *R. amethystoides* biomass increasing by 109.5% and 63.4%, respectively. The combination of gamma irradiation sterilization with vermiculite addition most accurately reflected soil microbial feedback effects on plant growth. A subsequent pot experiment comparing mixed plantings in irradiated soil with vermiculite versus non-sterilized soil with vermiculite revealed that soil microbes significantly enhanced the competitive advantage of *A. adenophora* over *R. amethystoides*, increasing its relative competitive advantage by 16.0%. These results demonstrate that gamma irradiation combined with vermiculite amendment is the most effective approach for pot experiments investigating soil microbial feedback effects, and that soil microbes exert positive feedback that facilitates the invasion process of *A. adenophora*.

Keywords: Invasive plant; *Ageratina adenophora*; Soil biota; Sterilization method; Feedback

Soil microbial communities constitute a critical biological component of plant rhizosphere ecosystems, forming a dynamic resource pool. Numerous studies have demonstrated that aboveground and belowground ecosystem components are intimately linked and interdependent, with plant-soil microbial interactions determining the direction and trajectory of plant community succession [1–4]. Once established, invasive plants can rapidly form monodominant communities through competitive expansion, altering aboveground community structure and causing biodiversity loss while profoundly affecting belowground soil microbial communities and functions [5–6]. This ecological process and its deep ecosystem-level changes inevitably feed back to directly or indirectly influence competition between invasive and native plants [4]. Consequently, interactions between exotic plants and soil microbes in invaded habitats, and their role in facilitating competitive displacement of native vegetation, have become focal points of international research [7–14].

Soil sterilization is a critical methodological tool for verifying the influence of soil microbes on plant community competition. In greenhouse pot experiments examining microbial feedback effects on plant growth and competition, comparing plants grown in sterile versus non-sterile soils is essential for accurately reflecting microbial feedback. The ideal sterilization method would eliminate all microorganisms without altering soil physicochemical properties [15]. Current sterilization techniques include dry-heating [16–17], autoclaving [10,18], gamma irradiation [18–20], and chemical fumigation [16,18]. However, different methods can affect the apparent strength of microbial feedback, potentially leading to divergent results and conclusions, yet few studies have systematically compared these approaches.

China suffers severely from biological invasions, with invasive plants accounting for 51% of all invasive species [21]. *Ageratina adenophora* (Sprengel) R. King & H. Robinson (synonym: *Eupatorium adenophorum* Sprengel) is among the most damaging invasive plants in China and a globally significant weed [21–23]. It is widely distributed across Yunnan, Guizhou, Sichuan, Chongqing, and Guangxi, and continues to spread rapidly eastward and northward [24–25]. *A. adenophora* quickly outcompetes native plants to form monospecific stands, severely reducing biodiversity, degrading ecosystem function, and causing substantial economic losses to agriculture, forestry, and livestock production [22–23,26]. Previous research has demonstrated that soil microbes play an important role in the competitive expansion of *A. adenophora* [27–37]. This study uses *A. adenophora*-invaded soil as a case study to: (1) compare three common sterilization methods (dry-heating, autoclaving, and gamma irradiation) to provide guidance for methodological selection in microbial feedback research; and (2) further elucidate the role of soil microbes in mediating competitive exclusion of native plants by *A. adenophora*, thereby providing theoretical insights into the microbial mechanisms underlying its invasion and establishment, laying foundations for control strategies and ecological restoration, and offering reference for

other invasive plant studies.

1. Materials and Methods

1.1 Soil Collection Soil was collected from *A. adenophora*-dominated communities in a valley near Qilin Bridge, Chengjiang County, Yuxi City, Yunnan Province (24°42 N, 102°52 E; elevation 1,840–1,873 m; mean annual precipitation 869 mm). The region features a transitional climate among mid-subtropical, north subtropical, south temperate, and mid-temperate zones, with lateritic red soil representative of central Yunnan—an optimal habitat for *A. adenophora*. In early September 2013, we collected soil from 20 sampling points following methods described by Niu et al. [28] and Xiao et al. [35]. Each 3 m × 3 m plot was spaced approximately 10 m apart. After removing surface litter, we collected 0–10 cm topsoil (approximately 4 L per plot). Soils were sieved through a 20-mesh screen, thoroughly mixed, and stored as potting substrate.

1.2 Plant Materials and Soil Treatments

1.2.1 Test Plants Seeds of *A. adenophora* and the native plant *Rabdosia amethystoides* (Benth.) Hara were collected from Chengjiang County, Yunnan. *R. amethystoides* is a common native associate in *A. adenophora*-invaded areas.

1.2.2 Soil Treatment Setup Eight soil treatments were established: (1) non-sterilized, (2) dry-heated, (3) autoclaved, (4) gamma-irradiated, (5) non-sterilized + vermiculite, (6) dry-heated + vermiculite, (7) autoclaved + vermiculite, and (8) irradiated + vermiculite. Vermiculite was added to mitigate sterilization-induced changes in soil physicochemical properties, particularly soil porosity, which could affect the strength of microbial feedback. Non-sterilized soil served as the microbial control. Dry-heating followed Trevors [16]: soil was spread in trays and heated at 180 °C for 4 h, with stirring at 2 h. Autoclaving followed Callaway et al. [10]: soil in bags was autoclaved three times at 121 °C and 0.105 MPa for 30 min each, with 24 h intervals. Gamma irradiation followed Berns et al. [20]: soil was irradiated at 36 kGy (4 kGy • h ^{1} for 9 h) using a BFT- Co- apparatus. For vermiculite treatments, irradiated vermiculite was mixed with soil at a 9:1 mass ratio.

1.2.3 Pot Experiment Design To assess microbial feedback effects on plant growth and competition, we conducted two planting types: monoculture and mixed culture of *A. adenophora* and *R. amethystoides*, with 10 replicates per treatment.

For monocultures, the eight soil treatments were used separately for each species. *A. adenophora* seeds were germinated in sterilized sand after 3% H O disinfection (3 min) and three sterile water rinses. Seedlings (~3 cm tall) were transplanted to the center of pots (12 cm diameter × 10 cm height). For *R. amethys-*

toides, five disinfected seeds were sown centrally and thinned to one seedling per pot.

For mixed cultures, based on preliminary trials, we used non-sterilized soil + vermiculite and irradiated soil + vermiculite. One *A. adenophora* seedling was planted at one-third of the pot radius from the edge, with five *R. amethystoides* seeds sown opposite at the same distance. Seedlings were thinned to one per species. This yielded 18 total planting treatments. Pots were randomly arranged in a greenhouse at ~25 °C with a 14:10 L:D photoperiod and watered as needed. Plants were harvested after three months.

1.2.4 Measurement Indicators and Methods Soil physicochemical properties were measured for four treatments (non-sterilized, dry-heated, autoclaved, irradiated). pH was determined by electrode (WTW pH 340) in a 1:2.5 soil:water suspension. Organic carbon was measured by dichromate oxidation. Total nitrogen was determined by Kjeldahl digestion. Nitrate-N and ammonium-N were extracted with copper sulfate and potassium chloride, respectively, and analyzed by flow injection (Zellwegger Analytical Milwaukee WI). Total phosphorus was measured by NaOH fusion-molybdenum blue colorimetry, and available P by NaHCO extraction-molybdenum blue method. Total potassium was determined by NaOH fusion-flame photometry, and available K by ammonium acetate extraction-flame photometry. Three replicates were analyzed per treatment.

Plant biomass was determined after three months. Plants were harvested, washed, oven-dried at 70 °C for 72 h to constant weight, and weighed. In mixed treatments, species were separated. The relative competitive advantage of *A. adenophora* was calculated as:

$$\text{Relative competitive advantage of } A.\text{ adenophora} = \frac{\text{Biomass of } A.\text{ adenophora}}{\text{Total biomass of both species}} \times 100\% \quad (1)$$

1.3 Statistical Analysis Relative competitive advantage was analyzed by one-way ANOVA. Soil nutrient comparisons among sterilization methods used one-way ANOVA with Fisher' s LSD test. Plant biomass comparisons across sterilization methods and vermiculite treatments used two-way ANOVA with Fisher' s LSD test. Analyses were performed using SAS 10.0 (Inc., Chicago, USA).

2. Results

2.1 Effects of Sterilization Methods on Soil Physicochemical Properties Sterilization methods differentially affected soil properties, with autoclaving having the greatest impact, followed by dry-heating, and irradiation the least (Table 1). Compared with non-sterilized soil, dry-heating significantly reduced

pH and available K by 4.5% and 11.5%, respectively. Autoclaving significantly decreased total N, total K, nitrate-N, ammonium-N, and available K by 9.7%, 2.6%, 7.1%, 18.7%, and 20.1%, respectively. Irradiation only significantly reduced total N (by 9.7%). Dry-heated soil had the lowest pH, significantly lower than other treatments. Its available K was significantly lower than non-sterilized and irradiated soils but higher than autoclaved soil. Autoclaved soil had the lowest total K, nitrate-N, and ammonium-N, significantly lower than non-sterilized and dry-heated soils but not different from irradiated soil. Available K in autoclaved soil was significantly lower than all other treatments. Organic carbon, total P, and available P did not differ among treatments.

2.2 Effects of Sterilization Methods and Vermiculite on Plant Biomass Regardless of vermiculite addition, all three sterilization methods significantly reduced biomass of both *A. adenophora* and *R. amethystoides* grown in soil from *A. adenophora*-dominated communities. However, the magnitude of reduction varied by species, sterilization method, and vermiculite amendment (Figure 1 [Figure 1: see original paper]).

In monoculture, non-vermiculite soils sterilized by dry-heating, autoclaving, and irradiation reduced *A. adenophora* biomass by 98.9%, 99.1%, and 97.8%, respectively, compared with non-sterilized soil. With vermiculite, the corresponding reductions were 84.8%, 80.7%, and 74.7%. In non-vermiculite soils, no significant differences occurred among sterilization methods. However, in vermiculite-amended soils, irradiated soil produced significantly greater *A. adenophora* biomass than dry-heated (30.8% increase) and autoclaved (66.5% increase) soils.

For *R. amethystoides* monoculture, all seedlings died within one month in non-vermiculite sterilized soils. In vermiculite-amended soils, biomass was reduced by 81.2%, 75.9%, and 60.7% in dry-heated, autoclaved, and irradiated soils, respectively, compared with non-sterilized soil. Among sterilized treatments with vermiculite, irradiation yielded the highest biomass, significantly exceeding dry-heated (109.5% increase) and autoclaved (63.4% increase) soils. Autoclaved soil produced 28.2% more biomass than dry-heated soil, though not significantly.

Vermiculite addition had contrasting effects: in non-sterilized soil, it did not significantly affect biomass of either species, but in all sterilized soils, it dramatically increased biomass. Compared with their non-vermiculite counterparts, vermiculite addition increased *A. adenophora* biomass by 12.9-fold, 20.1-fold, and 10.5-fold in dry-heated, autoclaved, and irradiated soils, respectively, and enabled *R. amethystoides* survival where it had previously died. Two-way ANOVA revealed significant interactions between sterilization method and vermiculite addition for both *A. adenophora* ($F = 3.59$, $df = 3$, $P = 0.0015$) and *R. amethystoides* ($F = 4.59$, $df = 3$, $P = 0.0056$) (Table 2).

2.3 Effects of Soil Microbes on Competition The relative competitive advantage of *A. adenophora* differed significantly between non-sterilized soil with vermiculite and irradiated soil with vermiculite ($F = 7.31$, $df = 1$, $P = 0.0145$)

(Figure 2 [Figure 2: see original paper]). In non-sterilized soil, *A. adenophora* achieved a relative competitive advantage of 60.94%, compared with 52.54% in irradiated soil, indicating that soil sterilization reduced its competitive dominance by 16.0%. This demonstrates that soil microbes significantly enhance the competitive superiority of *A. adenophora* over native plants.

Discussion

Numerous studies confirm that soil microbes play crucial roles in mediating competitive succession between invasive and native plants [10,39–43]. Soil sterilization and subsequent microbial inoculation are key approaches for validating microbial feedback effects [44–46]. Our results show that soil microbes exert significant positive feedback on both *A. adenophora* and native *R. amethystoides* growth, and enhance the invader' s competitive advantage, consistent with previous findings [27–28,35], further underscoring the importance of soil microbes in facilitating *A. adenophora* invasion.

Globally, soil sterilization methods for assessing microbial feedback include dry-heating, autoclaving, gamma irradiation, and chemical fumigation. Autoclaving is widely used due to simplicity and low cost, followed by irradiation. Our results indicate that sterilization method significantly affects plant growth, with gamma irradiation being optimal and dry-heating unsuitable. Several factors explain these differences. First, sterilization alters nutrient availability: dry-heating reduces available K, autoclaving decreases available N and K, while irradiation has minimal effects on fertility. Previous studies show autoclaving changes soil properties [47], reducing pH and aggregates, increasing soluble manganese, decreasing iron, and producing toxic organic compounds [16]. While gamma irradiation can alter nitrate:ammonium ratios [48], comparative analyses suggest autoclaving has greater impacts on physicochemical and biological properties than irradiation [19,20,47,50]. Second, physical changes matter: dry-heating desiccates soil particles [16], while autoclaving increases soil compaction [20,48–49], whereas irradiation minimally affects soil structure. Smith et al. [51] found that mixing sterilized soil with untreated soil significantly affected plant growth and mycorrhizal colonization compared with untreated controls. Our finding that vermiculite addition increased biomass in sterilized but not non-sterilized soils likely reflects improved soil porosity and aeration, benefits that are normally provided by microbial activity in non-sterilized soils.

Because sterilization methods differentially affect plant growth, inappropriate methods (e.g., dry-heating or autoclaving relative to irradiation) may overestimate microbial feedback effects. Our results demonstrate that gamma irradiation with vermiculite amendment is the superior approach for studying microbial feedback in plant competition. Since vermiculite did not affect plant growth in non-sterilized soil—likely because microbial activity already optimizes soil physical properties—this treatment combination provides the most accurate assessment of microbial influences on invasive plant competition.

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Note: Figure translations are in progress. See original paper for figures.