Study species

Closely related native and nonnative species were used for this experiment to control for species responses that are attributable to phylogeny and isolate the response of plant origin (native, nonnative) to climate change and soil biota. We chose species from Eugenia, in the Myrtaceae family, because there are closely-related native and nonnative species of the same genus and functional group which co-occur in subtropical hammock habitat of Florida (Liu et al. 2007). All three species are relatively abundant throughout Florida (Wunderlin et al. 1996). These small tree and shrub species are found in subtropical habitats in Florida, central and South America, and the Caribbean. Eugenia uniflora, or Surinam cherry, is native to Brazil and has been introduced to much of South America outside of Brazil, in addition to Asia, Australasia-Pacific Region, Europe, and North America (Wunderlin et al. 1996; ISSG 2016). Eugenia uniflora associates with arbuscular mycorrhizal fungi (Zangaro et al. 2005). Eugenia uniflora was introduced to Florida as an ornamental and for its edible fruit prior to 1931, and has been widely planted in central and south Florida, especially for hedges (Langeland et al. 2008). Eugenia uniflora has a high impact on ecological communities (FLEPPC 2017), is able to invade upland habitat, and is located south of the freeze line in Florida. Eugenia uniflora is considered Category I, as designated by the Florida Exotic Pest Plant Council (FLEPPC 2017), which is a species that causes large ecological damage through the displacement of native species, changing community structures or ecological functions, or hybridizing with natives.

Growth chamber Experiment set up

Changes in growth and germination of our three Eugenia study species was monitored in pots placed in growth chambers, using upland hammock soils from their current range in Florida and from their potential climatically-induced expanded range. Central Florida is the current northern limit of Eugenia species in Florida (Wunderlin and Hansen 2003), and so we chose a site with hammock habitats that was north and well outside of their current range, as predicted by the poleward expansion of species (Parmesan and Yohe 2003). Future temperature conditions of the northern site were estimated with a Low, B1 emission scenario; for a range of SRES emissions scenarios, and using global climate projections from the Fourth IPCC Assessment (IPCC 2007; Girvetz et al. 2009). Pots were placed in growth chambers where diurnal variation in daylength and temperatures were simulated, with the high and low daily temperatures determined by the average daily maximum and minimum temperatures for the month of May in Jacksonville, FL (Florida Climate Center, Center for Ocean-Atmospheric Prediction Studies), the northernmost site from where soil was collected. The pots experienced environmental conditions simulated for current (2010) and future (2050) conditions, with 10 hours of light per day and 30/17 °C and 31/18 °C and day/night temperatures (Table 1a & b).

Seed Sampling

Seeds were haphazardly collected from populations located within Hugh Taylor Birch State Park, in south Florida, for the one nonnative and two native study species. Seeds were collected for each species at the peak of seed production for their species. Seeds for the native species were collected on December 17th, 2011, and seeds for the nonnative Eugenia species were collected on April 28th, 2012. The fruit covering from each seed was removed by hand and the seeds were surface sterilized in 5% bleach solution for fifteen minutes, and washed with de-ionized water, prior to planting.

Soil Sampling

Soil was collected from three hammock habitat sites within each of the central, south, and north Florida sites. Soil biota was collected in the form of fresh field-collected soil from one of two sources: the current home range [Central Florida (Cape Canaveral, FL), South Florida (Hugh Taylor Birch State Park)] or within the projected new range [North Florida (Timucuan Ecological and Historic Preserve, Florida)]. Soils were collected from all three Florida source regions within one week prior of the potting date, to ensure viability of the soil microbiota. In the south and central Florida sites, we collected soil from hammock habitats within natural areas which were at least 20 meters from Eugenia shrubs or seedlings. In the north Florida site, we collected soil from randomly placed transects (using random point generator feature of ArcMap, ESRI, Redlands, CA) within hammock habitats. Within each of these three sites, two, 10-meter transects were laid within hammock habitat, at least 5m away from roads. Every two meters, 10cm deep soil samples were collected and placed into a Ziploc bag. The two, 10 m transects were parallel and at least 10 m apart. Soil samples were combined within each site, sieved to 2 mm, and added to the pots within one week of collection (as in, Hawkes et al. 2011). We pooled soils within each site to provide a soil inoculum treatment representing all possible soil microbes in that site and the average density found within that site (Cahill et al. 2017), which is a common treatment used to understand the effect of soil microbes on plant germination and growth (Grman and Suding 2010; Lau and Lennon 2011; Farrer and Suding 2016), however this method can artificially decrease variation in plant-microbe interactions (Reinhart and Rinella 2016; Rinella and Reinhart 2017; Rinella and Reinhart 2018). While variation in plant-microbe interactions is decreased with pooling samples, this method of soil pooling is preferable when the objective is to understand if the average pathogen density found in each of two regions differentially effects plant growth (Cahill et al. 2017). The soil biota treatment is one of several treatments, where we evaluate plant-microbial interactions in relation to those treatments.

The soil biota treatment was fresh field-collected soil from each of the central, south, and north Florida sites. For the control treatment, we sterilized half of these field-collected soils from the current and new ranges. The sterile soil inoculum was autoclaved three times, and we mixed the soil in between autoclave events, to ensure sterilization of the soils. The soil biota and sterile control inocula comprised 5% of the mass of the pot, to ensure sufficient inoculation of the soil biota to the pot while also maintaining the same nutrient conditions and soil characteristics across all treatments (as in Reinhart and Callaway, 2004).

For each species, eight seeds were planted into a minimum of seven, sterile replicate pots (4 x 4 x 6") filled with sterile potting mix (MetroMix 366 sterile potting soil) and one of two soil inoculum treatments (sterile, nonsterile), two temperature treatments 2010 (e.g. ‘current’) and 2050 (e.g. ‘future’ temperature conditions at our northernmost site) and three site treatments (south, central, and north), for a total of 86 pots for the nonnative species and 105 pots each for the native species. Eight to nine replicate pots per treatment were made for the native species (Table 1a), and seven replicate pots were made per treatment for the nonnative species (Table 1b), as the native species have lower germination rates, relative to the nonnative Eugenia species (Stricker and Stiling 2013). The potting dates were the 27th of January, 2012, for the native species and the 9th of May, 2012, for the nonnative species, in accord with their fruiting phenology and when the seeds were collected. After germination, pots were kept in a growth chamber for the next 12 weeks, to assess growth. They were watered daily with equal amounts of water, approximately 15-20 ml. Pots were rotated daily within the growth chamber, to control for positional effects. Care was taken to ensure that the soil biota were not cross-contaminated between pots by using sterile techniques. Germination was monitored weekly until after the appearance of the first germinant, at which point monitoring occurred daily. Daily monitoring ceased after the pots were monitored daily for two weeks with no new germination. Two weeks after germination ceased for each species, we selected a maximum of four seedlings to remain, and removed all other seedlings from the pot, taking care not to disturb the soil. Twelve weeks following initiation of germination, the remaining plants were harvested for total above and below-ground biomass. Shoots were cut at ground level and oven-dried separately in paper bags at 60 °C for 2 days. The roots were carefully washed to remove soil particles and also oven-dried at 60 °C in paper bags. After drying, shoots and roots were weighed with a precision balance to determine dry weight.

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