Frozen green leaves as potential nutrient subsidies in North American mangrove ecosystems

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Abstract

Avicennia germinans, the black mangrove, has shifted its range limit northward, and increased in abundance, in the southeastern United States. In January 2018, a three-day freeze event caused substantial defoliation of green leaves in *A. germinans* near its northernmost range limit in St. Augustine, Florida. During their recovery, plants that lost their leaves grew similarly to plants fertilized with nitrogen, leading to the hypothesis that freeze-killed green leaves may have acted as a fertilizer. To assess the value of frozen-green litter as a nutrient subsidy, we performed an experiment in which *A. germinans* seedlings were grown in sand with green, frozen-green, senescent, and control leaf litter. We measured growth response in seedlings using the following criteria: total plant height, internode elongation, and plant biomass. All litter treatments stimulated growth in seedlings to a greater extent than that of controls. Seedlings treated with green or frozen-green leaves grew taller and had longer internodes, than those treated with senescent leaves. Biomass was greater in seedlings treated with green or frozen-green litter, than in the control treatments. Frozen and green leaves lost more mass in a leaching experiment than senescent leaves or controls. These results support the hypothesis that green leaves that freeze can act as a nutrient source within the ecosystem.

Highlights

- Seedlings of the mangrove species *Avicennia germinans* were grown with leaf litter from one of four treatments: frozen green, green, senescent leaves or control.
- Over six months, seedlings grown with frozen green and green leaf litter grew taller and had more biomass than seedlings grown with senescent leaf litter or controls.
- Frozen green leaves and non-frozen green leaves leached more mass than senescent leaves.

Introduction

Coastal ecosystems are among the most highly productive on Earth, with mangrove forests alone contributing an estimated 4.6 kg C ha^{-1} $year^{-1}$ and 1.9 kg C ha^{-1} $year^{-1}$ in gross and net primary productivity (Alongi et al. 2018). To account for such high productivity rates, coastal wetlands require consistently available nutrients, and thus, largely depend upon nutrient cycling efficiency. Within mangrove ecosystems, such efficacy is primarily achieved through mangrove leaf litter, which upon decomposition, yields large quantities of essential nutrients in the form of nitrogen, carbon, and phosphorus (Singh et al. 2005, Alongi et al. 2018).

In mangroves, decomposition of fallen leaves by soil microbiota yields important sources of dissolved inorganic nutrients (Sousa and Dangremond 2011). Leaf litter, which accounts for an estimated 70 percent of net annual mangrove productivity (NPP) (Kristensen et al. 2017), is the connecting vessel through which macronutrients stored in leaf biomass return to the soil below. Therefore, the source and quality of leaf litter is of considerable significance to carbon and nitrogen cycles within mangrove ecosystems and in neighboring marine and estuarine ecosystems. In these ecosystems, litter dynamics, and consequent nutrient cycling, have important consequences for growth in higher plants and phytoplankton (Ray et al. 2015). As mangroves expand into coastal saltmarshes in northern Florida, coastal food webs are potentially changing with the input of mangrove leaf litter.

Mangroves occur globally throughout tropical and subtropical coastlines, and trends in climate change will likely alter current species distributions. In North America, mangrove range limits have historically been restricted to subtropical coastlines, due to species sensitivity to cold (Stuart et al. 2006, Cavanaugh et al. 2014) such that freeze events are associated with mangrove dieback. The intensity and scale of freeze-induced mangrove dieback is dictated, in part, by minimum temperature and freeze duration (Cavanaugh et al. 2019). Lethal freeze events are defined as those causing mortality rates substantial enough to reduce mangrove populations at their range limits, whereas non-lethal freeze events are those which do not cause substantial mangrove dieback and do not significantly shape mangrove range limits. With climate change, freeze events are likely to decline in intensity and duration, allowing for regions adjacent to and beyond range limits to maintain mangrove populations (Cavanaugh et al. 2019). Thus, as northern regions in the southeast US warm, mangrove forest range limits are projected to shift poleward — potentially disrupting mangrove litter dynamics and affecting subtropical nutrient cycling.

Non-lethal freezing events, though infrequent, may play an integral role in coastal nutrient cycling, as green litter produced during such events should possess a higher nutritional quality than that of senescent leaves. The bulk of leaf litter annually produced in a mangrove ecosystem consists of senescent leaves, which fall naturally as a result of the tree’s life cycle. Senescent leaves in mangroves do not fall seasonally, but gradually throughout the year. During leaf senescence, nutritional content is reallocated back to the tree itself. Because of this, senescent leaves retain essential nutrients such as nitrogen, phosphorus, and potassium (Lin and Wang 2001) to a much lesser extent than do green leaves (litter from tropical storms and cyclones), or frozen-green leaves (litter resulting from freeze-events) (Fonte and Schowalter 2017). Thus, green leaves produced during a tropical storm or freeze should provide a surplus of nutrients compared to the senescent litter typically produced — potentially leading to nutrient-enrichment and elevated plant growth.

In January 2018, a three-day freeze event caused substantial defoliation of green leaves in a fertilization experiment of *A. germinans* near its northernmost range limit in St. Augustine, Florida (see Dangremond et al. 2020 for description of the experiment), except in N-fertilized plants. Trees that were defoliated experienced complete recovery within 1.5 years of the freeze, and their interim growth mirrored the growth of N-fertilized trees (Feller et al. 2023). Thus, we predicted that frozen green leaves can serve as a source of nutrients for mangrove growth. Here, we tested the hypothesis that *A. germinans* seedlings fertilized using either frozen-green or green leaves would experience higher growth rates than those fertilized with senescent leaves. We used a laboratory experiment with leaves collected from the field to assess seedling growth responses to four leaf litter treatments: green, frozen green, senescent and control.

Methods

Study system: *Avicennia germinans*, the black mangrove, is a neotropical halophyte. It occurs in Western Africa and along the Atlantic and Pacific coastlines of the Americas (Dodd and Afzal 2002). Though over 80 species of mangroves are found globally (Singh et al. 2022), *A. germinans*, along with *Rhizophora mangle* (the red mangrove) and *Laguncularia racemosa* (the white mangrove) comprise the only three species native to the United States — likely due to restricting factors such as aridity and dispersal limitation (Bardou et al. 2020). *Avicennia germinans* is considered the most freeze tolerant of the three naturally occurring North American species (Cook-Patton et al. 2015) and is the most abundant mangrove species in northern Florida. Due to its freeze tolerance, *A. germinans* is also the most likely of the three North American species to migrate northward past currently defined range margins.

On the Atlantic coast of North America, northernmost mangrove range limits bewteen St. Augustine and Jacksonville, Florida, at roughly 30°N (Williams et al. 2014, Cavanaugh et al. 2015, personal observation). Along the Florida coastline, winters are typically mild and experience average temperatures ranging from 8.2 to 9.5°C (Stuart et al. 2016). However, occasional frosts as severe as − 10°C occur in Florida, taking place on average once every eight years over the past century (Stuart et al. 2016). Climate change has been associated with the poleward expansion of mangroves into temperate regions, by virtue of fewer severe freezes in recent decades. Though historically mangrove dominance into these regions has fluctuated, climate shifts may induce a persistent state of mangrove dominance (Cavanaugh et al. 2019).

Experimental Design: *Avicennia germinans* propagules were collected from Ft. George (30.4194° N, 81.4389° W) and Amelia Island, Florida (30.6266° N, 81.4609° W), near where *A. germinans* reaches its northernmost range limit, in December 2020. Propagules traveled in plastic ziplock bags to the laboratory at Roosevelt University, Schaumburg, Illinois, via postal mail. Propagules were soaked in water for a minimum of 7 days or until their cotyledons opened, and then were placed into their growing medium.

Sand was used as a growing medium so that media-based nutrients would be minimal. Propagules potted directly into the sand and assigned experimental treatments (January 2021 - July 2021). Once potted into their growing medium, plants were watered using deionized water once a week. The light source was fluorescent grow lights on 12-hour timers. Seedlings were assigned to one of four litter treatments: green, frozen-green, senescent and control, with 12 replicates in each litter treatment for the experiment. Each seedling received 2g of shredded dried leaves as its litter treatment. Controls received 2g of shredded brown packing paper.

Litter treatments came from *A. germinans* leaves collected at the same sites as propagules, near the northernmost range limit of North American mangroves (Cavanaugh et al. 2019). Green and senescent leaves were collected in December 2020. The frozen leaves spent three days at -4ºC in a laboratory freezer.

Mangrove growth was assessed in seedlings, according to the following three criteria: (1) Total plant height, (2) Elongation of the first 5 internodes, and (3) Dried plant biomass. A leaching experiment was performed to track biomass loss from leaf litter. Seedlings were potted in January, and measurements were taken after hypocotyl elongation, from March through July 2021.

Plant height and internode elongation: Plant height and elongation rate of the first internode were measured weekly. Plant height was measured from the edge of the pot to the apical-most leaf pair. Before seedlings developed true leaves, height was measured to the cotyledons. Internode elongation was measured as the distance between the topmost leaf pairs.

Biomass: Following the completion of all experiments, plants were harvested, and roots and shoots were separated. Roots and shoots were placed into individual paper bags and oven-dried at 60 C° for three days. At the end of the three-day period, dried roots and shoots were separately weighed and their masses recorded, though total biomass is reported in the results section. Cotyledons were excluded from the biomass.

Leaching Experiment: A leaching experiment was conducted to compare mass loss over time among the different types of litter. Approximately 2g of each leaf type were added to glass beakers and treated with DI water over the course of a six-week period. Each treatment had four replicates. Mass (g) of the leaf material were recorded twice — once at the beginning of the experiment and once following the six-week period. Mass loss was calculated by subtracting the final mass from the initial mass.

Statistical Analysis: In the seedling growth experiment, the independent variables were litter types. Dependent variables were height, internode elongation and biomass. One-way analysis of variance (ANOVA) was used to compare differences in growth metrics across litter treatments. When an ANOVA found significant effects, Tukey's HSD test was used to compare treatment groups. In the leaching experiment, the mass lost through the leaching experiment was analyzed with a one-way ANOVA. ANOVA assumptions were tested with a Bartlett test.

Results

Plant height

Seedlings grew significantly taller in the frozen-green (mean height ± SE was 11.2 cm ± 1.3) and green litter treatments (14.0 cm ± 1.14) than in senescent (6 cm ± 0.9) or controls (1.55 cm ± 0.4) (one-way ANOVA, F3,43 = 37.47, $p < 0.001$, Fig. 1).

Internode elongation

Internode length varied by treatment type (ANOVA, F3,43 = 9.53, $p < 0.001$). The mean internode length for seedlings in the frozen green treatment was 0.71 cm ± 0.06, which was significantly greater than seedlings grown in the senescent (mean internode = 0.38 cm ± 0.06) and control treatments (0.23 cm ± 0.06), but not the green leaf treatment (0.50 cm ± 0.06) (Fig. 2).

Biomass:

There was a significant effect of treatment on seedling biomass (ANOVA, $F_{3,43} = 21.31$, $p < 0.001$). Seedling biomass was significantly greater in the green (1.02 g ± 0.06) and frozen green (0.88 g ± 0.09) treatments than controls (0.35 g ± 0.05). The green litter treatment resulted in seedlings with greater mean biomass than the senescent (0.66 g ± 0.05) treatment, but biomass in the frozen and senescent treatments was not significantly different (Fig. 3).

Leaching Experiment:

Over the course of six weeks, frozen leaves lost 15.2% ± 0.69 of their mass, which was statistically different than the percentage lost from senescent leaves (6.99% ± 0.35) and controls (2.63% ± 1.30), but not different than green leaves (11.1% ± 1.52) (ANOVA, $F_{3,12} = 25.4$, $p < 0.001$; Fig. 4).

Discussion

We predicted that frozen green leaves would act as a fertilizer for *A. germinans* seedlings. We found support for this hypothesis from height and biomass data, as seedlings growing with frozen green and non-frozen green leaves grew taller, had longer internodes and had higher biomass than controls. In the leaching experiment, frozen green leaves and non-frozen green leaves lost more biomass than senescent leaves or controls.

Our results showed growth variation between litter treatment types over the course of this experiment, and most notably, results indicated that green and frozen-green leaf litter stimulated growth in sand-grown seedlings to a higher extent than that of senescent or control treatments. All seedlings were grown in a lab, using an artificial growing medium (sand). Using sand, a nutrient deprived soil medium, in addition to only distilled water in a laboratory setting helped to distinguish effects of litter type. However, this was not representative of soil microbes in a natural mangrove ecosystem. Understanding differences between decomposition rates in leaf litter types and the effects of microbes could be a direction for future studies.

This study shows support for the idea that green leaf litter, like that falling after a disturbance, can aid plant growth. However, growth responses seen in the 1.5 years after the 2018 freeze (Feller et al. 2023) could also be due to other sources of nutrients that increased after the freeze. Kominoski and others (2020) found that TN and TP increased following cold events, likely because freeze-induced mortality of plants or animals causes increased detritus. The extent of freeze damage to other parts of the coastal landscape in Jaunary 2018 are not well described, and but it is possible that other nutrient sources entered the ecosystem after the freeze.

Our results agree with other studies that show that not all leaf litter is nutritionally equal. Various studies have demonstrated that green leaves have higher nutrient content, particularly nitrogen, than senescent leaves (Lin and Wang 2001, Feller et al. 2003). Frozen leaves, in particular, have been found to be richer in nitrogen and carbon with lower C:N ratios than other litter sources (Ellis et al. 2006). Although green leaves removed from trees during a tropical storm retain a higher amount of nutrient content than do senescent leaves, often they are dispersed due to high winds. We believe that leaves that fall after a freeze are more likely to drop close to the tree they came from and their nutrients would therefore stay within the ecosystem.

Freezing may act to break up the leaf structure in a way that aids decomposition. Taylor and Parkinson (1988) found that the amount of mass leached doubled after repeated freeze and thaw cycles, compared to litter frozen only once. In this experiment, leaves were frozen only once. However, in a real-world freeze such as the one that occurred in St. Augustine, Florida, in January 2018, ambient temperatures often drop below freezing, then rise during the day and then drop below freezing again at night, thus exposing leaves to multiple freezes and thaws.

Few studies have addressed the impacts of climate change on mangrove nutrient cycling, and even fewer, the implications of freeze event-declination, as it pertains to projected mangrove encroachment.

Mangroves are expected to increase in abundance in regions formerly too cold for long-term survival (Cavanaugh et. al 2019). These regions, though warmer than typical conditions over the last century, will still likely encounter freeze events due to their northern geography compared to coastal regions historically associated with mangroves. These freezing events have the potential to cause great damage to mangroves by causing top kill and loss of leaves (Cavanaugh et al. 2019; Kaalstad et al. 2023). Our results are one line of evidence demonstrating the resilience of mangrove ecosystems to extreme cold temperatures, a knowledge gap identified by Osland and others (2021). We suggest that managers of coastal ecosystems consider the effects of disturbance when undertaking monitoring and management activities.

Declarations

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Figures

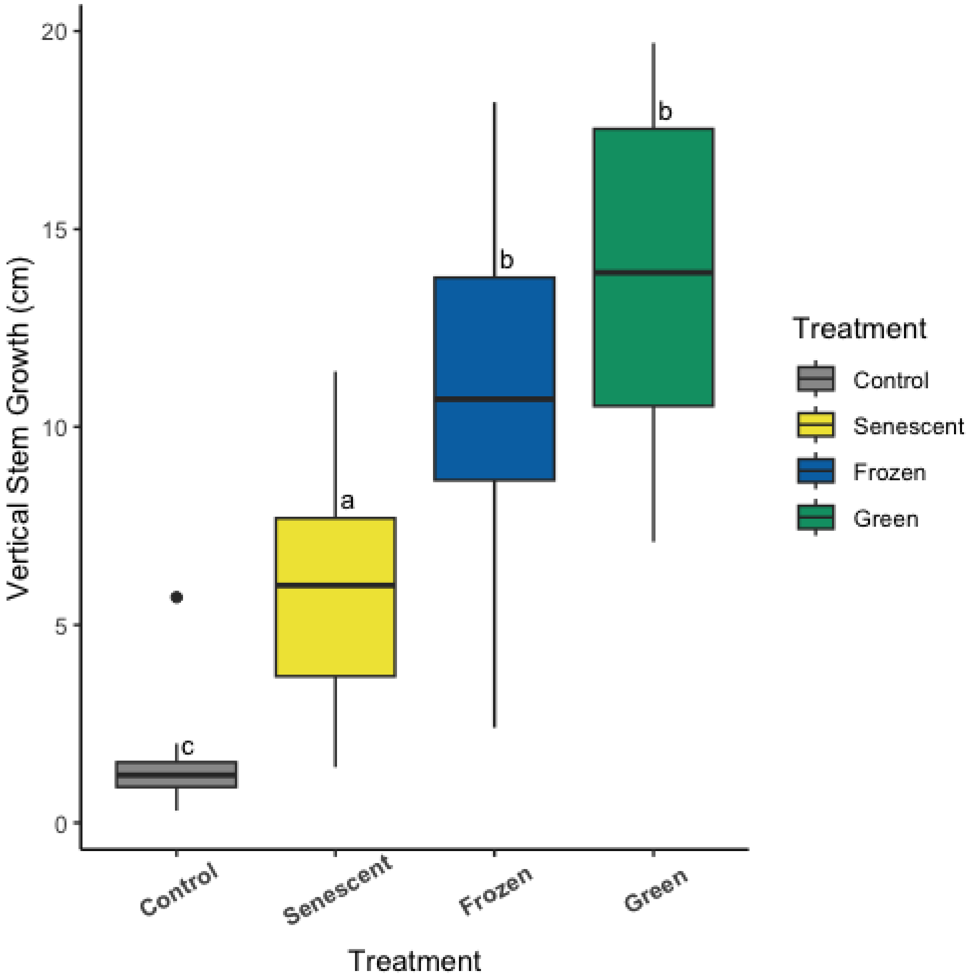

Figure 1

Vertical growth (height) of *Avicennia germinans* seedlings varied by leaf litter type. Treatments that share a letter are not statistically different from each other according to a Tukey post-hoc analysis.

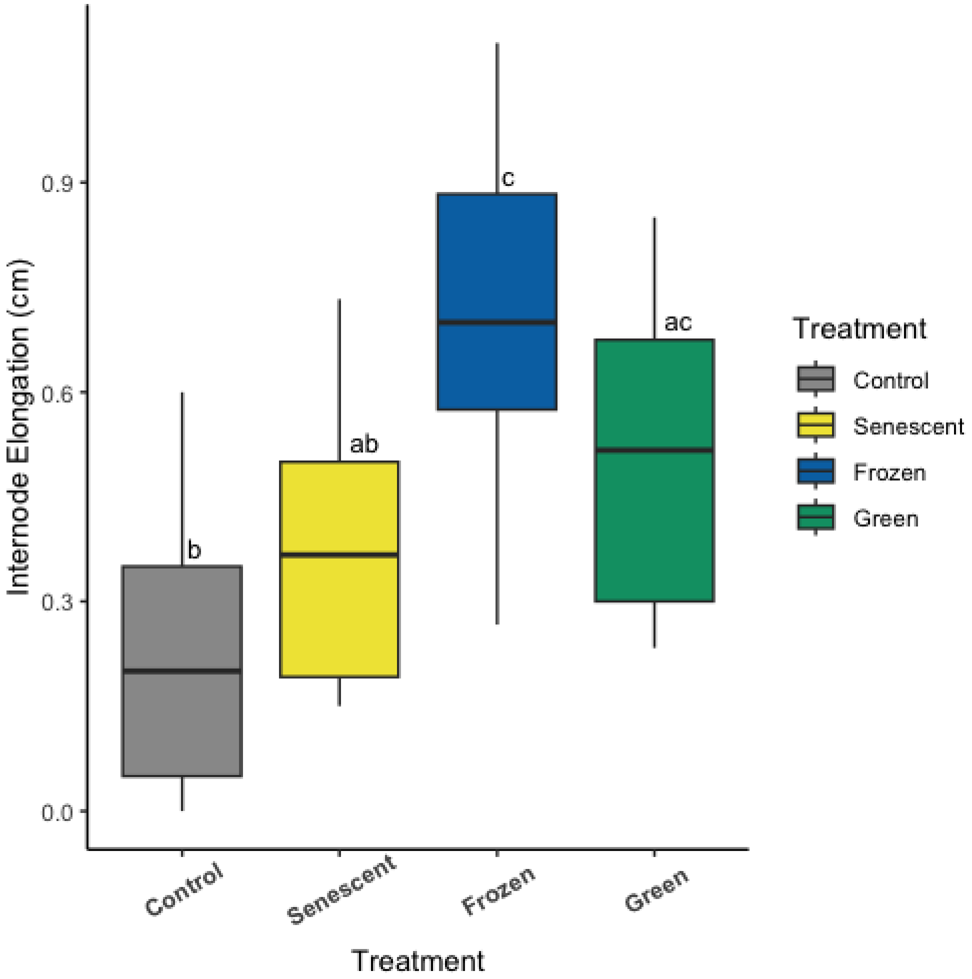

Figure 2

Internode elongation of *Avicennia germinans* seedlings varied by leaf litter type. Treatments that share a letter are not statistically different from each other according to a Tukey post-hoc analysis.

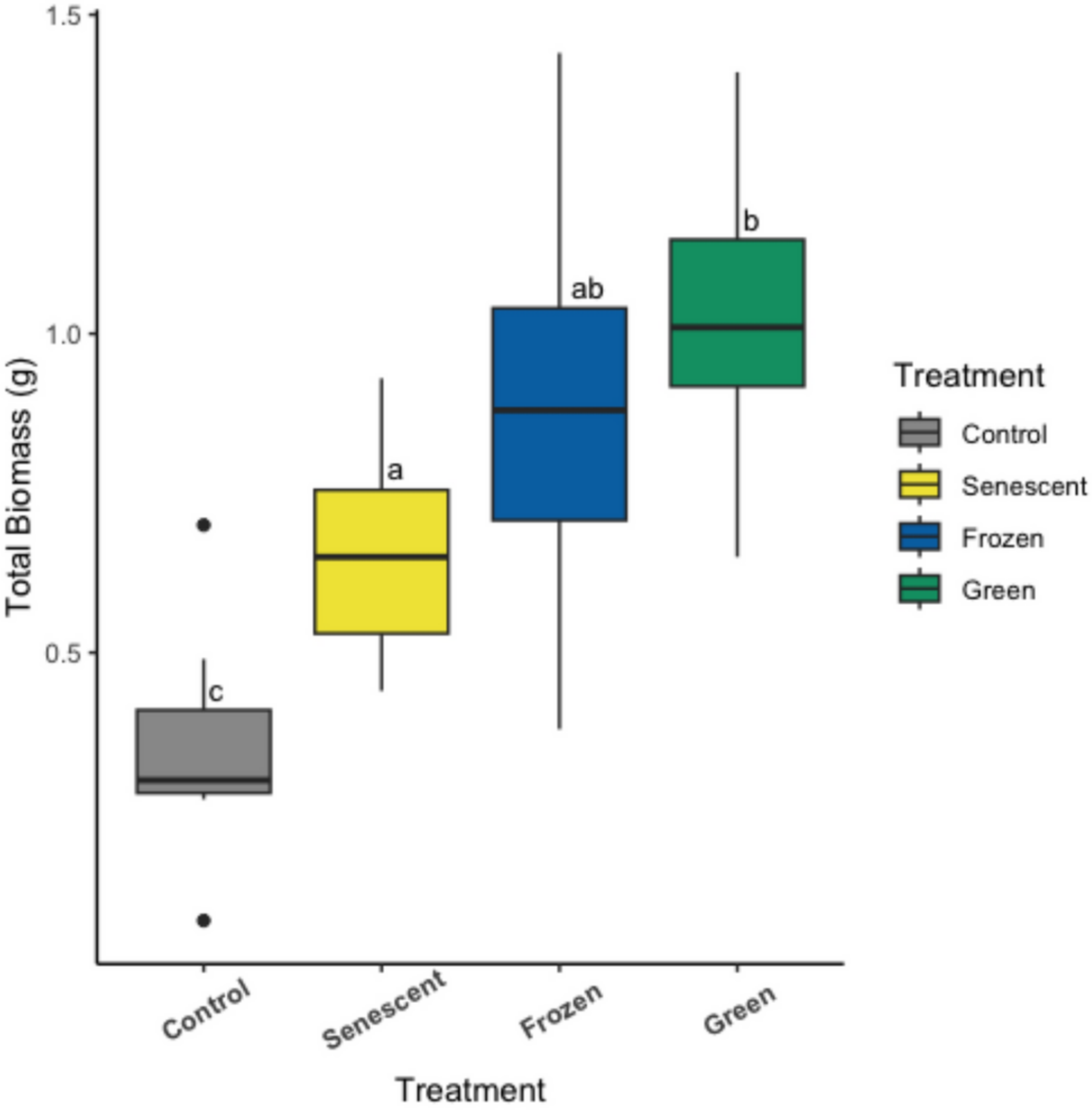

Figure 3

Biomass of *Avicennia germinans* seedlings varied by leaf litter type. Treatments that share a letter are not statistically different from each other according to a Tukey post-hoc analysis.

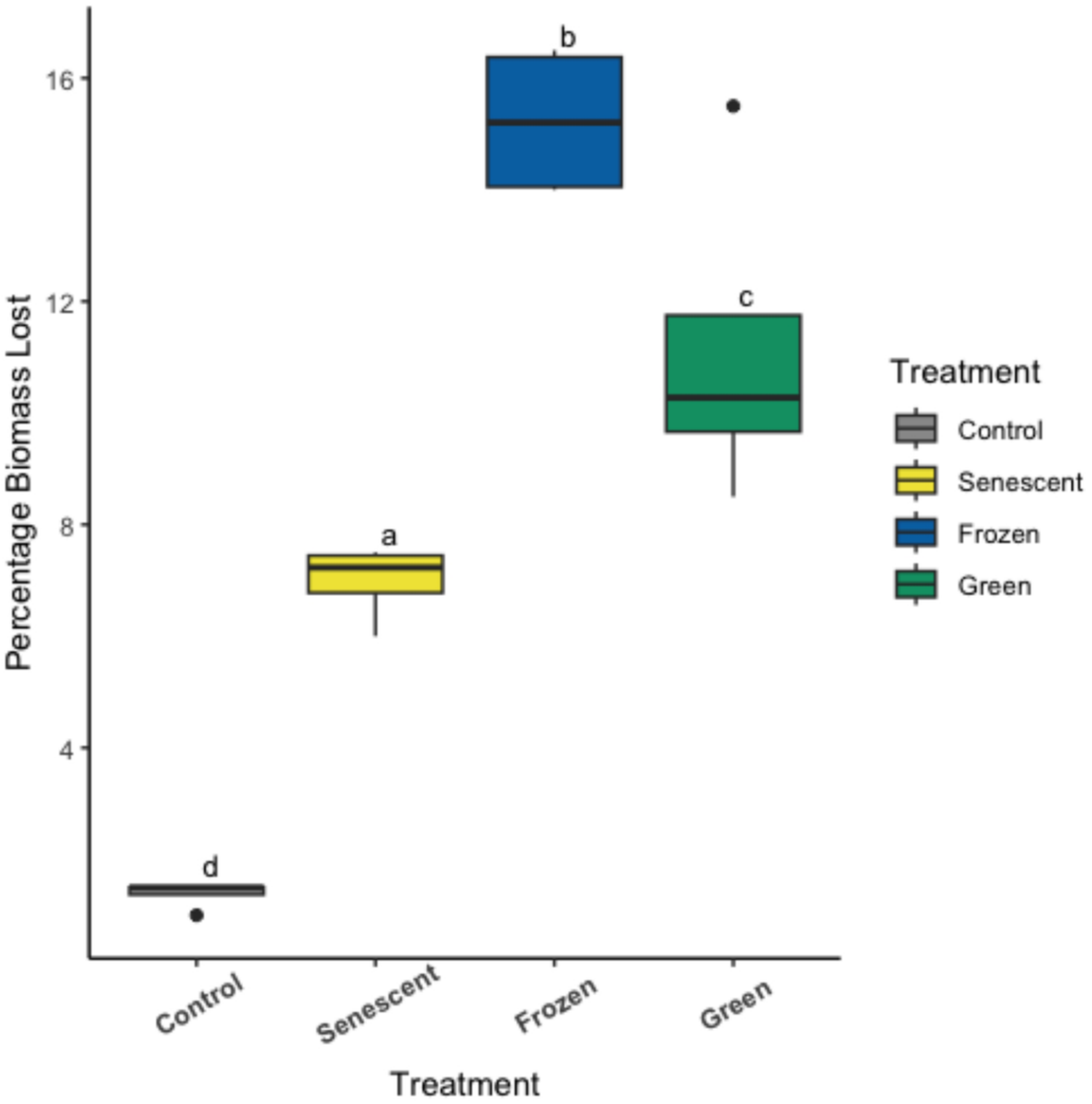

Figure 4

Total biomass loss from *Avicennia germinans* leaves by litter type in a leaching experiment. Treatments that share a letter are not statistically different from each other according to a Tukey post-hoc analysis.