Fire effects on growth of the invasive exotic fern *Lygodium microphyllum* and implications for management

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Abstract

The extremely invasive Old World Climbing Fern, *Lygodium microphyllum*, has invaded New World tropical and subtropical habitats. *Lygodium microphyllum* has indeterminate twining leaves that grow up on and shade out host shrubs and trees, their epiphytes, and the understory. This invasive plant threatens numerous native habitats in Florida USA and the Caribbean, including internationally valued conservation lands such as Everglades National Park. Fire, which can reduce or stimulate growth and/or reproduction in different plant species, is one intervention used to manage *L. microphyllum*, but the effects of burning on this species’ growth are unknown. We experimentally burned greenhouse-grown plants, then monitored their growth in response to burning for 18 months. We also clipped a subset of the greenhouse plants to determine whether fire effects were explained by aboveground biomass removal. In our experiment, fire either killed or reduced the regrowth of *L. microphyllum* plants. Burning killed 25% of the burned plants and slowed growth of the remaining burned plants for up to 18 months post-burn. Size had a significant effect on plant mortality: smaller burned plants had greater mortality than larger plants. Mechanically removing the aboveground biomass (leaves) by clipping did not kill any plants but reduced their growth as compared to controls in the first six months after treatment. Clipped plants, however, had substantially recovered after 12 months. Burned plants were more sensitive to drought than clipped or control plants. The results show that burning does more than remove biomass, compromising the root system and perhaps damaging the hydraulic support system, similar to the latent mortality effects of fire on forest trees. We discuss how fire could affect invasion by this species, incorporating fire effects on spore viability and dispersal, as well as on subsequent plant regrowth. These results show that fire is a useful management tool for *L. microphyllum* invasions that both kills plants and reduces plant regrowth for up to 18 months.

Key words: invasive plant management, fire-induced latent mortality, fire vs. mechanical control, non-native plants, post-fire mortality, spore dispersal by fire

Introduction

Invasive exotic plants alter the environments that they invade, and millions of dollars per year are spent in the United States attempting to halt and turn back invasions (Pimentel et al. 2000). The suite of tools available in this effort are physical, chemical, and biological interventions. Typical
physical means are mechanical control, such as mowing or pulling, and burning. Both of these tools are disturbances that have analogs in the natural environment, e.g., wind, water, or herbivore damage for mechanical control and natural fires for burning. Because the real-world presence of these disturbances may have selected for adaptations to them, the invasive plants being controlled might respond differently than anticipated to the control measure. This is especially true for fire. Plants respond to burning in diverse ways, depending on a species’ life history strategy in relation to fire, the type, intensity and duration of the burn, pre- and post-burn environmental factors such as season and hydrology, and fire frequency (Bond and van Wilgen 1996; Pausas et al. 2004; Bond and Keeley 2005). Fires increase light and nutrient availability in burned habitats, and some species respond through increased sexual reproduction post-burn (Bond and van Wilgen 1996; Bond and Keeley 2005). Some plants recover from fire by resprouting, while others recover through enhanced propagule establishment, both of which could exacerbate an exotic plant invasion (Pausas et al. 2004). Because of these possibilities, it is important to understand how fire affects a target species when using it as a control measure.

Old World climbing fern, *Lygodium microphyllum* (Cav.) R.Br., is an invasive non-native plant that colonizes wetlands and forests in the US southeastern bioregion (Stocker and Hupp 2008; Stocker et al. 2008), including internationally valued conservation lands such as Everglades National Park. Native to the wet tropics and subtropics of Africa, Asia and Oceania (including Australia), the species was introduced to Florida in the late 1950s and has spread rapidly across the state by wind-dispersed spores (Nauman and Austin 1978; Pemberton and Ferriter 1998; Hutchinson et al. 2006; Ferriter and Pernas 2006; Langeland and Hutchinson 2010; Philipp and Richards 2007; Volin et al. 2004).

*Lygodium microphyllum* has a horizontally growing, stoloniferous stem that branches dichotomously and, as an adult, produces vertically growing, tripininate compound leaves (Mueller 1982). The compound leaves twine and have indeterminate growth. These twining leaves climb other plants, forming thick canopies that shade out host and understory plants, out-competing native species. In addition, leaf rachises persist, producing thick mats that build up fire fuel (Ferriter 2001; O’Brien et al. 2010; Stocker et al. 2008). The species has the potential to invade a much wider area of the New World than its current extent. Using the CLIMEX model to predict potential habitat for *L. microphyllum* based on its native habitat, Goolsby (2004) found that, in addition to southern Florida, central Florida and large areas in the Caribbean and Central and South America provide potentially suitable habitat for this invasive species.

One way that this species threatens native ecosystems is through altering fire behavior. *Lygodium microphyllum* can serve as a ladder fuel, conveying ground fire into the host canopy and causing intense crown fires, increasing
fire damage to native vegetation (Ferriter 2001; Hutchinson et al. 2006; O’Brien et al. 2010). It can also produce flaming brands that carry fire across areas such as wetlands that are normally barriers to spread (Stocker et al. 2008; O’Brien et al. 2010). Despite the threat that this species poses through its interaction with fire, little is known about its responses to burning. In their review of gaps in our scientific knowledge about fire and non-native invasive species, Zouhar et al. (2008) found no information or information made on assertion alone for the invasive *Lygodium* species. We lack documentation of *L. microphyllum*’s post-fire vegetative response, as well as information on its response to aspects of the fire regime (Zouhar et al. 2008), although Stocker et al. (2008) reported data on *L. microphyllum* regrowth after fire or fire-plus-herbicide treatment.

Since 2000, prescribed fire with a planned 2- to 3-year return interval (J. Taylor, ENP, *personal observation*) has been used in Everglades National Park (ENP) as a treatment to manage *L. microphyllum* invasion. Fire is effective in reducing *L. microphyllum* aboveground biomass, which consists primarily of leaves (Stocker et al. 2008), but does not completely destroy the rhizomes and roots. Thus, plants can resprout after fire (Goolsby et al. 2003; Hutchinson et al. 2006; Stocker et al. 2008), and fires create disturbed patches that may be conducive to sporeling establishment (Call et al. 2007; Lynch et al. 2009, 2011). Additionally, because *L. microphyllum* reproduces sexually from wind-dispersed spores and can produce up to 28,600 spores per fertile pinna (Volin et al. 2004), fire may enhance spore dispersal if spores are released into fire-induced rising air currents (Stocker and Hupp 2008, O’Brien et al. 2010). We do not have quantitative data for *L. microphyllum* with respect to these fire responses.

Because fire is used to manage spread of *L. microphyllum* but there is no data on whether fire suppresses or enhances growth and spread of this species, we studied the effects of fire on *L. microphyllum* growth in a greenhouse experiment. The purpose of this study was to determine the effects of burning on growth of individual *L. microphyllum* plants. In addition to burning, we included a mechanical treatment where biomass was removed down to the soil surface, to determine whether effects of fire could be attributed to removal of aboveground biomass alone. We hypothesized that burning and clipping would have similar effects and that the re-growth of these two treatments would be reduced as compared to untreated control plants.

**Materials and methods**

*Plant Material*

**Source populations and growing conditions:** Plants of *Lygodium microphyllum* used in this study were collected from two invasive populations: the Florida Turnpike population and the Mowry Canal population. Plants at
the Florida Turnpike site were collected in the vicinity of 25°57'41.80"N; −80°20'13.52"W on Nov. 6, 2013. These plants were grown in the FIU greenhouse until repotting in Feb. 2014. Mowry Canal plants were collected in the vicinity of 25°28'18.74"N; −80°21'10.95"W on Feb. 16, 2014. Plants from both populations were planted in compost (EPS Organics, Hialeah Gardens, FL) in 25 × 23 cm (d × h), round, pressed-cellulose, pulp pots (Western Pulp Products Co., Corvallis, OR) between Feb. 20, 2014 and Mar. 2, 2014 with a total of 160 plants potted. Nutrients in the compost were analyzed in the FIU Freshwater Biogeochemistry Lab (Dr. Len Scinto, FIU, Miami, FL) for total C, N, and P for three samples randomly collected from the pots. Total C was 124 ± 21 mg/g, total N was 3.15 ± 0.56 mg/g, and total P was 489 ± 46 μg/g; N:P was 6.43 ± 0.80, indicating nitrogen rather than phosphorus limitation. Plants were not fertilized during the experiment.

**Growing Conditions**

Plants were grown in a limited-access greenhouse built inside of the FIU Modesto Maidique Campus Biological Sciences greenhouse in order to isolate this invasive species from the surrounding environment. A 25% shade cloth was installed during the summer wet season and removed during the winter dry season. Pots were set on the gravel-covered floor of the greenhouse. Each pot had a twine string for leaves to climb. Plants had from 2 to 2.5 m of twine to climb, depending on their position in the greenhouse. Plants were visited at one- to three-day intervals and elongating leaves were trained back onto the parent plant, so that plants remained isolated from each other. As leaves overgrew their twine, they were looped around so that they continued to climb on the parent plant. An automatic drip irrigation system (DIG Corporation, Vista, CA) with each pot individually plumbed was used to water plants, with each plant receiving approximately 1 L of water twice per day. This watering system failed in January 2015 for an unknown period, but plants did not visibly wilt during this failure. Subsequently, however, some leaflets, especially on large plants, browned and abscised. This artificial drought occurred approximately five months post-treatment, and the dry conditions resulted in additional mortality in both treated and control plants.

**Pre-treatment growth**

Growth of plants was monitored beginning Apr. 3, 2014, when all plants had initiated leaves. Many of these leaves were determinate; the number of indeterminate twining leaves per plant averaged 1 (range = 0–5) at this initial measurement, and 38% of plants lacked twining leaves. Height of twining leaves on Apr. 3 averaged 30 ± 13 cm. Twining leaf height and number of twining leaves was measured every 2 weeks for 3.5 months, then monthly thereafter.
**Treatments**

In order to compare the growth of burned and unburned plants and to determine how burning compared to mechanical biomass removal, plants were divided into three groups: control, clipped, and burned. Each group had 48 plants. Before assigning plants to experimental groups, 12 plants were randomly chosen from plants in the greenhouse for a pre-treatment harvest. Control and clipped plants remained in the greenhouse. Clipped plants had all leaves clipped to the soil surface between Sept. 17–20, 2014. Burned plants were transported to Everglades National Park on Sept. 3, 2014, burned under controlled conditions, and returned to the greenhouse on the same day. Plants were burned in three blocks with 16 plants per block.

Plants were assigned to a treatment group depending on plant size, as determined by a size index derived from the pre-treatment growth measurements. The size index, which was developed to incorporate both leaf number and leaf height into the estimates of size, was calculated as 

\[(\ln(\text{no. twining leaves > 0.5 m} + 1.1) \times \text{height of longest twining leaf}).\]

Plants were ordered by their size index, assigned consecutively to groups of three, and treatments randomly assigned within each group of three. Analysis of variance comparing plant size by treatment indicated that plants did not differ in size among treatments. The size index was again used to randomly assign plants to harvests within treatments, and ANOVA showed that within each treatment, plants did not differ among harvests by pre-treatment size. For plants in the burn treatment, the size index was used to distribute plants and harvests among one of three burn blocks, so that blocks were balanced for size of plants and for harvest time within each block. ANOVA indicated that these criteria were met.

**Burn conditions**

Burning occurred approximately six months after plants had been planted in the pulp pots. Plants were burned in 122 × 244 × 122 cm (w, l, h) plywood burn boxes. Boxes had a plywood base that sat 30 cm off the ground and had 16 holes in which the pulp pots were inserted, so that the pots sat on the ground, but the fire fuel was supported by the plywood base at approximately the plant/soil surface. The narrow ends of the burn boxes were open, while the longer sides had 91 cm vertical walls to contain and channel the fire. A thin layer of sand was put over the plywood base in order to prevent the plywood from burning. In order to mimic the natural southern Florida fire environment, dried, dead leaves of sawgrass (*Cladium jamaicense*) were used as fuel. These leaves were collected at the end of the southern Florida dry season in May 2014 and stored inside in plastic bags until the burn date. Plants were burned in three blocks. Each burn block had 4.0 kg of dried leaves spread over the burn box base after the plants were set in the boxes. Fuel depth averaged 11 ± 3 cm across the surface.
Mortality post-burn did not differ among burn blocks and the size index for living plants post-harvest at 107 days did not differ significantly among blocks (data not shown), so statistical analyses did not use the blocking factor.

Plants were transported to the ENP Fire Cache on Sept. 3, 2014, where they were burned outdoors between 10 AM and 1 PM. Soil moisture was measured on plants before burning using a Decagon Devices ECH20 EC-5 moisture sensor (SPW Industrial, Laguna Hills, CA). Soil moisture was 28.6 ± 4.1% (range = 19.4–34.3%).

Fire intensity was monitored with heat sensitive paints. Each pot had two copper tags that were painted with strips of Tempilaq (LA-CO Industries, Inc., Elk Grove Village, IL) temperature sensitive paints. Paints covered 200 to 1550 °C in 50 °C intervals, exclusive of 950 and 1150 °C. Both tags were painted with 10 strips of Tempilaq paints covering the temperature range, but paint strips on the two tags were offset from each other by 50 °C. The melting of a given Tempilaq paint strip indicated temperatures had reached at least that temperature, so the burn temperature could be determined within 50 °C.

Local weather at the time of the burn was recorded by a portable RAW (Remote Automated Weather) quick deploy station that was set up within 50 m of the burn boxes. The RAW station recorded wind speed and direction, air temperature and relative humidity, and solar radiation. Between 10:47 and 12:34 (the times that the second and third blocks were burned), the wind averaged 11.3 ± 3.4 kph from the east, the temperature was 28.4 ± 2.1 °C, the relative humidity was 73.2 ± 8.7%, and the solar radiation averaged 493.5 ± 425.1 W/m². There was light precipitation between the second and third burns.

Post-treatment growth

Burned and clipped plants were monitored weekly for resprouting after treatment. All plants were measured monthly beginning Oct. 16, 2014, which was 43 days after burning and between 26 to 29 days after clipping. We recorded the number of leaves > 0.5 m in height by counting rachis numbers at 0.5 m, the height of the tallest twining leaf, and whether sporangia were present. When leaves exceeded 2 m, this measurement became less accurate, as we were measuring the lengths of leaves that we had coiled back on themselves in order to keep plants separate in the greenhouse.

Plant Harvests

The pre-treatment harvest (H0) occurred between Sept. 9 and 13, 2014. Plants were subsequently harvested at six-month intervals post-burn (February 2014 (H1), September 2015 (H2), and March 2016 (H3)). Aboveground leaves and belowground biomass were harvested separately. The leaf harvest took from two to three weeks. The belowground biomass
harvest took from three to eight weeks. Aboveground twining leaves were removed from each plant in the greenhouse by trimming them at the soil surface, then they were transported to the lab. In the lab, the length of the longest leaf was measured in cm and the number of individual rachises was counted at 0.5 m intervals along the length of the leaf mass. Leaves were dried in an oven at 60 °C to constant weight and weighed on an Adventurer OHAUS balance (OHAUS Corp., Parsippany, NJ) with precision of 0.000 grams. Material with a mass less than 1 gram was weighed on a METTLER AE240 balance (Mettler-Toledo, Hialeah, FL) with a precision of 0.00000 grams.

Harvested belowground biomass included buried petioles and rachises, rhizomes and roots, which were separated during the harvest. These different plant parts were dried and weighed as described for leaves. Roots were harvested either by clipping roots from rhizomes or by separating roots from soil and organic debris through repeated washing of harvested material through sieves, re-suspending material in fresh water, and separating clean roots from debris. For very large plants in the final harvest, roots were removed and cleaned until only a mixture of small root pieces and debris remained. The mass of roots in this root/debris mixture was estimated by drying the entire mixture, weighing, then subsampling three representative portions, separating into roots and debris and weighing to calculate the proportion of roots per subsample. This proportion was used to estimate the root weight for the entire mixture. This number was added to the weight of the individually harvested roots to determine the entire root mass.

Additional data were taken on harvested, clean rhizomes before they were dried. Live apices on the rhizomes were counted under a dissecting microscope. Live apices were defined as terminal portions of the rhizome and rhizome branches that had healthy green color and firm turgor. The length of three internodes on recently produced sections of the rhizome were measured with a ruler and recorded. Rhizomes were then placed in labeled paper bags, dried, and weighed as described above.

Statistical Analysis

Morphological measurements were analyzed with Kruskal-Wallis (KW) non-parametric tests. Differences in mortality over time were analyzed with a Cox proportional hazard model. Effects of burning and clipping on plant growth were analyzed with generalized linear mixed effects models with repeated measures. Fixed effects were treatment and days, while random effects were days within plants. A first-order, autoregressive correlation structure was used. Significance of difference in slopes was tested with ANOVA using model simplification, looking initially at regression models with interactions included and comparing those to models with interactions excluded. Differences in biomass among treatments were compared with ANCOVA, using the size index at time of treatment as the random covariate. Post-hoc comparisons among treatments
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Analyses and data visualization were done in the R statistical environment (R Core Team 2019). In addition to the base package, we used packages ggplot2 (Wickham 2016), reshape2 (Wickham 2007), lattice (Sarkar 2008), plyr (Wickham 2011), survival (Therneau 2015), PMCMRplus (Pohlert 2018), nlme (Pinheiro et al. 2018), and agricolae (de Mendiburu 2019).

**Results**

**Fire Temperatures**

Melted indicator paint strips gave temperatures reached at the soil surface for each pot. These temperatures ranged from 200 °C to 600 °C. The average temperature was 439 ± 111 °C, and the median was 450 °C. The melted maximum paint strips on the two tags in each pot agreed closely with each other in 40% of the pots.

**Plant growth**

**Early post-burn growth and mortality:** Twenty-five percent of the burned plants never resprouted following burning. Some burned plants (60%) had begun to put out new leaves at 14 days post-burn. Seventy-three percent of burned plants had begun to expand new leaves at 23 days post-burn. Plants that were relatively small at the time of burning had greater mortality than larger plants. The pre-burn size index for dead plants was significantly less than that for plants that did resprout (mean ± SD = 213 ± 113 dead vs. 387 ± 118 living; *p* < 0.01, KW test). Plants that died had an average of 3 ± 2 leaves > 0.5 m and were 154 ± 45 cm tall pre-burn. Plants that lived averaged 6 ± 3 leaves > 0.5 m and were 205 ± 26 cm tall. Soil moisture content was not associated with plant mortality (*p* = 0.48, KW test).

Clipped plants all resprouted by 14 days post-clipping. When the number of leaves at 14 days was compared between clipped and burned plants, clipped plants had produced significantly more leaves, even when the plants that did not resprout post-burning were removed from the comparison (mean ± SD = 3 ± 2 burned vs. 9 ± 8 clipped; *p* < 0.01, KW test).

By the end of the experiment, 50.0% of burned plants, 22.9% of clipped plants, and 6.3% of control plants had died (Figure 1). Control and clipped plant death occurred between 77 and 223 days, after the watering system failure, and mortality was greater and occurred earlier in the clipped than the control plants. Burned plants, however, were dying before the drought event and continued to die up through day 400 (Figure 1). Survival was significantly different among burned, clipped and control plants (χ² = 29.2, *df* = 2, *p* < 0.01, log-rank test), and all groups differed significantly in pair-wise comparisons (*p* < 0.02 for all comparisons). Both treatment and original plant
size, but not their interaction, affected survival (Cox model comparison \( \chi^2 = 0.92, df = 2, p = 0.63 \)). Similar results were obtained when analysis excluded the initial mortality in the burned plants.

**Monthly growth, post-burn, 2014–2016:** Growth of control, clipped, and burned plants differed (Figure 2). Control plants were larger than burned or clipped plants immediately post-treatment, and they continued to grow. Clipped plants added leaves at a rate comparable to control plants and produced taller leaves at a greater rate (Figure 2A, B), so that leaf height for control and clipped plants converged in the final two months of the experiment (Figure 2B). Burned plants did not add new leaves or increase in leaf height at the same rate as clipped plants (Figure 2A, B).

Differences in growth between control, clipped and burned plants are summarized by differences over time in their size indices (Figure 2C). A model for change in size index as a function of treatment and time that included interactions was significantly better than one without interactions \( (p < 0.01, \text{ANOVA}) \), indicating growth differences (differences in slopes) among treatments. The size index of clipped plants increased more rapidly, as these plants added new leaves and their leaves grew taller. The size index of burned plants also increased, but more slowly than that of clipped or control plants. Comparisons of the size index over time showed that control and burned plants had similar slopes \( (p = 0.07) \), whereas the size index slope for clipped plants differed from that of both control \( (p < 0.01) \) and burned \( (p = 0.02) \) plants.

Only two plants, both controls, produced sporangia during the experiment. In both cases, the sporangia were on only a small portion of a single twining leaf.
Figure 2. Growth of *Lygodium microphyllum* plants in the greenhouse after treatment; each point represents a single plant, with plants being re-measured monthly. Regression lines summarize trends, while points show the variation among treatments and controls: A. Number of twining leaves greater than 0.5 m in height; B. Height of the tallest twining leaf; C. Size index.
Figure 3. Total plant dry weight of harvested *L. microphyllum* burned, clipped and control plants immediately prior to treatment (H0) and at harvests at 6 (H1), 12 (H2) and 18 (H3) months post-treatment. burned (Bu), clipped (Cl) and control (Co) plants.

Table 1. Mean ± standard deviation for *L. microphyllum* morphological and biomass variables for the initial harvest (H0 Initial, pretreatment) and for the final harvest (H3) for burned (H3 Burn), clipped (H3 Clip) and control (H3 Control) plants.

| Variable                      | H0 Initial | H3 Burn | H3 Clip | H3 Control |
|-------------------------------|------------|---------|---------|------------|
| Leaf Height (cm)              | 226 ± 28   | 300 ± 179 | 242 ± 48 | 369 ± 179  |
| Number of Leaves > 0.5m       | 14 ± 7     | 34 ± 31  | 44 ± 32  | 64 ± 32    |
| Number of Shoot Apices        | 24 ± 17    | 83 ± 68  | 110 ± 75 | 92 ± 54    |
| Internode Length (mm)         | 22 ± 8     | 27 ± 11  | 30 ± 10  | 31 ± 6     |
| Size Index                    | 600 ± 167  | 1061 ± 958 | 876 ± 323 | 1521 ± 807 |
| Leaf Dry Mass (g)             | 12.5 ± 8.4 | 44.4 ± 62.6 | 106.2 ± 78.1 | 131.2 ± 80.2 |
| Rhizome Dry Mass (g)          | 1.8 ± 1.3  | 5.0 ± 7.9 | 13.3 ± 10.5 | 15.0 ± 9.6 |
| Root Dry Mass (g)             | 3.6 ± 3.2  | 5.8 ± 10  | 26.2 ± 24.8 | 28.7 ± 23.3 |
| Plant Dry Mass (g)            | 17.8 ± 12.5 | 55.1 ± 80.4 | 145.6 ± 109.8 | 174.9 ± 110.2 |

Sexual reproduction was not found on any of the other control, clipped, or burned plants.

**Plant biomass**

Total plant biomass changed over time and with treatment (Figure 3). Control plants increased biomass throughout the experiment (Figure 3, Table 1). Initially, burning and clipping severely reduced total plant biomass. Both treatments had significantly less biomass than control plants at the six months post-treatment harvest (H1) but did not differ from each other (Figure 3, Table 2). Total biomass of one-year post-treatment (H2) plants differed significantly between burned and control plants, but total biomass of clipped plants did not differ from either (Figure 3, Table 2). The large variation and presence of an extreme outlier in the burned plants resulted in only marginally significant differences among treatments for...
Table 2. Results of Kruskal-Wallis (KW) tests and post-hoc comparisons among *L. microphyllum* variables for each harvest (H1, H2, H3). For the overall KW comparison, values in white are not significant; those in orange are significant at \( p \leq 0.05 \). Within a harvest, variables with the same letter are not significantly different at \( p \leq 0.05 \). cont = control.

|                      | H1     | H2     | H3     |
|----------------------|--------|--------|--------|
| **Leaf Height (cm)** | \(< 0.01\) | a a b  | \(< 0.01\) | a a b  | 0.28 a a a  |
| **Number of Leaves > 0.5m** | \(< 0.01\) | a a b  | 0.01 a ab b | 0.08 a a a  |
| **Number of Shoot Apices** | \(< 0.01\) | a ab b | \(< 0.01\) | a ab b  | 0.8 a a a  |
| **Internode Length (mm)** | \(< 0.01\) | a ab b | 0.1 a a a | 0.81 a a a  |
| **Size Index**       | \(< 0.01\) | a a b  | \(< 0.01\) | a a b  | 0.04 a a a  |
| **Leaf Dry Mass (g)** | \(< 0.01\) | a a b  | 0.08 a a b | 0.07 a a a  |
| **Rhzome Dry Mass (g)** | 0.08 a a a | a a b  | 0.01 a ab b | 0.1 a a a  |
| **Root Dry Mass (g)** | 0.01 a a b | \(< 0.01\) | a ab b  | 0.06 a a a  |
| **Plant Dry Mass (g)** | \(< 0.01\) | a a b  | \(< 0.01\) | a ab b  | 0.06 a ab b  |

Table 3. Regression statistics for regressions of *L. microphyllum* plant total dry mass vs. original size index (Tot DM ~ Orig SI), root dry mass vs. original size index (Rt DM ~ Orig SI) and root dry mass vs. leaf dry mass (Rt DM ~ Lf DM) for burned, clipped and control plants. Superscripts indicate differences among treatments in post-hoc Scheffe tests; treatments with the same superscript are not significantly different.

|                      | Slope Intercept | \( R^2 \) | F     | p    |
|----------------------|-----------------|----------|-------|------|
| **Tot DM ~ Orig SI:** |                 |          |       |      |
| Burned               | 0.015           | 19.038   | 0.002 | 0.04 | 0.853 |
| Clipped              | 0.223           | −7.718   | 0.099 | 3.73 | 0.062 |
| Control              | 0.331           | 13.950   | 0.260 | 11.60| 0.002 |
| **Rt DM ~ Orig SI:** |                 |          |       |      |
| Burned               | −0.001          | 3.033    | 0.000 | 0.01 | 0.940 |
| Clipped              | 0.063           | −9.285   | 0.172 | 7.06 | 0.012 |
| Control              | 0.090           | −6.811   | 0.370 | 19.40| < 0.001 |
| **Rt DM ~ LfDM:**    |                 |          |       |      |
| Burned               | 0.775           | −1.707   | 0.575 | 28.42| < 0.001 |
| Clipped              | 1.110           | −2.030   | 0.805 | 140.00| < 0.001 |
| Control              | 1.489           | −3.844   | 0.817 | 147.20| < 0.001 |

Total plant biomass at the third harvest (KW \( p = 0.06 \)), but the burned and control plants were still significantly different (Figure 3, Table 2).

Like total biomass, biomass of leaves, roots, and rhizomes increased for the controls, as compared to the initial harvest (Table 1). Leaf and root biomass of burned and clipped plants did not differ significantly at any harvest, but root biomass differed significantly from controls at both H1 and H2 (Table 2). Clipped plant leaf biomass differed from controls at the first harvest but not the second, while clipped plant root biomass was not significantly different from controls at either the first or second harvest. All treatments were similar for leaf and root biomass at the final harvest (Table 2). Rhizome biomass did not differ significantly among the treatments at H1, differed only between burned and control plants at H2, and was similar among all treatments at H3 (Table 2).

Total plant biomass was significantly correlated with both the original plant size index and treatment (Figure 4A, Table 3). A post-hoc Scheffe test for differences among treatments showed that total biomass of clipped and burned plants differed from control plants, but not from each other (Table 3). When total biomass of burned, clipped, and control plants was regressed
Figure 4. Regressions of *L. microphyllum* total dry weight (A.) and root dry weight (B.) vs. the original size index and of ln root dry weight vs. ln leaf dry weight (C.) for the post-treatment harvests of the burned (Burn), clipped (Clip) and control (Control) plants. Regression equations and statistics are given in Table 3.
against their original size index, clipped and control plants both had a positive slope with a significant correlation (Figure 4A, Table 3). Burned plants, however, had no significant relation to the original plant size (Figure 4A, Table 3).

Total root biomass was also significantly correlated with the original plant size index and treatment (Figure 4B). A post-hoc Scheffe test for differences among treatments showed that root biomass differed significantly among burned, clipped and control plants (Table 3). Regressions of burned, clipped and control plant root biomass against the original size index showed that root biomass of clipped and control plants were positively correlated with the original size index, whereas burned plants were not (Figure 4B, Table 3).

Ln root dry weight was significantly correlated to ln leaf dry weight for all treatments (Figure 4C, \( R^2 = 0.82, p < 0.01 \)). Slopes of the relationship, however, differed significantly among treatments, with control > clipped > burned (Figure 4C, Table 3).

Like biomass, leaf height, leaf number, number of shoot apices, internode length, and size index of harvested plants had early post-treatment differences between burned and clipped plants vs. controls, with later convergences (Table 2). The third harvest had no significant differences among treatments and controls for morphological variables except for differences in the size index (Table 2).

**Discussion**

Both burning and clipping immediately reduced the size of *L. microphyllum* plants, but effects of these treatments differed. Burning caused mortality in some plants, while clipping did not. The fire treatment varied from 200 to 600 °C with a relatively light fuel load. These temperatures are in the range of a temperature of 400 °C estimated from ash nutrients for a cattail/sawgrass marsh in southern Florida (Qian et al. 2009). They also are similar to the 488 ± 227 °C maximum temperature at the soil surface measured in central Florida scrub (Wally et al. 2006) and resemble what is found in other grasslands (e.g., 239 ± 61 to 410 ± 43 °C in *Themeda triandra* grasslands in Australia (Morgan 1999)) and shrublands (e.g., 140 to 480 °C in Scottish heath (Hobbs and Gimingham 1984)).

In the field, *L. microphyllum* plants grow especially large when they climb shrubs and trees. Fire temperatures can vary between grasslands and woody vegetation: temperatures in Alberta, Canada, grasslands were lower (186 ± 10 °C) than in shrublands (398 ± 16 °C) or aspen forests (393 ± 28 °C) and varied with fuel load as well as wind direction (Bailey and Anderson 1980). *Lygodium microphyllum*’s climbing can also alter temperature/fuel load relationships by accumulating more woody fuel (dead rachis mats) that can burn longer. Invaded woody canopies build up significant *L. microphyllum*
rachis and leaf biomass (O’Brien et al. 2010), possibly increasing fire duration and intensity. Canopy-derived fuels have also been shown to increase fire intensity on the ground in long-leaf pine forests in northern Florida (O’Brien et al. 2016). In southern Florida, woody sites that have *L. microphyllum* are often slightly elevated from the surrounding marsh and thus could be drier and burn more readily. Field burns, therefore, could be hotter and kill more and larger plants than our controlled experimental burn.

Plants, if still alive after treatment, began to re-grow within two weeks in both burned and clipped treatments. Burned plants recovered more slowly than clipped plants, while clipping stimulated growth, with clipped plants growing more rapidly than both burned and control plants (e.g., Figure 2C). The different responses of burned and clipped plants indicate that the effect of burning is not simply a reduction in leaf biomass and thus photosynthetic capacity. Fire must cause mortality through additional effects. Because aboveground biomass was completely removed by both burning and clipping, these additional effects were on the belowground rhizomes and roots. *Lygodium microphyllum* roots are formed adventitiously from the rhizomes during growth at the shoot apex (Mueller 1982). Plant roots are typically killed between 48 and 54 °C (Neary et al. 1999) and living cells, in general, die above 60 °C (Michaletz and Johnson 2007). Although in moderate to cool fires, heat does not penetrate more than 3–5 cm into the soil (Neary et al. 1999; Valette et al. 1994), temperatures at 2.5 cm level can be sufficient to kill roots, which would sever *L. microphyllum*’s root system from the parent rhizome, curtailing the plant’s access to water and nutrients. In addition, burning in woody plants has been shown to alter hydraulic conductivity by deforming the viscoelastic cell walls of xylem between 60 °C and 90 °C, thus reducing water conductance (Bar et al. 2018; Michaletz et al. 2012). In trees, these effects may not kill immediately but can cause decreased water flow and increased sensitivity to subsequent environmental stresses, especially drought (Bar et al. 2018; Michaletz et al. 2012), leading to fire-induced latent mortality.

Our burned plants showed increased mortality and reduced growth over time as compared to clipped and control plants, especially after the artificial drought induced by the watering system failure. Because ferns lack significant amounts of secondary growth, the root and rhizome can replace damaged xylem only by production of new growth from the shoot apices. If the old xylem had increased impedance to flow, access to previously available water and nutrients would be restricted. Both burned and clipped plants began to put out new leaves within two weeks of treatment, but the clipped plants put out more leaves that increased in height more rapidly than those of burned plants. Thus, plants in both treatments were initiating leaves, rhizomes, and roots from viable shoot apices, but growth of burned plants was restricted. The comparison of root biomass, as well as
comparison of the relative growth of roots to leaves (Figure 4C) both showed the reduced growth of the root system in the burned plants as compared to clipped and control plants. Additionally, the lack of correlation between total biomass and root biomass vs. the original size index (Figure 4A, B) suggests that burning at least partially disconnected the belowground root system from the shoot system. One hypothesis to explain differences in growth rate between clipped and burned plants is that clipped plants were able to draw on intact roots and rhizomes to supply water and nutrients to new growth, whereas burned plants lacked an intact hydraulic support system and therefore grew more slowly.

Should fire be used to manage L. microphyllum invasions?

The research reported here shows that a relatively cool fire (200–600 °C) kills 25% of L. microphyllum plants, reduces growth of most of the remaining plants, and increases their susceptibility to environmental perturbations. Sebesta et al. (2016) showed that heat above 65 °C reduces spore viability, while short (5 or 30 seconds) exposures to heat of at least 100 °C renders most spores non-viable. The use of fire as a management tool has been questioned because fire has been postulated to contribute to L. microphyllum invasion by drawing spores up into the air, thus increasing their dispersal potential (O’Brien et al. 2010; Stocker and Hupp 2008). We can infer from the distribution of L. microphyllum in invaded habitats, however, that the species is successfully spreading via long distance spore dispersal without the use of fire as a management tool (Ferriter and Pernas 2006; Volin et al. 2004; Wu et al. 2006; Philippi and Richards 2007). Additionally, spores dispersed by updrafts in fires probably have a large proportion of non-viable spores because of the heat effect on spore viability (Sebesta et al. 2016), reducing the effect of the postulated enhanced aerial dispersal. Additionally, spores collecting at or near the ground surface over time potentially form a spore bank, with spores retaining some viability for several years (Hutchinson 2010). This surface spore bank would be heated by a fire, likely destroying exposed spores and decreasing viability of spores near the soil surface (Ramírez Trejo et al. 2010), further reducing chances of recolonization.

The chance of colonization by a spore depends on the number of aerial spores dispersed, as well as on their viability. This number, in turn, depends on the number of spores produced and released, so burning reduces the chance of colonization by reducing the number of sporulating plants. For example, consider two L. microphyllum patches with the same size and same rate of spore production. If one patch is burned while the other remains unburned, the probability of producing and dispersing viable spores is greater in the unburned patch than the burned patch. In addition, the research described here shows that after burning, some plants
will have been killed, while the surviving plants will be less vigorous and more vulnerable to environmental perturbations for at least 1.5 years after burning. Thus, burning reduces the probability of spore spread from a patch and decreases the viability of spores released into the air, as compared to unburned sporulating patches of the same size. Finally, the effect of fire on spore production should also be integrated over time – i.e., spore production will be reduced in a burned patch until plants return to reproductive size and resume pre-fire levels of sporulation. Plants in an unburned patch will continue producing spores at the normal rate, potentially building both local and distant spore banks.

This landscape-level argument about the interaction of fire and spore dispersal by L. microphyllum integrates effects of fire on spore viability as well as on plant mortality and recovery from burning. The reasoning can be applied more broadly to similar concerns about dispersal by fire for any invasive plant that has very small diasporas, such as other invasive ferns (Robinson et al. 2010), or invasive orchids (Ackerman 2007; Kolanowska and Konowalik 2014). Upscaling concerns about lofting diasporas in fires should consider the larger-scale effects of burning vs. not-burning at the community or ecosystem level, as well as the effects of heat on the viability of the diaspore.

The slower growth of burned L. microphyllum plants indicates that burning, even if it does not kill plants, inhibits their growth for more than a year after burning. Thus, our initial hypothesis of growth equality between burning and mechanical removal was wrong: fire inhibits L. microphyllum re-growth well into a 2-to 3-year fire regime, whereas recovery from mechanical removal is more rapid. Our results support using fire to control L. microphyllum invasions. Remaining management questions are how native host communities respond to this fire frequency, as well as how these results from a controlled greenhouse experiment and from lab experiments on the effect of heat on spore viability apply under field conditions.

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